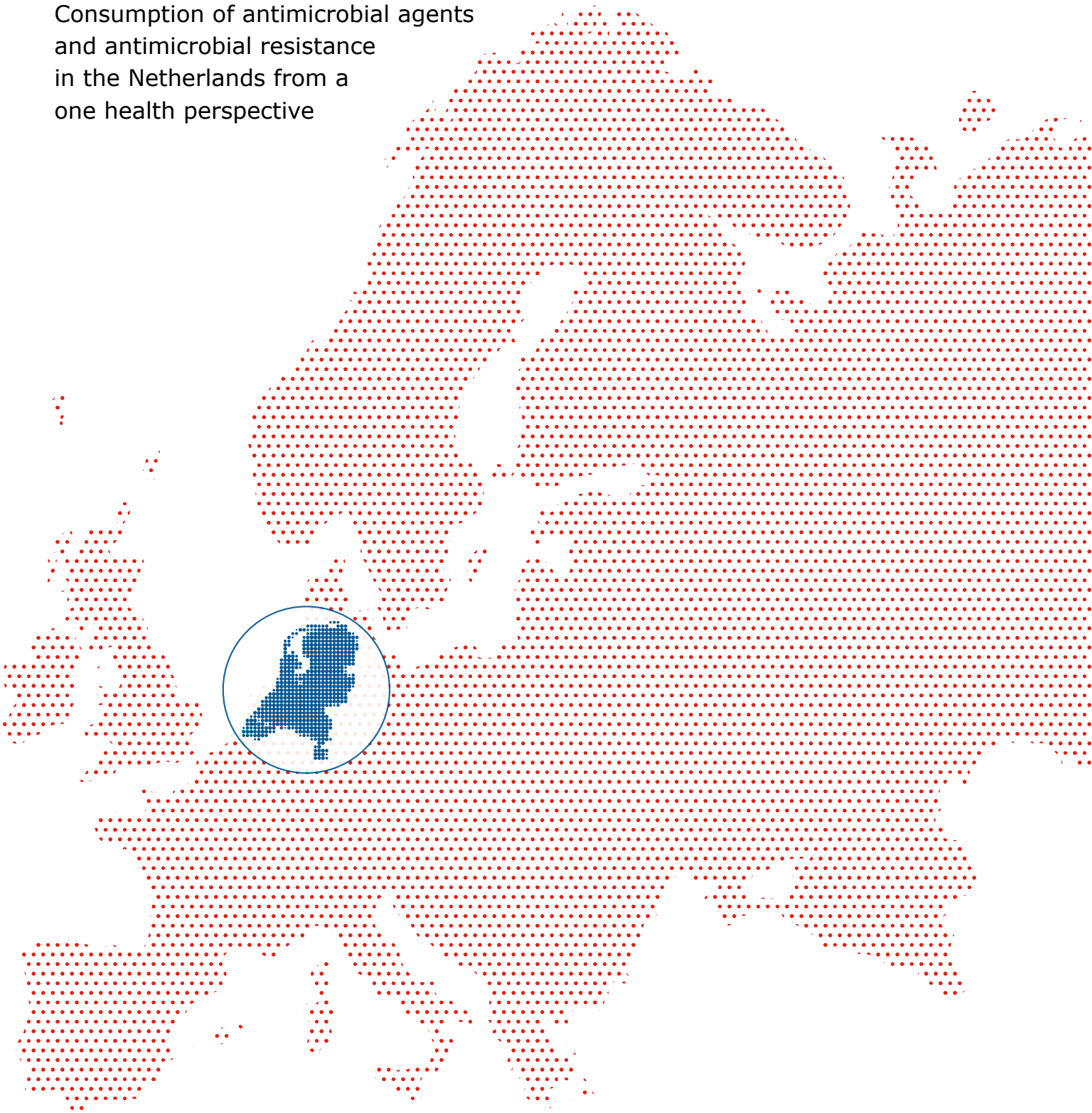
NethMap One Health 2025

Consumption of antimicrobial agents and antimicrobial resistance in the Netherlands from a one health perspective



National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

SWAB Stichting Werkgroep Antibioticabeleid

WAGENINGEN UNIVERSITY & RESEARCH

NethMap One Health 2025

RIVM report 2025-0122

Colophon

This report NethMap One Health is published by the Centre for Infectious disease control (CIb) of the National Institute for Public Health and the Environment of the Netherlands (RIVM) in collaboration with the Dutch Foundation of the Working Party on Antibiotic Policy (SWAB), and Wageningen Bioveterinary Research (WBVR).

NethMap One Health 2025 and earlier versions are also available from the website of the SWAB (www.swab.nl), RIVM (www.rivm.nl) and WBVR (https://www.wur.nl/maran).

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Synopsis

NethMap One Health 2025

In NethMap One Health 2025, a number of organisations present information on the use of antibiotics among people and animals in the Netherlands, as well as antibiotic resistance against pathogenic bacteria. Antibiotic resistance is a threat to people's health because treating an infection with resistant bacteria is usually harder. People and animals carry resistant bacteria, and they are also present in food and the environment. This is why it is crucial for all experts involved to work together to combat antibiotic resistance. Starting this year, this one health approach will be emphasised more strongly in the report.

Every year, for each type of bacteria, the percentage of bacteria resistant to antibiotics in the Netherlands is calculated. Antibiotic resistance is still uncommon in the Netherlands, but in 2024, a greater percentage of some bacteria was found to be resistant. This was the case in particular among bacteria that can, for example, cause severe urinary tract infections. There are several reasons for this, and this development can be observed throughout Europe. Resistance was also observed more frequently for bacteria that cause, among other things, skin infections (*Staphylococcus* bacteria).

In 2024, in GP practices, hospitals and nursing homes, antibiotics were prescribed at approximately the same rate as in the previous year. On the other hand, the amount of antibiotics used did differ within types of care institution, for example between different nursing homes. Moreover, more last-resort antibiotics were used in hospitals in recent years. These are only used for severe infections once standard antibiotics no longer suffice.

In Dutch livestock farming in 2024, antibiotics were used at approximately the same rate as in previous years, following a significant decrease since 2009. Antibiotic resistance among animals also remained the same. Only in exceptional cases livestock is treated with antibiotics that are essential to humans. As a result, resistance against these antibiotics is uncommon among animals.

Incorrect and unnecessary use of antibiotics should be prevented as much as possible, because this could make bacteria resistant to antibiotics. For this reason, responsible use of antibiotics for people and animals remains crucial. In addition, measures to prevent the spread of resistant bacteria, such as washing our hands and other hygienic measures, remain necessary.

Keywords: one health, AMR, antimicrobial stewardship, antibiotics use, bacteria, infection

Publiekssamenvatting

NethMap One Health 2025

In NethMap One Health 2025 presenteren verschillende organisaties informatie over het gebruik van antibiotica en antibioticaresistentie tegen ziekmakende bacteriën bij mensen en dieren in Nederland. Antibioticaresistentie is een bedreiging is voor de gezondheid van mensen omdat een infectie met een resistente bacterie vaak minder goed is te behandelen. Resistente bacteriën komen bij mensen en dieren voor, in voeding en in het milieu. Daarom is het noodzakelijk dat alle betrokken experts samenwerken om antibioticaresistentie tegen te gaan. Deze *one health*-benadering is vanaf dit jaar meer benadrukt in de rapportage.

Elk jaar wordt in Nederland per bacteriesoort berekend welk percentage van de bacteriën resistent is tegen antibiotica. In Nederland komt antibioticaresistentie nog steeds vrij weinig voor, maar in 2024 is van sommige bacteriën een groter deel resistent geworden. Dat is vooral zo bij bacteriën die bijvoorbeeld ernstige urineweginfecties kunnen veroorzaken. Dit heeft verschillende oorzaken en is in heel Europa te zien. Verder is vaker resistentie gezien bij bacteriën die onder andere huidinfecties veroorzaken (Staphylococcus-bacterie).

In huisartsenpraktijken, ziekenhuizen en verpleeghuizen is in 2024 ongeveer even vaak antibiotica voorgeschreven als in het jaar ervoor. Wel verschilde het gebruik bij hetzelfde type zorginstellingen, bijvoorbeeld tussen verpleeghuizen. Verder zijn de laatste jaren in ziekenhuizen meer laatste-redmiddel-antibiotica gebruikt. Deze soorten worden pas gebruikt als de standaard antibiotica niet meer helpen bij een ernstige infectie.

In de Nederlandse veehouderij zijn in 2024 ongeveer evenveel antibiotica gebruikt als in afgelopen paar jaren, na een flinke daling sinds 2009. Ook is de antibioticaresistentie bij dieren hetzelfde gebleven. Landbouwhuisdieren krijgen alleen bij hele hoge uitzondering antibiotica die voor mensen onmisbaar zijn. Hierdoor komt resistentie tegen deze antibiotica bij dieren weinig voor.

Onjuist en onnodig gebruik van antibiotica moet zo veel mogelijk worden voorkomen, omdat bacteriën hierdoor resistent kunnen worden tegen antibiotica. Daarom blijft het belangrijk om antibiotica bij mensen en dieren op de goede manier te gebruiken. Ook blijven maatregelen noodzakelijk die voorkomen dat resistente bacteriën zich verspreiden, zoals handen wassen en andere hygiënemaatregelen.

Kernwoorden: one health, ABR, antimicrobial stewardship, antibioticagebruik, bacteriën, infectie

Contents

1 Introduction

This is NethMap One Health, the annual report presenting trends in the consumption of antimicrobial agents and antimicrobial resistance in the Netherlands from a One Health perspective. Previously, we presented trends in the human domain (NethMap) back-to-back with trends in the veterinary domain (Maran), but this year we have changed the structure of the report to achieve a more integrated overview of antimicrobial resistance (AMR) and antimicrobial consumption (AMC) in humans and animals in the Netherlands.

The main aim of surveillance of AMR and AMC in the Netherlands is to halt the emergence and limit the spread of AMR. Key objectives of NethMap One Health are to deliver essential data to medical doctors, veterinarians, and other health professionals to support the development and continuous improvement of evidence-based antibiotic treatment guidelines. Secondly, it acts as a comprehensive knowledge base for authorities, academia, and policymakers, supporting risk assessment and management, and thus facilitating informed decision-making in the prevention and control of resistant bacterial infections. Lastly, AMR and AMC surveillance identifies areas for further research, such as antimicrobial resistance transmission dynamics or possible associations between antimicrobial consumption and resistance.

NethMap One Health is compiled under the editorial supervision of the Dutch Working Group on Antibiotic Policy (SWAB; Stichting Werkgroep Antibiotica Beleid), Wageningen Bioveterinary Research (WBVR) and the National Institute for Public Health and the Environment (RIVM).

The SWAB was founded in 1996 as an initiative of The Netherlands Society for Infectious Diseases, The Netherlands Society of Hospital Pharmacists and The Netherlands Society for Medical Microbiology. SWAB is fully funded by a structural grant from the CIb, on behalf of the Ministry of Health, Welfare and Sports. The SWAB plays a central role in promoting the rational use of antimicrobial agents in the Netherlands. SWAB's primary task is to develop evidence-based guidelines for antimicrobial therapy in humans, thereby supporting clinicians in making informed decisions and contributing to the containment of antimicrobial resistance. In addition, SWAB coordinates antibiotic stewardship activities, facilitates interdisciplinary collaboration and advises policymakers on issues related to antimicrobial stewardship, antimicrobial consumption and antimicrobial resistance.

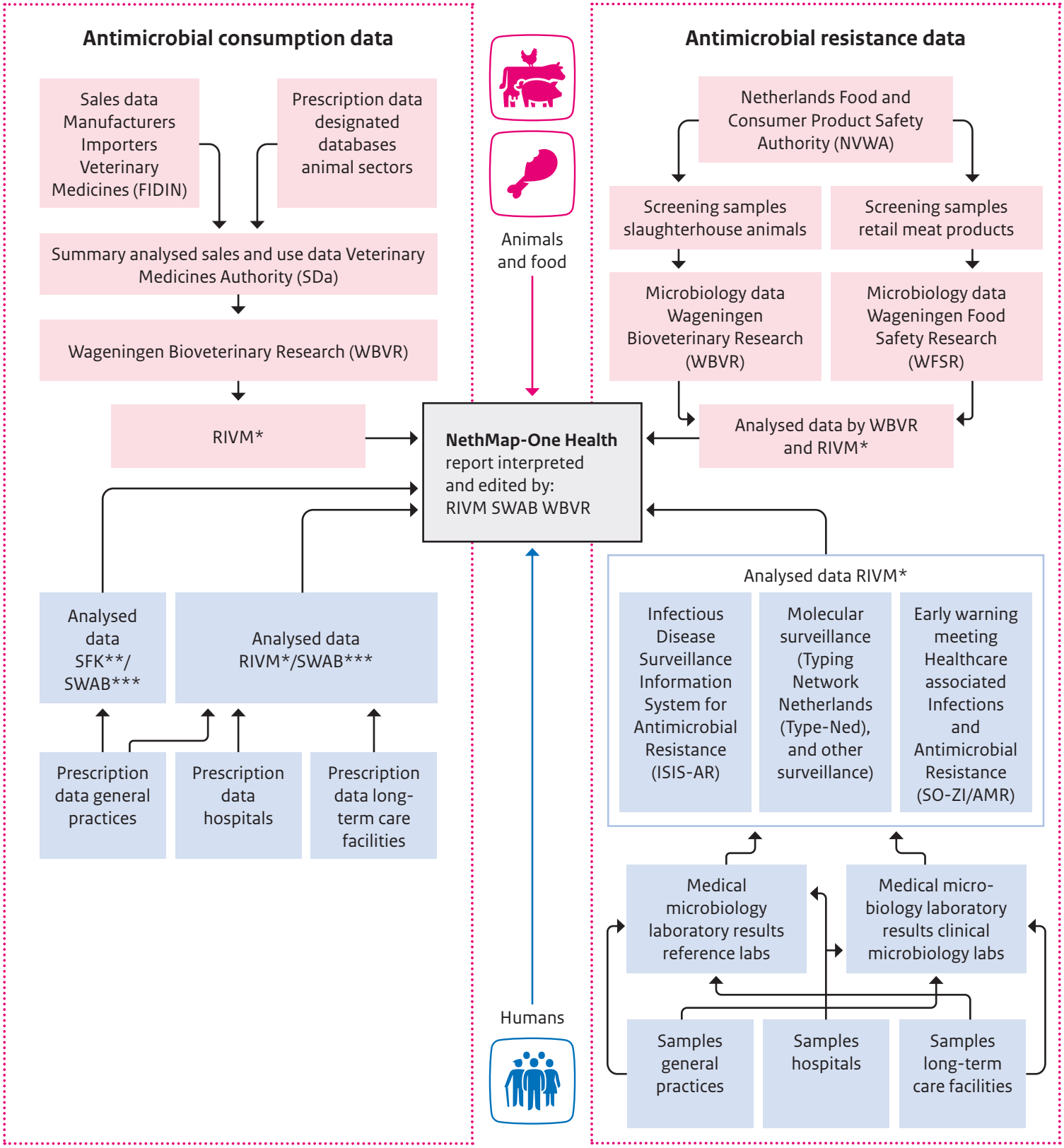
Wageningen Bioveterinary Research (WBVR) monitors and researches antimicrobial resistance in animals in the Netherlands. WBVR conducts surveillance to track resistance trends, investigates the development and spread of resistance, and advises veterinarians and policymakers on antibiotic stewardship. The results from the veterinary domain presented in this report have been generated through collaboration between WBVR, the Food and Consumer Product Safety Authority (NVWA), Wageningen Food Safety Research (WFSR), Veterinary Medicines Institute (SDa), the Faculty of Veterinary Medicine, Utrecht University

(FD) and the Centre for Infectious Disease Control Netherlands (CIb) at the RIVM.

The CIb at the RIVM coordinates surveillance on antimicrobial resistance and consumption in humans in the Netherlands to inform the general public, professionals and policy makers about potential national health threats with regard to antimicrobial resistance and to guide healthcare professionals and policymakers on the control of AMR. Furthermore, the CIb subsidizes surveillance programs that focus on the monitoring of specific pathogens, or specific resistance mechanisms. By collaborating with national and international partners, CIb works to prevent the spread of AMR and protect public health.

Figure 1 provides a schematic overview of the data flows from the general surveillance systems from which the results are presented in this report. All results presented in this report have been made possible through the collaboration of numerous partners in both the human healthcare and veterinary sectors. We are very grateful for the valuable contributions and commitment of all involved parties and express our hope that they are willing to continue their important clinical and scientific support to NethMap One Health and thereby contribute to the general benefit and health of the people.

Figure 1 Flowchart NethMap-One Health



*RIVM Dutch National Institute for Public Health and the Environment
**SFK Foundation for Pharmaceutical Statistics
***SWAB Dutch Working Party on Antibiotic Policy

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2 Extensive summary

This chapter provides a summary of the findings described in this report and relevant conclusions with respect to antimicrobial use, policy and resistance surveillance in both humans and farm animals.

2.1 Most important trends in antimicrobial consumption in humans

Outpatient use

- Total outpatient antibiotic use increased by 2.7%, from 8.78 DID in 2023 to 9.02 DID in 2024.
- The most frequently prescribed antibiotics were **doxycycline, amoxicillin,** and **nitrofurantoin**.
- Since 2018, the most pronounced increases have been observed in the use of **lincosamides, sulfonamides combined with trimethoprim,** and **beta-lactamase-resistant penicillins**.
- The evaluation of antibiotic prescribing quality at general practitioner (GP) practice level shows that there is a wide variation between GP practices in the quantity and quality of antibiotic prescribing.

Inpatient use

- For the first time since 2021, the DID has decreased. The trend of shorter hospital stays and more intensive treatment regimens per patient during hospitalization seems to have come to a halt for now.
- The use of systemic antibiotics in inpatient care decreased by 1.1%, from 92.0 defined daily doses (DDD) per 100 patient-days in 2023 to 91.0 in 2024.
- **Flucloxacillin** remained the most commonly used antibiotic in hospital, followed by **ceftriaxone** and **cefazolin**.
- Over time, an upward trend is evident for **flucloxacillin**, **all generations of cephalosporins, meropenem**, and **vancomycin**.
- The use of **amoxicillin/clavulanic acid** appears to be decreasing.

AWaRe classification

- 71.7% of antibiotics consumed in outpatient and inpatient settings in the Netherlands are classified within the 'Access' group, indicating that the WHO’s (65%) 2030 target has already been met. Continued vigilance is required to ensure that this achievement is sustained.

Long-term care facilities (LTCFs)

- Antibiotic use in LTCFs increased from 47.1 in 2023 to 53.1 DDD/1,000 residents/day in 2024.
- The most commonly used antibiotics were combinations of **penicillins including beta-lactamase-inhibitors, macrolides** and **nitrofuran derivatives**.

Antimicrobial stewardship in hospitals

- Since 2014, all hospitals have established antimicrobial stewardship teams (A-teams) responsible for implementing an antimicrobial stewardship program.
- Overall antibiotic use (DOT/100 patient days) varied across hospitals and was generally higher in academic hospitals than in non-academic hospitals, particularly for broad-spectrum antibiotics such as **carbapenems**, **quinolones** and **glycopeptides.** This likely reflects differences in patient case-mix, greater prevalence of multidrug resistant organisms and differences in prescribing practices.
- Usage of restricted antimicrobials varied notably across the 22 hospitals, with non-academic hospitals showing the greatest variability, particularly **amoxicillin-clavulanic acid** use (with a maximum relative use up to 17.9%).
- Use of (last resort) antibiotics **aztreonam, cefiderocol** and **ampicillin-sulbactam** remained low (< 0.1% of all courses) throughout 2022-2024 in both academic and non-academic hospitals.
- In the coming years, the Antimicrobial Stewardship Monitor (AMSM) will provide increasingly active feedback to support and encourage improvement initiatives at the hospital level.

2.2 Most important trends in antimicrobial consumption in animals

- In 2024, a total of 121 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is an increase of 4.0% compared to 2023. However, the antibiotic reduction policies in the Netherlands has resulted in 75.5% reduction of sales of Antimicrobial Veterinary Medicinal Products (AVMPs) for veterinary use since 2009, leveling off during recent years.
- Although a small increase of mass active substances in the sales of AVMPs in the Netherlands in 2024 is noted, this increase is not observed in used mass of active substances calculated based on the use monitoring data. The calculation of consumption, based on national conversion factors (DDDAs) of authorized veterinary medicinal products shows that use has stabilized in most sectors.
- The use of antibiotics of critical importance to human health care (especially **cephalosporins** of 3rd and 4th generation) is low, even in the unmonitored sectors. Use and sales of **polymyxins** decreased again in 2024, overall decrease since 2011 is 86% in sales. Of the **fluoroquinolones**, 48% is applied in sectors not yet monitored; an overall decrease of 92.1% since 2011 is observed.

2.3 Most important trends in antimicrobial resistance and implications for treatment in humans

2.3.1 General AMR surveillance

2.3.1.1 General practices

Urinary tract infections - Enterobacterales

- In *Escherichia coli*, resistance levels for **nitrofurantoin** and **fosfomycin**, i.e. respectively first and second choice antibiotics for the treatment of uncomplicated UTI in adults in primary care, were stable and low (≤2%) over the last 5 years. For **trimethoprim**, third choice antibiotic for the treatment of uncomplicated UTI in adults, resistance was 21%.
- Resistance levels for **ciprofloxacin**, first choice antibiotic for the empirical treatment of complicated UTI in adults in primary care, was stable at 11% or lower for all Enterobacterales and *Pseudomonas aeruginosa*. Resistance levels for **co-amoxiclav**, second choice antibiotic for the empirical treatment of complicated UTI in primary care, was 26% in *E. coli* and 14% in *Klebsiella pneumoniae*. Resistance levels for **co-trimoxazole**, third empirical choice antibiotic for this indication, was 19% in *E. coli* and 9% in *K. pneumoniae* and remained stable over the last five years.
- Combined resistance for **co-amoxiclav, ciprofloxacin,** and **co-trimoxazole** in all Enterobacterales was low (≤3%).

Skin and soft tissue infections – *S. aureus* and *β*-haemolytic *Streptococci*

- Antibiotic resistance levels in *Staphylococcus aureus* were relatively low except for **clindamycin** (including inducible resistance, 14%), **erythromycin** (15%) and **fusidic acid** (26%).
- Interestingly, resistance to **clindamycin** without considering inducible resistance was 1.7%. According to EUCAST, **clindamycin** may still be used for short-term therapy in less severe skin and soft tissue infections if tested susceptible, even in the presence of an inducible resistance mechanism.
- A worrisome increase in resistance to **fusidic acid** (26%) and **mupirocine** (3%) was found in *S. aureus* over the last 5 years. This likely reflects a real rise in resistance; however, the relatively high percentages may also be influenced by a selective sampling population, as cultures are often obtained from patients who have failed **fusidic acid** or **mupirocine** therapy. In addition, for primary care we selected only *S. aureus* isolates from skin infections, whereas in hospital populations isolates from a wider range of clinical specimens were included.
- **MRSA** was found in 4% of isolates of primary care patients which was stable over the previous 5 years.
- Resistance to **doxycycline/tetracycline** in *β*-haemolytic *Streptococcus* group A (24%) and group B (76%) is concerning. In combination with the high rate of **clindamycin** resistance in *β*-haemolytic *Streptococcus* group B (including inducible

resistance, 17%), this complicates treatment options for patients with a beta-lactam allergy.

Respiratory infections – *S. pneumoniae*

- Resistance to **doxycycline/tetracycline** (13%) and **erythromycin** (17%) in *S. pneumoniae* was high in GP patients, most probably due to the more frequent use of these antibiotics in primary care and selective sampling of isolates.

2.3.1.2 Outpatient departments

Urinary tract and abdominal infections - Enterobacterales and *P. aeruginosa*

- For both Enterobacterales and *P. aeruginosa*, resistance levels for **all tested antibiotics** were higher in isolates of OPD patients compared to resistance levels in isolates from primary care patients.
- For the three most important oral antibiotics used in OPD setting, **co-amoxiclav, co-trimoxazole** and **ciprofloxacin**, no significant increase in resistance levels were found. Resistance to **co-amoxiclav** was 29% in *E. coli* and 18% in *K. pneumoniae*. Resistance to **co-trimoxazole** was 23% in *E. coli* and 14% in *K. pneumoniae*. Resistance levels for **ciprofloxacin** were 15% in *E. coli*, 14% in *K. pneumoniae* and 10% in *P. aeruginosa*. In the majority of cases, at least one oral treatment option remained with these three antibiotics, given the relatively low (≤6%) combined resistance rates.
- In *K. pneumoniae*, resistance to **third generation cephalosporins** increased significantly over the last 5 years (10%).
- Although **meropenem/imipenem** resistance in *Klebsiella pneumoniae* was less than 1%, there was an increase over the last five years from 0.05% to 0.2%.

Skin and soft tissue infections – *S. aureus*

- In *S. aureus* isolates of OPD patients, resistance to **levofloxacin, doxycycline**, and **co-trimoxazole** was less than 4%.
- Resistance to **clindamycin** (including inducible resistance) was 18%. Interestingly, **clindamycin** resistance without considering inducible resistance was 3%, which makes **clindamycin** still useful for short-term therapy in less severe skin and soft tissue infections if tested susceptible, even in the presence of an inducible resistance mechanism.
- Resistance to **fusidic acid** (9%) and **mupirocine** (0.9%) was lower in *S. aureus* isolates of OPD patients than in *S. aureus* isolates of GP patients (26% and 3%). This difference might be attributed to two factors: first, isolates in OPD patients are obtained not only from skin infections but also from a variety of other clinical specimens; and second, in primary care a relatively larger proportion of cultures are derived from patients in whom **fusidic acid** or **mupirocine** therapy has failed, which increases the likelihood of detecting resistant strains.

- **MRSA** was found in 2% of isolates of OPD patients which was stable over the previous 5 years.

2.3.1.3 Hospital departments

Inpatient hospital departments (excl. ICU)

Blood, abdominal, urinary tract, skin and respiratory infections – Enterobacterales, *P. aeruginosa* and *Acinetobacter baumannii/calcoaceticus* complex

- For both Enterobacterales and *P. aeruginosa*, resistance levels for **all tested antibiotics** were comparable to resistance levels in isolates of OPD patients.
- For *E. coli* resistance to **second and third generation cephalosporins** seemed to have plateaued over the past five years. Resistance to **cefuroxime** was 12% and to **cefotaxime/ceftriaxone** 7%.
- For *K. pneumoniae*, there was a significant increase in resistance to **cefotaxime/ceftriaxone** (10%). Resistance to **cefuroxime** was 15%. This means that patients that are infected *with K. pneumoniae* have a considerable risk of non-adequate empiric treatment with a **second or** (to a lesser extent) **third generation cephalosporin**. In case of severe infection, empiric combination therapy with **aminoglycosides**, reduced likelihood of resistance to 4%.
- Although **meropenem/imipenem** resistance in *Klebsiella pneumoniae* was less than 1%, there was a significant increase over the last five years from 0,1% to 0.4%.
- For *P. aeruginosa*, resistance was relatively low and stable for **all antibiotics** over the last five years.
- A significant increase in resistance to **meropenem/imipenem** (2%), **ciprofloxacin** (5%) and **tobramycin** (3%) in *Acinetobacter baumannii/calcoaceticus* complex isolates were found over the last five years.

Blood, urinary tract, abdominal, skin and respiratory - *S. aureus*, β-haemolytic *Streptococcus* spp. groups A, B and C/G

- In *S. aureus* resistance was high for **clindamycin** (including inducible resistance, 18%) and increased significantly over the last five years. This indicates that culture and susceptibility testing are advised before starting treatment with this drug. However, when inducible **clindamycin** resistance was ignored, only 3% of isolates were resistant which indicates that **clindamycin** may still be used for short-term treatment in less severe skin and soft tissue infections.
- **MRSA** was found in 3% of *S. aureus* isolates of hospital patients, which remained stable over the previous 5 years.
- In β-haemolytic *Streptococcus* spp. group A, resistance to **clindamycin** and **erythromycin** remained stable at 6% and 9%, respectively. Resistance to **doxycycline/tetracycline** was 25%.
- In β-haemolytic *Streptococcus* spp. group B and C/G, resistance levels for **clindamycin** (19% group B, 14% group C/G), were higher than for group A.

Respiratory infections - *S. pneumoniae* and *Haemophilus influenzae*

- The proportion of *S. pneumoniae* isolates in the I-category for **(benzyl)penicillin** appears to be increasing and significantly in hospital patients to 9%.
- Resistance to **doxycycline/tetracycline** and **macrolides** in *S. pneumoniae* was 10% hospital patients, which was lower than in GP patients, most probably due to the more frequent use of these antibiotics and/or more selective sampling of isolates in primary care.
- Resistance to **co-amoxiclav** in *H. influenzae* isolates from hospital patients remained stable at 12%. However, resistance to **cefuroxime** increased significantly over the last five years to 14%.

Intensive Care Units

Blood, urinary tract, blood, abdominal, skin and respiratory - Enterobacterales*, P. aeruginosa*

- For Enterobacterales and *P. aeruginosa*, resistance levels for **beta-lactam antibiotics** were higher in intensive care unit (ICU) patients than in isolates from non-ICU patients.
- In *K. pneumoniae*, resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 21% and 17%, respectively, and increased slightly over the last five years. In *E. coli*, resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 17% and 11%, respectively. This means that ICU patients with infections due to *K. pneumoniae* and *E. coli* had considerable risk of non-adequate empiric treatment with **a second or a third generation cephalosporin**. In case of severe infection, in *E. coli*, empiric combination therapy with an **aminoglycoside**, reducing likelihood of resistance to 3%, which might be a suitable strategy. For *K. pneumonia*, empiric combination therapy with an **aminoglycoside** may result in 8% risk of treatment failure, which represents a substantial risk that warrants close monitoring over the coming years.
- A worrisome increase in resistance to **meropenem/imipenem** in *K. pneumoniae* was found. Resistance increased from 0.3% to 2% over the last five years.
- In *P. aeruginosa* isolates from ICU patients, resistance to **piperacillin-tazobactam** and **ceftazidime**, the two first choice agents for the treatment of severe *P. aeruginosa* infections, was 14% for **piperacillin-tazobactam** and 9% for **ceftazidime**, which is much higher than in isolates from other hospital departments. This might complicate empirical treatment of severe infections due to *P. aeruginosa* in the ICU.

Blood, urine, blood, wound/pus, and respiratory - *S. aureus* and *E. faecium*

- **MRSA** percentage in clinical *S. aureus* isolates of ICU patients increased to 4% over the last five years, but not significantly.
- The percentage of **vancomycin** resistance in clinical *E. faecium* isolates of ICU patients increased significantly to 1.2% over the last five years.

Blood isolates from inpatient departments (incl. intensive care units)

- In *K. pneumoniae*, resistance to **cefuroxime and cefotaxime/ceftriaxone** was 16% and 13%, and in *E. coli*, resistance to **cefuroxime and cefotaxime/ceftriaxone** was 13% and 9%. Patients with a bloodstream infection with *K. pneumoniae* or *E. coli* thus have a considerable risk of non-adequate empiric treatment with a **second** or (to a lesser extent) **third generation cephalosporin**. In case of severe infection, empiric combination therapy with an **aminoglycoside**, reducing likelihood of resistance to 2% in *E. coli*. which might be a suitable option. However, for *K. pneumonia*, empiric combination therapy with an **aminoglycoside** may result in 6% risk of treatment failure, a significant increase compared to last year (3%), which represents a substantial risk that warrants close monitoring over the coming years.
- Empirical combination therapy with **cephalosporins and ciprofloxacin** appears to provide little additional benefit over treatment with a second- or third-generation cephalosporin alone.
- *K. pneumoniae* from blood cultures showed a significant increase in resistance to **meropenem/imipenem** of 0.7%.
- After initial iv treatment, in patients with *E. coli* bacteremia, a switch to either **ciprofloxacin, co-trimoxazole, or co-amoxiclav** was most often possible given the relatively low (≤4%) combined resistance rates for these oral agents. However, in patients with *K. pneumoniae* bacteremia, there was a significant increase in **multidrug resistance** of these oral agents to 9% over the last five years.
- **MRSA** was found in 2% of *S. aureus* isolates in blood cultures which remained stable over the previous 5 years.
- **Vancomycin** resistance was found in 0.7% of *Enterococcus faecium* isolates in blood cultures which increased significantly over the previous 5 years.

Urology services

- Resistance levels in Enterobacterales and *P. aeruginosa* from patients in urology services traditionally have been higher than in non-urology patients.
- Resistance to **ciprofloxacin** (23%) and **co-trimoxazole** (28%) in *E. coli* from admitted patients remains a problem.
- A worrisome increase in resistance to almost **all antibiotics** was seen for *K. pneumoniae* from admitted patients over the last five years: resistance to **ciprofloxacin and co-trimoxazole** increased to 24% and 22%; resistance to **cefuroxime** increased

to 23% and **cefotaxime/ceftriaxone** to 19%; resistance to **meropenem/imipenem** increased to 1.1% and **gentamicin** to 10%.
- Resistance to **ciprofloxacin** in *P. aeruginosa* in admitted patients has increased 13% over the last five years. This is a problem as ciprofloxacin is the only available oral agent to treat *P. aeruginosa* infections.

2.3.1.4 Long-term care facilities

- Resistance levels in *E. coli*, *K. pneumoniae* and *P. aeruginosa* urine isolates from selected LTCF patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients.
- Resistance levels for **ciprofloxacin** have significantly decreased to 14% in *E. coli* and was 12% in *K. pneumoniae* and 9% in *P. aeruginosa*. Resistance levels for **co-amoxiclav** decreased to 30% in *E. coli* and to 17% in *K. pneumoniae*. Resistance levels for **co-trimoxazole** was 18% in *E. coli* and 9% in *K. pneumoniae*.
- Resistance levels in *S. aureus* isolates from LTCF patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients, with the exception of resistance to **ciprofloxacin** (17%), which was much higher in *S. aureus* from LTCF patients than in *S. aureus* from GP (3%), OPD (4%), hospital (4%) and ICU patients (3%).

2.3.2 Specific AMR surveillance

Extended spectrum beta-lactamases
- From 2015 to 2024, the proportion of extended spectrum β-lactamase (ESBL) *E. coli* increased in GP (4%), OPD (6%), inpatient departments (7%) and ICUs (10%).
- From 2015 to 2024, the proportion of ESBL-producing *K. pneumoniae* significantly increased in GP (6%), and OPD (9%). The percentage ESBL-producing *K. pneumoniae* in the inpatients department population shows a peak in 2024, which is for the first time above 10%. In the ICUs, the proportion of ESBL-producing *K. pneumoniae* remained high (15%).

Carbapenem-resistant and carbapenemase-producing Enterobacterales
- The prevalence of **carbapenem** resistance in diagnostic isolates of *E. coli* was 0.01% in 2024, which was comparable to previous years. **Carbapenem** resistance in *K. pneumoniae* is significantly higher compared to *E. coli*, and has increased from 0.04% in 2020 to 0.2% in 2024 for all healthcare settings combined.
- The RIVM received 696 Enterobacterales isolates that produced carbapenemase, which was considerably higher than in previous years.
- The most frequently identified carbapenemase encoding genes in Enterobacterales were *bla*$_{\text{OXA-48}}$-like (24%), *bla*$_{\text{NDM-5}}$ (18%) and *bla*$_{\text{OXA-48}}$ (15%).

- In 38% of patients, there was a relation with hospitalization abroad for more than 24 hours during the two months prior to sampling.

Carbapenem-resistant and carbapenemase-producing *P. aeruginosa*

- In 2024, 5% of *P. aeruginosa* in diagnostic isolates were resistant to **carbapenems**. The percentage of **carbapenem**-resistant *P. aeruginosa* isolates was higher among *P. aeruginosa* isolates from patients in ICU compared to other departments (10%). In ICU patients, this proportion increased significantly over the last five years.
- The RIVM received 76 *P. aeruginosa* isolates that produced carbapenemase.
- The predominant (41%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was $bla_{\text{VIM-2}}$. In contrast, in 2022-2023 the dominant carbapenemase encoding allele in CPPA was $bla_{\text{NDM-1}}$.

Carbapenem-resistant *Acinetobacter baumannii-calcoaceticus* complex

- In 2024, 1.2% of *A. baumannii-calcoaceticus* complex in diagnostic isolates were resistant to **carbapenems**. The percentage of **carbapenem**-resistant *A. baumannii-calcoaceticus* isolates from inpatient departments increased significantly over the past five years (4%).
- The RIVM received 63 *A. baumannii-calcoaceticus* complex isolates that produced carbapenemase.
- The 2024 results of the enhanced CRAB surveillance submitted via Type-Ned revealed a genetically highly diverse, and highly resistant CRAB population in the Netherlands.
- The predominant (48%, 29/61) carbapenemase-encoding gene combination in *A. baumannii-calcoaceticus* complex was $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$, and 11% (7/61) carried a bla_{NDM}-like carbapenemase.

Methicillin-resistant *S. aureus*

- The overall proportion of routinely collected diagnostic *S. aureus* isolates that were MRSA positive in 2020-2024 was still at a low level of 3%. A higher proportion of 4% was seen for cultures requested by GPs and for ICUs.
- LA-MRSA MC0398 is no longer the predominant MRSA clade. With MC0008, MC0005 and MC0398 constituting 19%, 16% and 10% of the MRSA isolates in 2024, the most frequently identified MLVA-complexes are more equally distributed.
- In 2024, 40% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 19% of the screening isolates were PVL-positive.

Vancomycin-resistant *E. faecium*

- The proportion of VRE in diagnostic isolates with *E. faecium* in various healthcare settings in 2024 was still low (below 1%), except from ICU patients (1.2%).
- The absolute number of positive screening VRE_{fm} isolates continued to increase compared to the pre-COVID-19-period.

Healthcare associated Infections and Antimicrobial Resistance

- In 2024, 50 outbreaks were reported to SO-ZI/AMR. This number was higher than in 2020-2023, and comparable to pre-COVID years.
- The number of VRE outbreaks in hospitals was notably higher compared to previous years (n=14).
- Most outbreaks were related to MRSA of which approximately half of the outbreaks were related to pediatric or neonatal wards.

Neisseria meningitidis

- The number of invasive *N. meningitidis* isolates in recent years has nearly reached pre-COVID-19 levels.
- **Penicillin** resistance in *N. meningitidis* isolates is rare (<3%) in the Netherlands.

Neisseria gonorrhoeae

- A significant increase of gonorrhea diagnoses was reported in 2024 (13.952 diagnoses) compared to 2020 and before (around 6.000 diagnoses)
- No resistance to **ceftriaxone**, the current first-line treatment for gonorrhoea, has been reported. However, the MIC distribution has shifted towards higher MICs since 2020.
- Resistance to **ciprofloxacin** yearly increases and more than doubled since 2016, to 59.2% in 2024, despite the fact that **ciprofloxacin** is not prescribed for gonorrhoea, according to guidelines.

Mycobacterium tuberculosis

- In 2024, 13% of *M. tuberculosis* isolates contained resistance to one or more antimycobacterial drug. This mainly concerned resistance to **isoniazid**.
- The number of **multi-drug resistant** isolates was stable in recent years and represented 2.4% of culture positive cases.

Helicobacter pylori

- Although probably biased towards higher resistance levels due to sampling policies, resistance in *H. pylori* remained high for **levofloxacin** (24%), **clarithromycin** (53%) and **metronidazole** (51%) over the last five years.
- Resistance to **amoxicillin/ampicillin** (7%) and **doxycycline/tetracycline** (2%) traditionally has been low.
- For the treatment of *H. pylori* infections, first choice combination treatment consists of **amoxicillin and clarithromycin**, of which combined resistance was 5% in 2024. If treatment fails, a combination of **tetracyclin plus metronidazole** (1%) or **amoxicillin plus metronidazole** (4%) is recommended.

Clostridioides difficile

- No *C. difficile* infection (CDI) outbreaks were notified to the national CDI Expert Center in 2024.
- The increase in severe CDI cases and community-onset CDI that was found in 2022 and 2023 was not sustained in 2024.

- The hypervirulent RT027 and the newly reported **metronidazole**-resistant RT955 strains were not found in the Netherlands.

Aspergillus fumigatus and *Candida auris*

- **Azole** resistance among *A. fumigatus* remains stable in the Netherlands with similar rates in 2024 compared to previous years. **Azole** resistance in 2024 was 9.4% in five UMCs and 4.6% in five teaching hospitals.
- Overall, 81.5% of **azole**-resistant *A. fumigatus* isolates harbored a TR-mediated resistance mechanism.
- The first *C. auris* case was identified in the Netherlands in March 2018, and since then 26 cases with *C. auris* were reported by March 2025. The cases were reported by 22 different hospitals, and two patients had invasive *C. auris* infection, while the remaining patients were colonized.

Shigella

- In 2024, *S. sonnei* and *S. flexneri* were the most frequently identified species, comprising 51.4% and 38.8% of the isolates, respectively.
- For most antibiotics, resistance levels were higher among the men-who-have-sex-with-men (MSM) population compared to the overall population.
- Over the past five years, resistance to **third generation cephalosporins** and **ciprofloxacin** in *S. sonnei* isolates has been increasing. Multi-drug resistance has increased from 0% in 2020 to 9% in 2024.
- Over the past five years, there has been an upward trend in *S. flexneri* resistance to **ciprofloxacin** (52%) and **third generation cephalosporins** (35%).

2.4 Most important trends in antimicrobial resistance in zoonotic pathogens in humans and animals

Salmonella

- Among **human** *S.* Enteritidis isolates resistance against **fluoroquinolones** is high (31% in 2024), and it has been increasing strongly since 2010 (10%).
- In contrast, resistance against **fluoroquinolones** was much lower in a small sample of *S*. Enteritidis isolates from **layers and broilers** (2% and 5%). Although not specified per serotype, resistance levels to **fluoroquinolones** among isolates from Dutch/EU broiler meat (which are an additional source of SE) was around 60%.
- Among **human** *S.* Typhimurium isolates resistance percentages increased in 2024 for **sulfamethoxazole** (24% to 38%) and **tetracycline** (18% to 37%), after a steady decrease for almost all classes since the peak in 2010. Correspondingly, in isolates from **animal** sources the levels of resistance of *S.* Typhimurium to these classes was high (ranging from 32% to 70%).
- In contrast to *S*. Enteritidis, resistance to **fluoroquinolones** was relatively low for *S*. Typhimurium (<10%).

- Over the past 5 years, the proportion of ESBL-producing Salmonella isolates from humans has remained stable, with a prevalence of 1.4% (20/1470) in 2024. None of the tested human and non-human *S.* enterica isolates were found to produce carbapenemases.

Campylobacter

- In **human** *Campylobacter* spp. isolates, the level of **ciprofloxacin** and **tetracycline** resistance remained high in 2024 (around 62% and 45%, respectively)
- Resistance to **erythromycin**, first choice antibiotic for campylobacteriosis in humans, remained low among human isolates (<5%)
- **Poultry and poultry meat** are the most important reservoir and transmission route of campylobacteriosis. Levels of resistance among human isolates correspond with similarly high levels among isolates from broilers and meat thereof.
- Among isolates from **veal calves erythromycin** resistance is notably higher (45%), however consumption of veal meat is relatively low.

STEC

- In **human** STEC isolates, resistance to **ampicillin**, **tetracycline**, s**ulfamethoxazole**, **trimethoprim** and **chloramphenicol** is moderate (<15%)
- Although, therapeutic treatment of STEC infections with antimicrobials is not advised, monitoring AMR in STEC from symptomatic human cases is useful in assessing the risk of transmission of resistant bacteria (especially other *E. coli*).

Yersinia enterocolitica

- In **humans**, the levels of resistance to **amoxicillin/ampicillin** in *Y. enterocolitica* have been very high (98%) over the past five years. In that same time period, resistance levels against **ciprofloxacin** for this species decreased from 6% in 2021 to 2% in 2024, while the proportions of resistance to **third generation cephalosporins**, **cotrimoxazole**, and **gentamicin** have remained stable at levels below 2% in the same period.

2.5 Most important trends in antimicrobial resistance in commensal bacteria from farm-animals and food

2.5.1 General AMR surveillance – using indicator bacteria

E. coli

- An indicator *E. coli* for the surveillance of antimicrobial resistance (AMR) in animals is a non-pathogenic, commensal strain of *E. coli* that is commonly found in the intestinal tract of healthy animals. These indicator *E. coli* strains are used as a representative organism to monitor and track trends in antimicrobial resistance within animal populations and on food products, as they can acquire and transfer resistance genes in Gram-negative bacteria.

- The highest resistance levels were observed in broilers, slaughter pigs and white veal calves, lower levels in rosé veal calves, and the lowest levels of resistance were observed in isolates from dairy cattle and dairy goats.
- Overall, the highest resistance levels were detected for **ampicillin** (15.2 – 34.1%), **tetracycline** (14.3 – 53.5%), **sulfamethoxazole** (14.3 – 34.1%), **trimethoprim** (5.4 – 28.1%) and specifically for **chloramphenicol** (22.7%) in white veal calves.
- Resistance to **fluoroquinolones** was still commonly present in indicator *E. coli* from caecal samples of broilers (21.9%) in contrast with the prevalence (<5%) in pigs, veal calves and dairy cattle. Resistance against **fluoroquinolones** was relatively high in retail chicken and turkey meat (22.2 – 24.9%) and even higher in imported meat (35.7 – 38.2%).

Enterococci

- Enterococci for the surveillance of antimicrobial resistance (AMR) in animals are non-pathogenic, commensal strains of *E. faecalis* or *E. faecium* that are commonly found in the intestinal tract of healthy animals. These indicator Enterococci are used as a representative organism to monitor and track trends in antimicrobial resistance within animal populations and on food products, as they can acquire and transfer resistance genes in Gram-positive bacteria.
- In 2024 Enterococci were included from caecal samples from broilers. In *E. faecium*, resistance levels were relatively high for **tetracycline** (30.5%), **erythromycin** (21.6%), and **ampicillin** (10.4%), while in *E. faecalis*, relatively high resistance levels were also observed for **tetracycline** (71.4%) and **erythromycin** (27.6%), with complete absence of resistance to **ampicillin**. Resistance to other antibiotics of critical or high importance to human health (**daptomycin, linezolid, teicoplanin**, and **vancomycin**) was low or not detected in both subspecies.

2.5.2 Specific AMR surveillance

- In 2024, ESC-resistant *E. coli* were rarely detected (<1%) amongst randomly selected *E. coli* on a non-selective plate from samples collected at slaughter houses and retail meat
- Amongst randomly selected *E. coli* on a non-selective plate from food products, ESC-resistant *E. coli* were only detected in imported poultry meat (12%).
- Selective culturing showed a significant increase in the prevalence of ESC-resistant *E. coli* in dairy cattle from 8.7% in 2014 to 22.9% in 2024.
- The prevalence of ESC-resistant *E. coli* observed with selective culturing was stable in pigs (13.6%), rosé veal calves (21.9%) and traditionally highest in white veal calves with (44.6%).
- Genetic analysis of ESC-resistant *E. coli* shows that the proportion of related ESC-resistant *E. coli* is higher in white veal calves than in other livestock sectors.
- In meat produced in the EU, poultry still contains the highest prevalence of ESC-resistant *E. coli*.

- ESC-resistant *E. coli* is detected at a higher prevalence from imported poultry meat than in poultry meat produced in the EU.
- In 2024, using selective culturing, two carbapenemase-producing *E. coli* isolates were detected for the first time from a broiler and pig sample at slaughter. In 2024, carbapenemase-producing *Enterobacter cloacae* and an *E. coli* isolate were also detected from imported shrimps and imported fresh herbs. Monitoring of clinical samples from dogs and cats resulted in detection of one carbapenemase-producing *E. coli* isolate from a dog.
- In 2024, only two *mcr*-producing *E. coli* were detected from broilers and one from a pig. In samples from retail and imported meat, only one *mcr*-producing *E. coli* was detected from fresh turkey meat and one from imported broiler meat.
- In 2024, MRSA samples were collected from pig farms. Conventional farms have a significantly higher prevalence (77%) than organic pig farms (21%). In general, pig farms have a higher prevalence than other farm types (0-25%) that were studied in previous years. The prevalence of MRSA in samples from farm workers on pig farms (35%) is higher than on other types of livestock farms (1-13%). Comparison of MRSA prevalence on fresh retail meat showed no significant differences to previous years, and pork, poultry meat and veal were shown to be most often contaminated (8-10%).

2.6 Implications for public health and health policy

By 2024, the direct impact of COVID-19 and the related measures have diminished, and most figures have returned to the levels seen in 2019 before COVID-19. In 2024, the total use of antibiotics and their distribution across classes in outpatients and long-term care facilities was similar to pre-pandemic levels. In hospitals there is a trend towards shorter hospital stays coupled with more intensive treatment regimens for patients.

For some highly-resistant micro-organisms (HRMO), such as carbapenem-resistant *K. pneumoniae*, the resistance percentage have clearly increased from 0.04% in 2020 to 0.2% in 2024. Moreover, the number of carbapenemase-producing Enterobacterales isolates submitted to the RIVM (n=696) was again considerably higher than in previous years and surpassed the numbers in pre-COVID years. The most identified risk factor for CPE carriage is still recent hospitalization abroad. However, most patients with a diagnostic isolate had no known risk factor or it was unknown whether the person had a risk factor (65%).
Other HRMO also showed increases in 2024 compared to the previous years. In 2024, there has been a significant increase in ESBL-producing *K. pneumoniae* in general practice (GP) and outpatient department populations, and in the inpatient department population, ESBL-producing *K. pneumoniae* percentages showed a peak above 10% for the first time. These resistance percentages were even higher on intensive care units (ICUs).
The overall proportion *S. aureus* isolates that were MRSA positive in 2020-2024 was stable at a low level of 3%, although the absolute

number of MRSA isolates submitted for the enhanced MRSA surveillance was higher compared to pre-COVID-19 pandemic levels. In addition, a strong increase was observed in phenotypic fusidic acid resistance among diagnostic *S. aureus* isolates including MRSA. This might lead to increased spread of MRSA within the community, since antibiotic treatment of impetigo caused by a fusidic acid-resistant MRSA-strain, would not respond to both the first (fusidic acid) and second choice (flucloxacillin) treatment according to the national guidelines, resulting in a longer duration of transmission risk. These developments are monitored closely by the national AMR surveillance systems.
Furthermore, resistance to other antimicrobials among some Gram-positive micro-organisms remains high, such as clindamycin resistance (including inducible resistance) among *S. aureus*, which warrants special attention for antibiotic stewardship programs and surveillance of these pathogens as well.
Despite increases in HRMO and resistance percentages, the number of HRMO outbreaks in 2024 in healthcare institutes reported to the SO-ZI/AMR was comparable to the pre-COVID numbers in 2017-2019.

In the past years several studies have been performed in the Netherlands to quantify the effect of reduction in antibiotic use in animals on AMR in humans. In 2024, the results of these studies were published.[1] The study indicates that potential zoonotic spread of AMR in *E. coli* causing human UTIs in primary care is rather limited. However, reduction in AMU in livestock has been effective to reduce resistance in commensal *E. coli* in livestock, thereby decreasing the potential risk in spread of resistance to humans or the environment. This underlines the importance of prudent use of antibiotics in both humans and animals.

Worldwide and in Europe, antibiotic resistance continues to be a serious threat to public health, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death. A Lancet paper[2], published in September 2024, describes a recent modelling study in which it is estimated that the burden of AMR is forecasted to increase to 1.91 million (95% uncertainty interval (UI) 1.56-2.26) attributable and 8.22 million (6.85-9.65) associated deaths in 2050.
In June 2023, the Council of the EU adopted a Council Recommendation[3] on stepping up EU actions to combat AMR using a One Health approach, which recommends targets to be achieved by the EU by 2030. These include three AMR targets to reduce the total EU incidence of bloodstream infections with MRSA, third-generation cephalosporin-resistant *E. coli* and carbapenem-resistant *K. pneumoniae*, by 15%, 10% and 5%, respectively, by 2030 compared to baseline year 2019. While the EU incidence of bloodstream infections with MRSA indicated that the EU target has been reached in 2024, the results for the other two EU targets were not pointing in the right direction. The estimated EU incidence of third-generation cephalosporin-resistant *E. coli* bloodstream infections with a 10% reduction target, increased by more than 5% compared to 2019. The estimated EU incidence of carbapenem-resistant *K. pneumoniae* increased by over 60% compared to 2019, which differs substantially from the target of a 5% reduction by 2030.
Wide variations in the occurrence of antimicrobial resistance across the EU/EEA exist.[4] Higher AMR was generally reported by countries in southern and central and eastern Europe. In the absence of stronger

public health action, it is unlikely that the EU will reach all its AMR targets by 2030.

Several AMR developments in Europe require extra attention. Firstly, increases in the estimated EU/EEA incidences of bloodstream infections with HRMO were not only observed for third-generation cephalosporin-resistant *E. coli* and carbapenem-resistant *K. pneumoniae*, but also for many other bacteria and antimicrobial groups under surveillance in 2020-2024. Based on EARS-net, the estimated EU/EEA incidences of *E. coli* bloodstream infections with AMR increased compared to 2020 and there was a significantly increasing trend for aminopenicillin resistance, fluoroquinolone resistance, third-generation cephalosporin resistance and carbapenem resistance. The same accounted for resistance to all antimicrobial groups in *K. pneumoniae*, and the incidence of combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides was also at the rise. Compared to 2020, the 2024 estimated EU/EEA incidences of bloodstream infections with resistant *S. pneumoniae* more than doubled and there was an increasing trend for all incidences. Combined resistance for macrolides and penicillin non-wild-type had also more than doubled compared to 2020.

Secondly, a risk assessment[5] of the ECDC in 2024 describes the emergence of *K. pneumoniae* isolates with combined hypervirulence and resistance to last-line antibiotics such as carbapenems in EU/EEA countries. This is of concern, as in contrast to 'classic' *K. pneumoniae* strains, hypervirulent *K. pneumoniae* (hvKp) strains can cause severe infections in healthy individuals, often complicated by dissemination to various body sites. With the convergence of virulence and antimicrobial resistance in hvKp strains, there is a possibility of potentially untreatable infections in previously healthy adults. An even higher morbidity and mortality must be expected if carbapenem-resistant hvKp strains spread in healthcare settings and affect a vulnerable patient population. Sustained transmission of the globally dominant hvKp ST23-K1 lineage carrying carbapenemase genes between healthcare facilities in a EU/EEA country has been confirmed. The probability of further spread and establishment of hvKp carrying carbapenemase genes in healthcare settings in EU/EEA countries with consequent significant impact on morbidity and mortality is therefore currently considered to be high. An investigation[6] in the EU/EEA showed that a plasmid with both resistance (blaOXA-48) and hypervirulence genes (aerobactin) carried by *K. pneumoniae*, mostly belonging to ST147, had been detected in 10 EU countries. The plasmid might spread across other Enterobacterales species as well. In the Netherlands however, until now there have been only sporadic cases of hvKp in the previous years, and up until now four cases with hvKp carrying carbapenemase genes.

Finally, a recent report[7] of the ECDC in September 2025 described the results of the *Candidozyma auris* (formerly *Candida auris*) survey 2024. This report showed that the number of cases with *C. auris* colonization or infection are increasing rapidly in the EU/EEA. Three countries report recent distinct *C. auris* outbreaks, and four countries report a situation of regional endemicity. This rapid dissemination is of serious concern and points to a high risk for continued *C. auris* spread throughout European healthcare systems and sustained control will become more

difficult. In the Netherlands, *C. auris* is only sporadically detected in patients, but the yearly number of *C. auris* detections has increased since 2022. All cases with available information are related to stay or hospital admission in endemic countries and until now, there has been no evidence for local transmission or outbreaks within healthcare settings in the Netherlands. Attention for infection control practices remains crucial, especially seeing the increasing numbers in surrounding European countries.

Conclusions and discussion

The data presented in NethMap One Health 2025 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals in the Netherlands but also in international context.

For some clinically important bacterial species, antimicrobial resistance is increasing, although in general, the resistance percentages in the Netherlands are still low compared to other countries. When comparing farm animals in the Netherlands to other countries, the resistance percentages are generally also below the average, but vigilance is prudent as the first detection of CPE in farm animals occurred in 2024. Worldwide, resistance and multidrug resistance in Enterobacterales (most notably *K. pneumoniae*) is of major concern, and needs ongoing close attention.

To control the spread of HRMO in October 2024 the new SRI (Surveillance and Reporting of Infections) HRMO guideline has been launched providing updated recommendations for the detection, reporting, and management of antimicrobial resistance in healthcare settings.[8] The SRI guidelines emphasize the importance of rapid laboratory diagnostics, standardized reporting, and clear communication pathways to effectively manage BRMO cases and minimize their impact on public health

In addition to control the spread of HRMO, antimicrobial stewardship programs have been implemented universally in Dutch hospitals and are more and more implemented in GP practices and LTCF to further optimize antibiotic prescription practices. The usage of antimicrobials in farm animals has been greatly reduced, benchmarks are implemented per sector and preparations are made for the future inclusion of currently unmonitored sectors. In addition, with adequate surveillance systems the impact of measures to control the prevalence and spread of antimicrobial resistance in human healthcare as well as the open population, the environment, food-producing animals and the food chain, can be monitored and if necessary adjusted. In 2024 the new National Action Plan (NAP) on Antimicrobial Resistance 2024-2030[9] was launched, in which the Netherlands proposes numerous actions. The Netherlands plays the role of a proactive connector and partner in this effort, not only within its own borders but also internationally. The NAP includes (in line with Regulation EU 2019/6) the extension of veterinary antimicrobial consumption monitoring to all animals kept or bred, to be completed in 2030. At this moment only AMC in the main food producing animals is monitored. Although not monitored yet, the use of antimicrobial medicinal products authorized for humans is restricted in dogs and cats in the Netherlands since 2014, which is now endorsed for all EU member states with the EU 2022/1255 regulation.

2.7 References

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3 Antimicrobial consumption in humans

3.1 Antimicrobial consumption in humans

3.1.1 Outpatient antibiotic use

Methods
Data on outpatient antibiotic use in the Netherlands for 2024 were obtained from the Foundation for Pharmaceutical Statistics (SFK),The Hague. The SFK database contains dispensing data from approximately 90% of community pharmacies in the Netherlands, which collectively serve around 93% of the Dutch population. These data are extrapolated to achieve full national coverage and include prescriptions issued by general practitioners, outpatient clinics, hospital outpatient departments, and dental practices. Antibiotic dispensing was measured using Defined Daily Doses (DDD) per Anatomical Therapeutic Chemical (ATC) 5 classification. Antibiotic use is expressed as DDD per 1,000 inhabitants per day (DID).

Results
Total outpatient antibiotic consumption slightly increased by 2.7%, rising from 8.78 DID in 2023 to 9.02 DID in 2024 (Table 3.1.1.1). The most frequently prescribed antibiotics were doxycycline, amoxicillin, and nitrofurantoin (Figures 3.1.1.1 and 3.1.1.2). Compared to 2023, the largest relative increases were observed for macrolides—particularly azithromycin (+9%)—as well as lincosamides (clindamycin) (+8%) and tetracyclines (+7%). In contrast, use of fosfomycin declined by 11%, and amoxicillin by 2%.
Since 2018, the most pronounced increases have been observed in the use of lincosamides, sulfonamides combined with trimethoprim, and beta-lactamase-resistant penicillins.

Discussion
A marked decline in antibiotic use was observed between 2019 and 2021, coinciding with the COVID-19 pandemic. This decrease is likely attributable to reduced transmission of infectious diseases, diminished healthcare utilization and the 'viral narrative' during that period. The subsequent increase in antibiotic use observed between 2022 and 2024 suggests a return to pre-pandemic patterns of healthcare delivery and prescribing. The *Mycoplasma pneumoniae* outbreak in 2023-2024 might also have contributed to this.[1,2]

Notably, the use of nitrofurantoin and trimethoprim has shown a consistent downward trend since 2018. This might reflect increased adherence to national prescribing guidelines, issued by the Dutch College of General Practitioners (NHG), which emphasize more judicious antibiotic use and potential for wait-and-see for uncomplicated urinary tract infections.

Table 3.1.1.1 *Seven years data on the use of antibiotics for systemic use (J01) in outpatients (DID), 2018-2024 (source: SFK)*

ATC Group	Therapeutic group	2018	2019	2020	2021	2022	2023	2024
J01AA	Tetracyclines	1.94	1.83	1.54	1.42	1.54	1.72	1.84
J01CA	Penicillins with extended spectrum	1.35	1.26	0.98	0.98	1.22	1.34	1.30
J01CE	Beta-lactamase sensitive penicillins	0.07	0.16	0.12	0.13	0.20	0.18	0.17
J01CF	Beta-lactamase resistant penicillins	0.49	0.48	0.47	0.48	0.53	0.58	0.60
J01CR	Penicillins + beta-lactamase-inhibitors	0.95	0.93	0.81	0.81	0.92	0.97	0.99
J01D	Cephalosporins & carbapenems	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.13	0.12	0.12	0.12	0.12	0.11	0.11
J01EE	Sulphonamides + trimethoprim	0.30	0.33	0.33	0.33	0.35	0.37	0.38
J01FA	Macrolides	1.22	1.22	1.13	1.07	1.14	1.20	1.30
J01FF	Lincosamides	0.23	0.23	0.23	0.24	0.27	0.27	0.29
J01GB	Aminoglycosides	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.73	0.67	0.64	0.64	0.67	0.69	0.68
J01XE	Nitrofuran derivatives	1.35	1.30	1.24	1.24	1.23	1.22	1.20
J01XX01	Fosfomycin	0.06	0.06	0.07	0.07	0.07	0.07	0.06
	Others	0.04	0.03	0.03	0.03	0.03	0.00	0.03
J01	**Antibiotics for systemic use (total)**	**8.90**	**8.68**	**7.77**	**7.61**	**8.32**	**8.78**	**9.02**

Figure 3.1.1.1 *Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2018-2024 (source: SFK)*

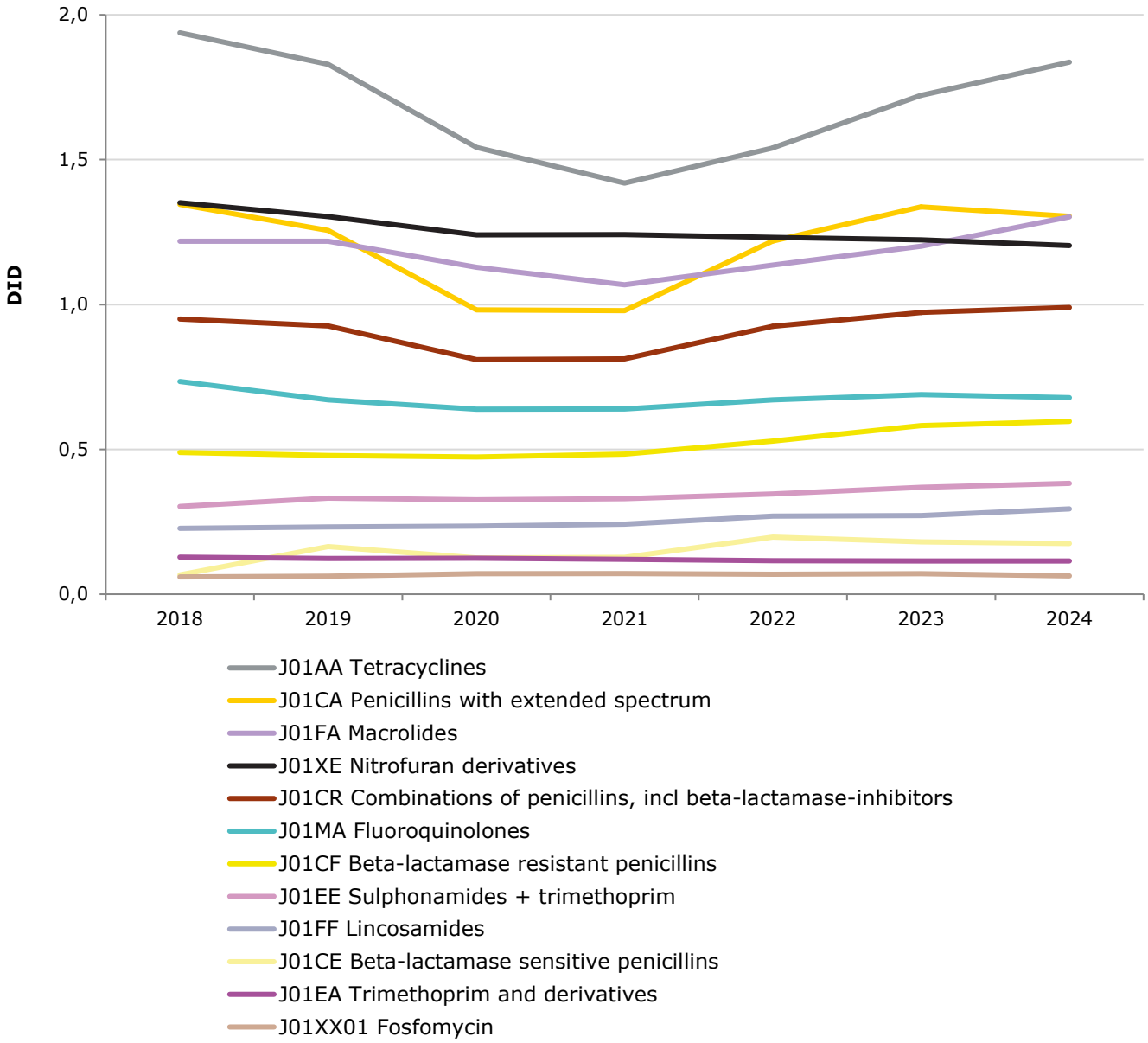
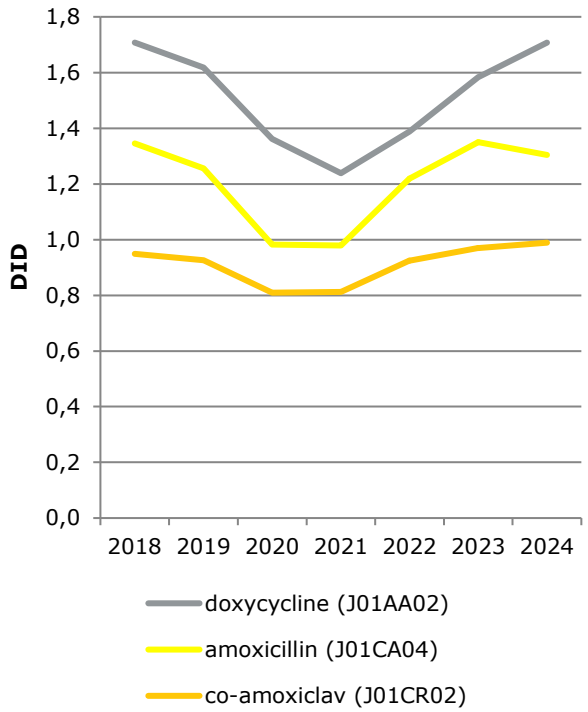
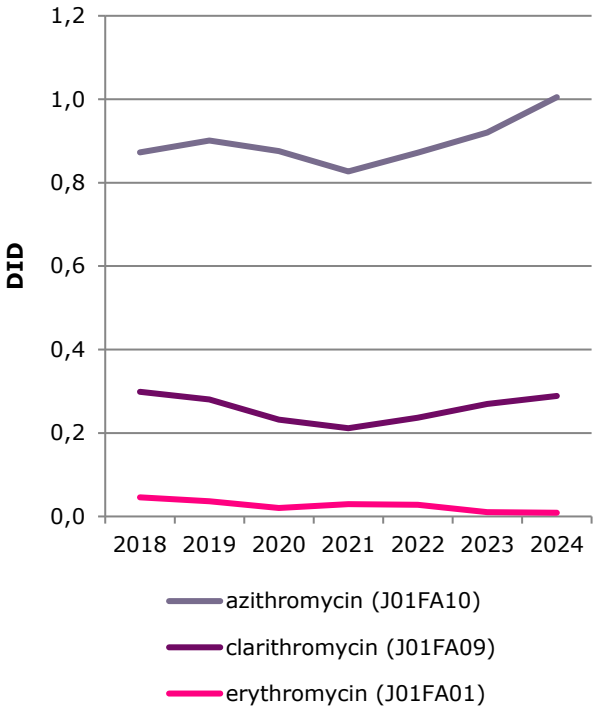
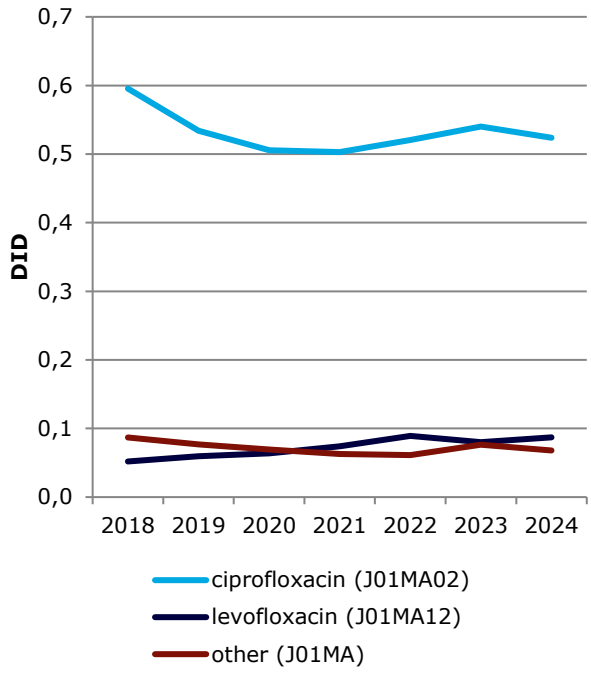
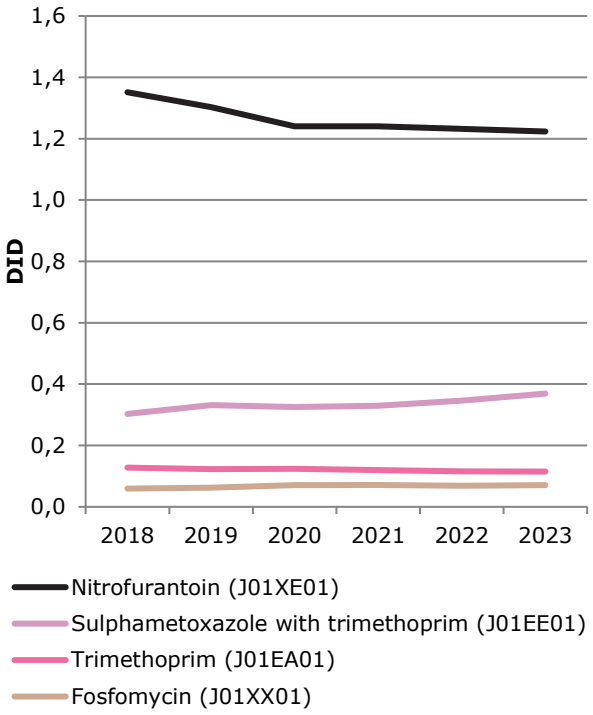


Figure 3.1.1.2 *Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2018-2024 (source: SFK)*









3.1.2 Antibiotic prescribing quality indicators in primary care

Introduction
To evaluate antibiotic prescribing quality at general practitioner (GP) practice level, the SABEL (Spiegelinformatie AntiBiotica EersteLijn) quality indicators (QIs) are used[1,2]. These QIs link antibiotic prescribing to clinical indications (illness episodes derived from International Classification of Primary Care (ICPC)-coded consultations from practices' electronic medical records).

Feedback reports on QI outcomes are produced and made available to GPs. GPs are encouraged to use these reports as a basis for discussion and evaluation of their results to optimise prescribing behaviour, for instance during pharmacotherapy audit meetings (FTOs) with colleagues, which are organised for FTO groups by the regional antimicrobial resistance care networks.

Methods
Each year, the RIVM receives the QI outcomes derived from anonymized routinely collected health care data from GP practices in the Netherlands, which agreed to collaborate with "Stichting Informatievoorziening voor Zorg en Onderzoek" (STIZON) and, from this year on, also data from practices that collaborate with VIPLive. Data are available from 2018 to 2024.

For all analyses, GP practices with <500 registered patients and with <100 or >750 oral antibiotic prescriptions per 1000 registered patients were considered outliers and therefore excluded. For 2024, data were available from 1825 GP practices in total (831 from STIZON and 994 from VIPLive), covering 38.7% of the Dutch population. The median score for each QI outcome was calculated with the interquartile range (IQR; 25–75 percentiles).

The fourteen QIs for primary care are:
General QIs (total prescriptions and prescribing percentages for specific subgroups of antibiotics):
1. Total number of oral antibiotic prescriptions/1000 registered patients/year (excluding prescriptions for prophylaxis and/or chronic use)
2. Percentage of amoxicillin/clavulanic acid prescriptions from total oral antibiotic prescriptions
3. Percentage of macrolide prescriptions from total oral antibiotic prescriptions
4. Percentage of quinolone prescriptions from total oral antibiotic prescriptions
5. Percentage of amoxicillin/clavulanic acid + macrolide + quinolone prescriptions from total oral antibiotic prescriptions

Antibiotic prescribing percentages (percentages of episodes of the specified diagnoses treated with antibiotics) for:
1. Otitis media
2. Upper respiratory tract infection (URTI)
3. Lower RTI (LRTI)
4. Impetigo

First-choice antibiotic prescribing percentages -according to the current Dutch guidelines[3]- (percentages of antibiotic-treated episodes that are treated with the first-choice antibiotic) for:
1. Otitis media (amoxicillin)
2. Tonsillitis (pheneticillin or phenoxymethylpenicillin)
3. Pneumonia (amoxicillin or doxycycline)
4. Cystitis in women (nitrofurantoin or fosfomycin)
5. Impetigo (flucloxacillin)

Results
In 2024, the median number of antibiotic prescriptions per 1000 registered patients was 280 (IQR: 234–338) (Table 3.1.2.1). Most QI outcomes show considerable variability between individual practices.

Trend analyses from 2018 to 2024 are shown in Figure 3.1.2.1. A sharp decline in overall antibiotic prescribing (QI1) was seen during the COVID-19 pandemic (Figure 3.1.2.1 A). In 2024, this antibiotic prescribing outcome increased to the level observed in 2022 but remained below pre-COVID-19 levels. The median percentages of amoxicillin/clavulanic acid, macrolide, and quinolone prescriptions were quite stable from 2018 to 2024 (Figure 3.1.2.1 B). The median antibiotic prescribing percentage for otitis media increased in 2022 but decreased again in 2023 and 2024 to pre-COVID-19 levels. The median antibiotic prescribing percentage for impetigo was quite stable but slightly increased in 2024. For URTI and LRTI sharp decreases were seen during the COVID-19 pandemic. The prescribing percentage for URTI increased again in 2021 and for LRTI in 2023 (Figure 3.1.2.1 C). In 2024, these prescribing percentages were slightly below the pre-COVID-19 levels. The QIs of first-choice antibiotic prescribing for otitis media, cystitis in women, and for tonsillitis were stable since 2019 (Figure 3.1.2.1 D). A small increase in first-choice antibiotic prescribing was seen for pneumonia, while impetigo showed the largest increase, from 50% in 2019 to 75% in 2024.

Table 3.1.2.1 *Outcomes of the antibiotic prescribing QIs for GP practices (n=1825) in 2024 (source: STIZON and VIPLive)*

Quality indicator	Median	IQR (25%–75%)
General		
1. Antibiotic prescriptions/1000 patients	280	234 - 338
2. Amoxicillin/clavulanic acid (%)	13	11 - 15
3. Macrolides (%)	7	6 - 10
4. Quinolones (%)	7	6 - 8
5. Amoxicillin/clavulanic acid + Macrolides + Quinolones (%)	28	25 - 31
Antibiotic prescribing (%)		
6. Otitis media	45	36 - 54
7. Upper respiratory tract infection	23	18 - 30
8. Lower respiratory tract infection	27	22 - 34
9. Impetigo	33	26 - 40
First-choice antibiotic prescribing (%)		
10. Otitis media	83	76 - 90
11. Tonsillitis	53	38 - 64
12. Pneumonia	80	72 - 86
13. Cystitis (women)	86	83 - 89
14. Impetigo	75	61 - 87

LRTI: Lower respiratory tract infection; URTI: upper respiratory tract infection

Figure 3.1.2.1 *Trends in antibiotic prescribing QI outcomes in primary care from 2018 to 2024, source: STIZON and VIPLive. See Table 3.1.2.1 for definition of the QIs. Median values are shown. (A) QI1, (B) QIs 2–4, (C) QIs 6–9, (D) QIs 10–14. Please note that the Y-axis has different ranges for Figures B, C, and D*

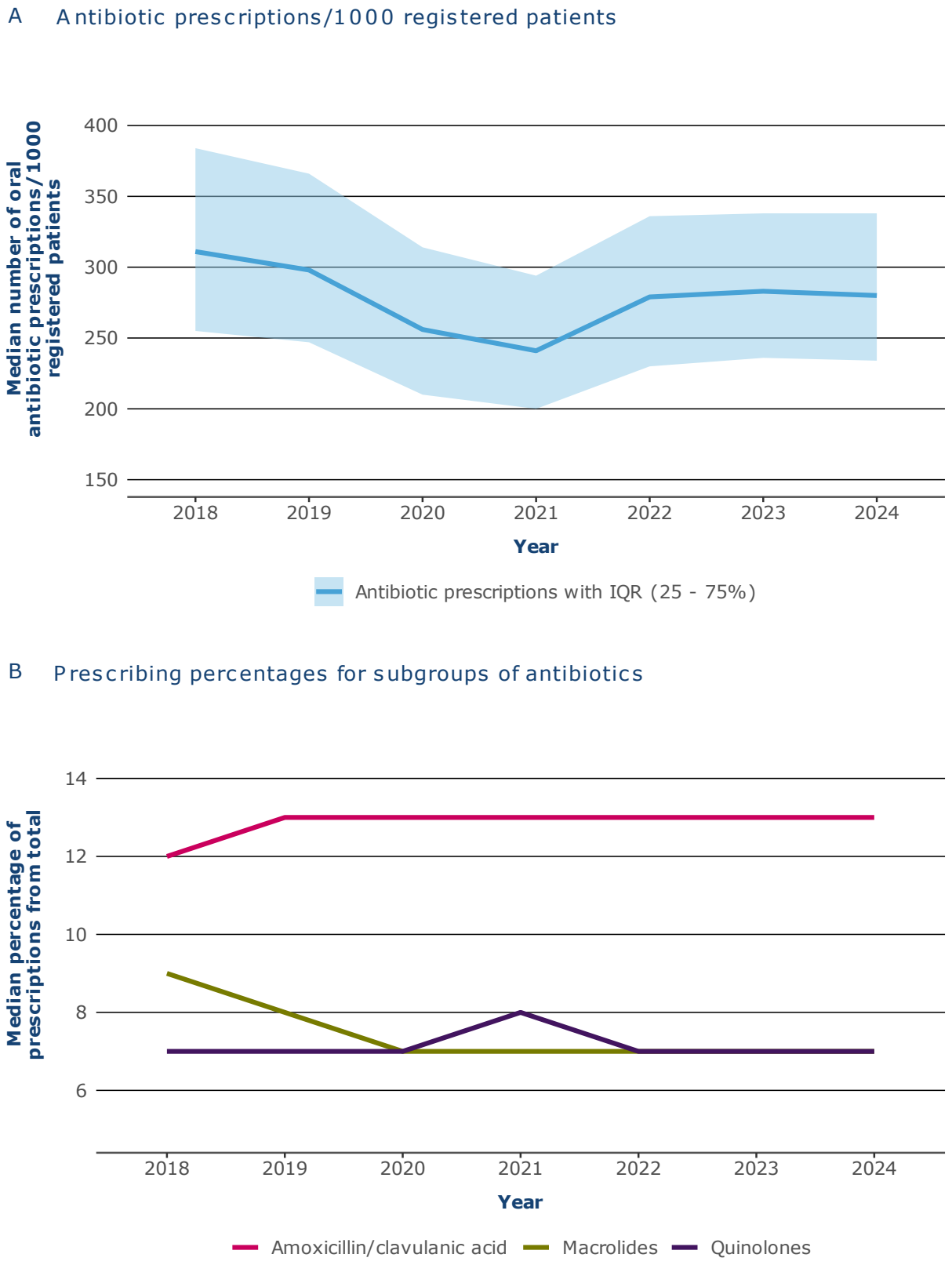
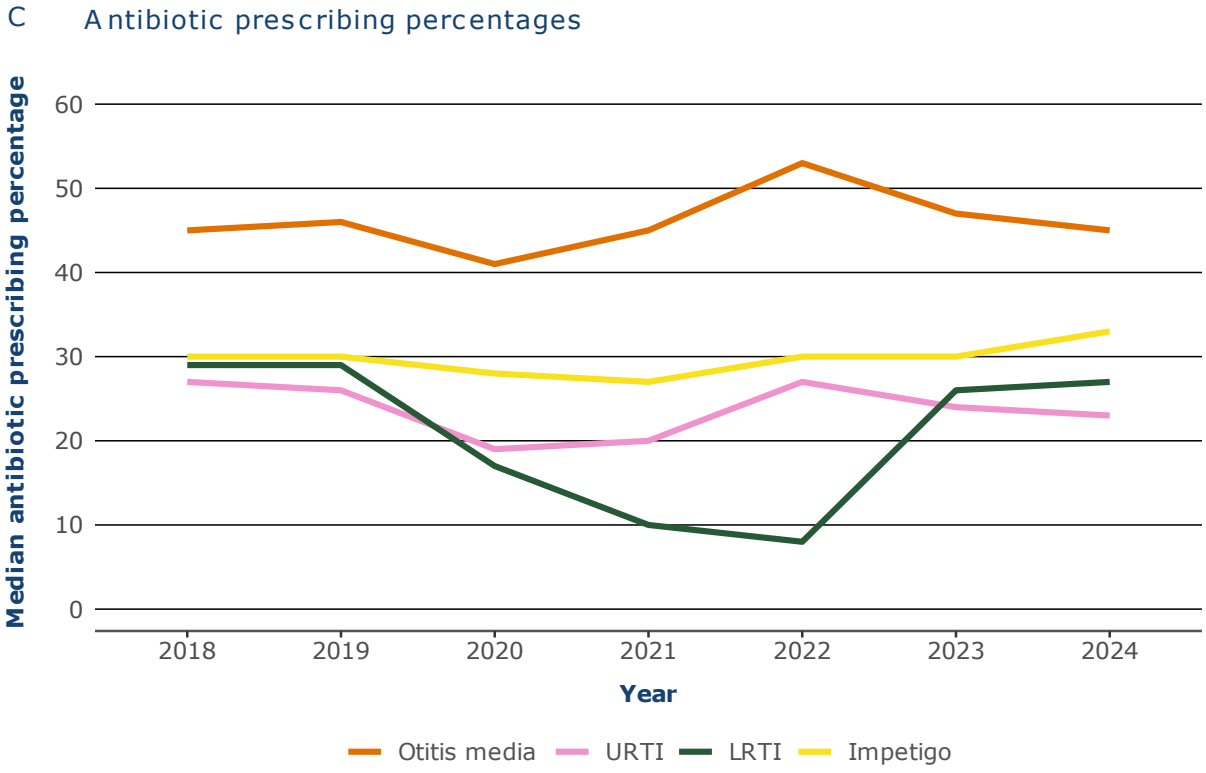
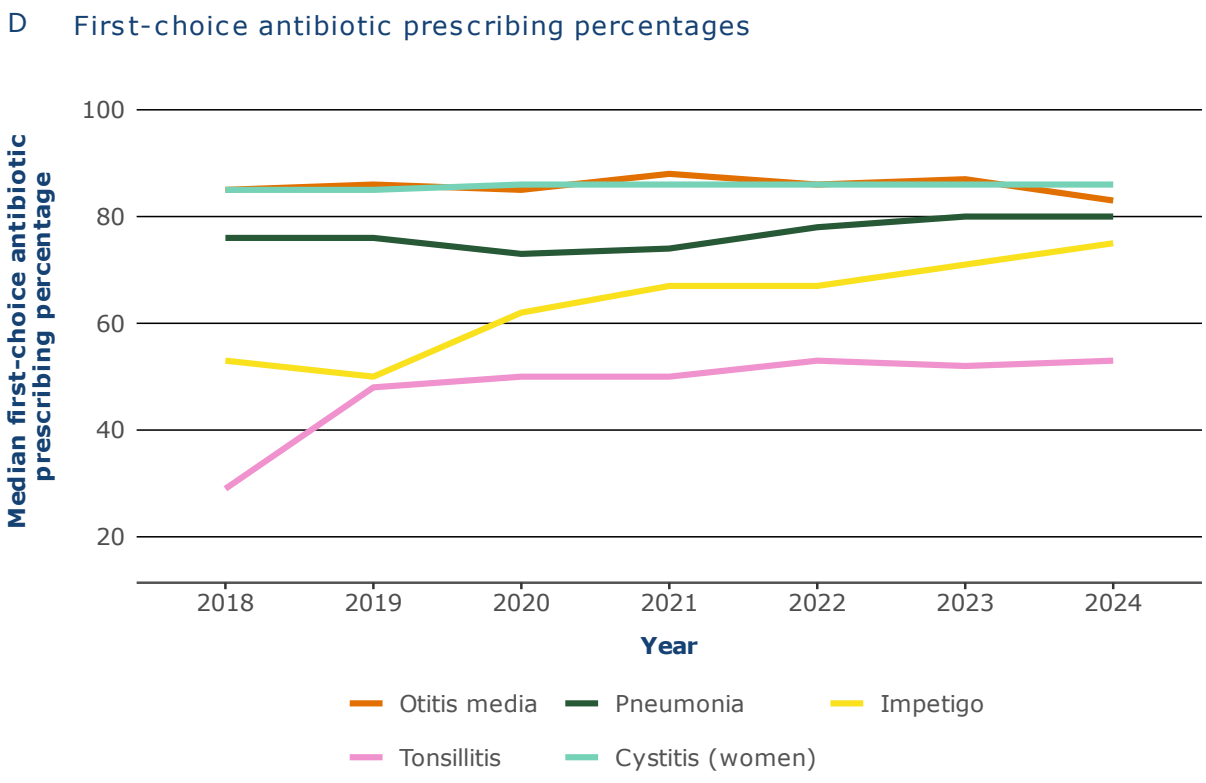


Figure 3.1.2.1 (continued) *Trends in antibiotic prescribing QI outcomes in primary care from 2018 to 2024, source: STIZON and VIPLive. See Table 3.1.2.1 for definition of the QIs. Median values are shown. (A) QI1, (B) QIs 2–4, (C) QIs 6–9, (D) QIs 10–14. Please note that the Y-axis has different ranges for Figures B, C, and D*





Discussion

These QI outcomes provide insight in the antibiotic prescribing quality by GPs in the Netherlands. Data are available at the GP practices level to benchmark prescribing behaviour through the STIZON and VIPLive dashboards. Feedback based on these QIs is also used in the pharmacotherapy audit meetings organised for FTO groups by the regional antimicrobial resistance care networks. Since 2018, at least 205 of the 1825 GP practices (11.2%) have participated in such an FTO.
This year, we were able to report on a larger number of GP practices because data from a second source were added, resulting in a coverage care for 38.7% of the Dutch population. This makes the benchmark results even more robust.
Of note, the huge decrease in the antibiotic prescribing percentage for LRTI in 2020 to 2022 was at least partially caused by an increase in the number of LRTI episodes (denominator) as a result introducing a subcode of the ICPC code R83 ICPC for SARS-CoV-2 and long-COVID.
Furthermore, prescribing first-choice antibiotics for impetigo showed the largest increase from 2019 to 2024. A potential explanation for this increase is the availability of flucloxacillin as first-choice antibiotic, which has become better in more recent years. On the contrary, availability of the first-choice antibiotics for throat infections remains an issue.

Conclusions

In 2024, a median of 280 antibiotics were prescribed per 1000 registered patients by GP practices for acute infectious diseases.
There is wide variation in prescribing habits between GP practices.
In 2020 and 2021, a sharp decline in the number of antibiotic prescriptions per 1000 registered patients was seen due to the COVID-19 pandemic. The rate of prescribing increased again in 2022 but remained below the pre-COVID level afterwards.
These QI outcomes provide insight in the quantity and quality of antibiotic prescribing by GPs in the Netherlands.
QI outcomes for FTO groups are made available to GPs by the regional antimicrobial resistance care networks during an FTO meeting to optimize antibiotic prescribing by GPs.

References

1. AW van der Velden, MI van Triest, AF Schoffelen, TJM Verheij. Structural Antibiotic Surveillance and Stewardship via Indication-Linked Quality Indicators: Pilot in Dutch Primary Care. Antibiotics (Basel). 2020;9(10):670. doi:10.3390/antibiotics9100670.
2. SEJD van den Eijnde, PD van der Linden, AW van der Velden. Diagnosis-linked antibiotic prescribing quality indicators: demonstrating feasibility using practice-based routine primary care data, reliability, validity and their potential in antimicrobial stewardship. J Antimicrob Chemother. 2024. 2024;79(4):767-773. doi.org/10.1093/jac/dkae017.
3. Dutch College of General Practitioners (NHG) guidelines. https://richtlijnen.nhg.org/#tab--nhgstandaarden.

3.1.3 Inpatient antibiotic use

Methods
Data on antibiotic use in Dutch hospitals were collected via questionnaires distributed to all Dutch hospital pharmacies. Defined Daily Doses (DDD) were extracted from the Dutch drug database 'Z-index' per ATC code with route of administration, at both the unit and product level. Data from 2018 to 2023 were analyzed by SWAB, using WHO DDD definitions of each respective year. Data from 2023 onwards were analyzed by RIVM, using DDD definitions as of May 2025 retrospectively for data from 2023 onwards. The change in method may have introduced an artefactual trend. Therefore, data from 2023 were analyzed according to both methods and displayed twice in tables and figures.

Inpatient antibiotic use is expressed as DDD per 100 patient-days and DDD per 100 admissions. The number of patient-days was estimated by subtracting the number of admissions from the number of bed-days to account for the fact that bed-day statistics count both the day of admission and the day of discharge as full days. Extrapolated data are expressed in DDD per 1,000 inhabitants per day (DID), as used in the international antibiotic consumption surveillance by the European Centre for Disease Prevention and Control (ECDC). Data on the annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS).

Results
In 2024, data were obtained from 57 out of 77 hospital pharmacies across the Netherlands. The use of systemic antibiotics in inpatient care decreased by 1.1%, from 92.0 defined daily doses (DDD) per 100 patient-days in 2023 to 91.0 in 2024 (Table 3.1.3.1). When expressed as DDD per 100 admissions, systemic antibiotic use declined by 5.3% since 2023, reaching 327 in 2024. Due to the revised analytical methodology, a slightly lower value was observed when comparing 2023 data calculated by the new method to previously reported values. Systemic antibiotic use per 1,000 inhabitant-days (DID) decreased since 2023, by 3.6% to 0.792 (Table 3.1.3.2). In this analysis, in contrast to results expressed as DDD per 100 patient-days or admissions, the revised analytical methodology led to a higher value for 2023 calculated by the new method compared to previously reported values. Although a new analytical approach has been adopted, the ability to assess and interpret long-term developments in antibiotic consumption remains intact.

Flucloxacillin remained the most commonly used antibiotic in hospitals (measured in DDD), followed by ceftriaxone and cefazolin (Figures 3.1.3.1 and 3.1.3.2). Compared to 2023, the most notable increases were seen in the use of first-generation cephalosporins (+19%), carbapenems (+14%), and imidazole derivatives (+9%, Table 3.1.3.1). The steepest declines were observed for lincosamides (−29%), combinations of penicillins including beta-lactamase inhibitors (−16%), and combinations of sulfonamides and trimethoprim (−14%).

Over time, an upward trend is evident for flucloxacillin, all generations of cephalosporins, meropenem, and vancomycin (Figure 3.1.3.2). In contrast, the use of amoxicillin/clavulanic acid appears to be decreasing. The downward trend in parenteral gentamicin use, observed in previous years, has stabilized since 2021.

As in previous years, systemic antibiotic use varied substantially between hospitals (Figures 3.1.3.3–3.1.3.5), with academic hospitals reporting the highest median consumption (Figure 3.1.3.4).

Cephalosporins
Ceftriaxone (third generation) was the most commonly used cephalosporin in university and large teaching hospitals, whereas cefuroxime (second generation) was predominantly used in general hospitals (Figure 3.1.3.6).

Antimycotics
Antimycotic use was highest in academic hospitals. Triazole derivatives were the most commonly used antifungals, however, their use showed a noticeable decline in 2024 (Figure 3.1.3.7).

Discussion
Due to the updated analytical method, antibiotics imported in response to supply shortages, as well as formulations prepared by hospital pharmacies, are currently not included in the analysis as of 2023. This likely results in an underestimation of actual antibiotic use. Nonetheless, the long-term development of antibiotic consumption can be meaningfully assessed and discussed.

For the first time since 2021, the DID has decreased. The DDD per 100 patient-days also decreased. The trend of shorter hospital stays and more intensive treatment regimens per patient during hospitalization seems to have come to a halt for now.

The use of meropenem continues to rise, despite ongoing initiatives aimed at sparing carbapenem use. Reasons may include increased number of infections with ESBL-producing bacteria requesting more aggressive empiric treatment strategies. The increasing consumption of cephalosporins remains difficult to explain.

Table 3.1.3.1 *Use of antibiotics for systemic use (J01) in hospitals, by year and therapeutic group, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*

ATC group	Therapeutic group	2018	2019	2020	2021	2022	2023	2023*	2024
J01AA	Tetracyclines	2.05	2.10	2.00	1.87	2.83	3.47	3.33	3.36
J01CA	Penicillins with extended spectrum	5.26	4.92	5.01	4.86	6.02	6.34	6.03	5.59
J01CE	Beta-lactamase sensitive penicillins	2.26	2.49	2.60	2.40	2.95	3.13	3.01	2.82
J01CF	Beta-lactamase resistant penicillins	10.80	10.60	12.00	11.30	14.50	13.50	12.96	13.12
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	12.00	10.10	10.60	8.84	10.00	10.50	10.10	8.45
J01DB	First-generation cephalosporins	6.43	6.68	6.55	7.13	7.58	7.80	7.37	8.76
J01DC	Second-generation cephalosporins	7.99	7.99	8.48	6.77	7.63	8.92	8.37	8.58
J01DD	Third-generation cephalosporins	6.88	7.73	9.93	11.41	10.94	11.20	10.79	11.59
J01DH	Carbapenems	1.32	1.41	1.53	1.63	1.69	1.74	1.66	1.89
J01EA	Trimethoprim and derivatives	0.23	0.20	0.23	0.17	0.19	0.15	0.14	0.14
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	2.15	2.41	3.00	2.59	2.96	3.40	3.28	2.81
J01FA	Macrolides	2.66	2.75	3.18	2.50	2.72	2.93	2.82	2.85
J01FF	Lincosamides	2.54	2.36	2.34	2.08	2.71	3.32	3.20	2.27
J01GB	Aminoglycosides	3.76	3.34	2.97	2.83	2.84	2.95	2.76	2.93
J01MA	Fluoroquinolones	7.67	6.99	7.39	6.57	7.69	8.01	7.66	7.02
J01XA	Glycopeptides	1.73	1.99	2.39	2.46	2.52	2.55	2.45	2.55
J01XB	Polymyxins	0.11	0.15	0.14	0.16	0.19	0.15	0.14	0.12
J01XD	Imidazole derivatives	3.20	3.21	3.28	3.44	3.72	3.97	3.68	4.00
J01XE	Nitrofuran derivatives	1.63	1.40	1.77	1.70	2.00	1.84	1.77	1.59
J01XX	Other antibacterials [1]	0.24	0.28	0.31	0.31	0.29	0.36	0.35	0.35
	Others [2]	0.10	0.13	0.10	0.12	0.15	0.15	0.16	0.22
J01	**Antibiotics for systemic use (total)**	**81.0**	**79.3**	**85.8**	**81.1**	**92.1**	**96.3**	**92.0**	**91.0**
	Expressed in DDD/100 admissions:								
J01	**Antibiotics for systemic use (total)**	**303**	**319**	**333**	**304**	**338**	**360**	**346**	**327**

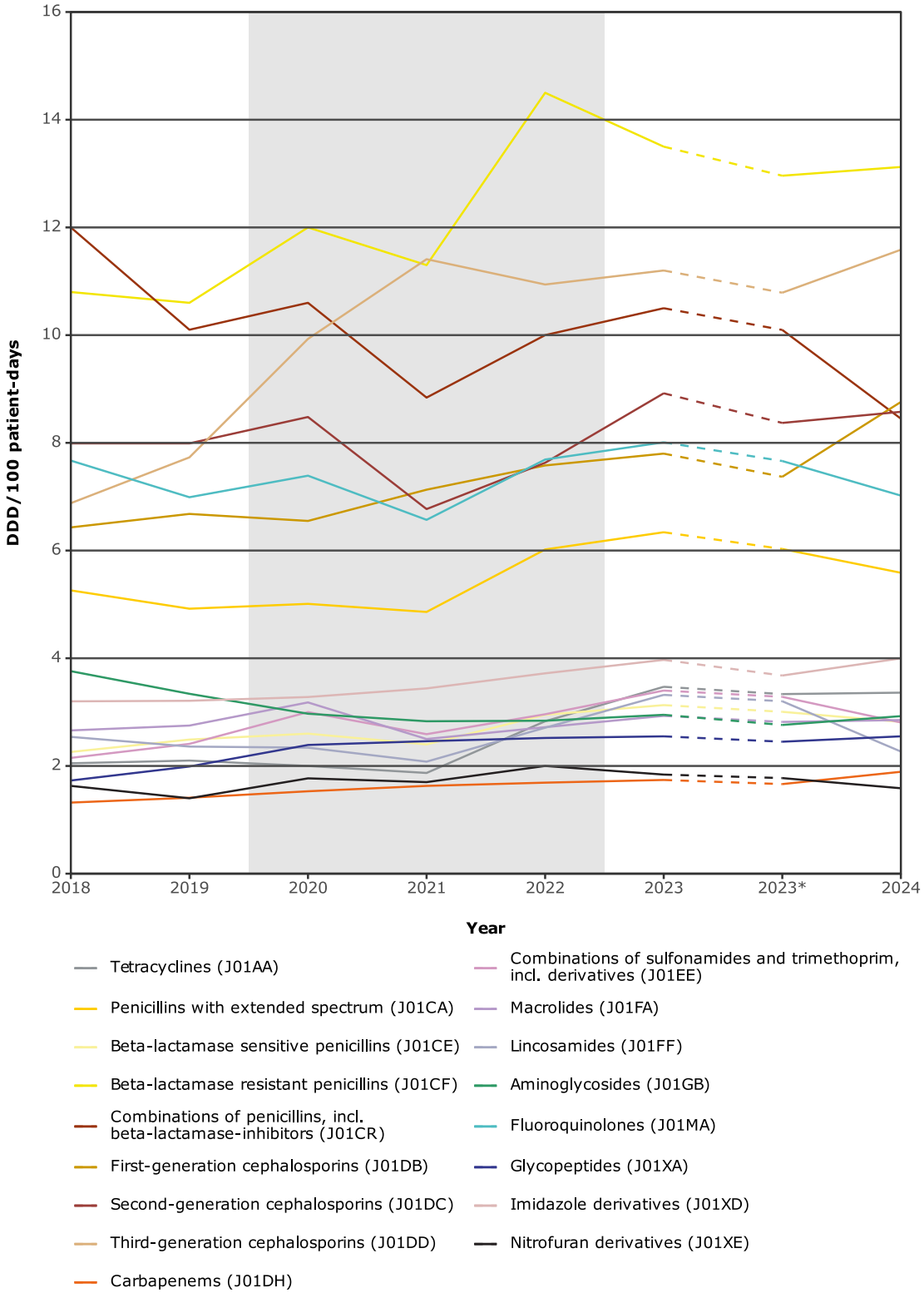
[1] Other antibacterials: fosfomycin, linezolid, daptomycin, methenamine
[2] Others: J01DE, J01DF, J01DI, J01EC
* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and displayed twice.

Table 3.1.3.2 *Use of antibiotics for systemic use (J01) in hospitals, by year and therapeutic group, 2018-2024*, presented as DDD/1000 inhabitant-days (source: SWAB, RIVM)*

ATC group	Therapeutic group	2018	2019	2020	2021	2022	2023	2023*	2024
J01AA	Tetracyclines	0.023	0.021	0.019	0.016	0.022	0.026	0.030	0.029
J01CA	Penicillins with extended spectrum	0.052	0.063	0.050	0.044	0.048	0.051	0.054	0.049
J01CE	Beta-lactamase sensitive penicillins	0.033	0.024	0.022	0.021	0.024	0.024	0.027	0.025
J01CF	Beta-lactamase resistant penicillins	0.105	0.104	0.103	0.097	0.116	0.108	0.116	0.114
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	0.128	0.109	0.098	0.078	0.083	0.083	0.090	0.074
J01DB	First-generation cephalosporins	0.070	0.066	0.056	0.061	0.060	0.064	0.066	0.076
J01DC	Second-generation cephalosporins	0.070	0.077	0.073	0.063	0.062	0.071	0.075	0.075
J01DD	Third-generation cephalosporins	0.072	0.074	0.085	0.093	0.093	0.091	0.096	0.101
J01DH	Carbapenems	0.014	0.014	0.013	0.014	0.013	0.014	0.015	0.016
J01EA	Trimethoprim and derivatives	0.003	0.002	0.002	0.002	0.002	0.001	0.001	0.001
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.022	0.022	0.024	0.021	0.023	0.026	0.029	0.024
J01FA	Macrolides	0.030	0.026	0.027	0.021	0.022	0.023	0.025	0.025
J01FF	Lincosamides	0.026	0.024	0.022	0.018	0.022	0.026	0.029	0.020
J01GB	Aminoglycosides	0.037	0.033	0.027	0.025	0.024	0.024	0.025	0.025
J01MA	Fluoroquinolones	0.079	0.071	0.066	0.057	0.061	0.063	0.068	0.061
J01XA	Glycopeptides	0.018	0.018	0.019	0.020	0.020	0.019	0.022	0.022
J01XB	Polymyxins	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001
J01XD	Imidazole derivatives	0.033	0.033	0.031	0.031	0.030	0.031	0.033	0.035
J01XE	Nitrofuran derivatives	0.017	0.015	0.016	0.014	0.017	0.014	0.016	0.014
J01XX	Other antibacterials[1]	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.003
	Others[2]	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
J01	**Antibiotics for systemic use (total)**	**0.836**	**0.799**	**0.760**	**0.700**	**0.747**	**0.766**	**0.822**	**0.792**

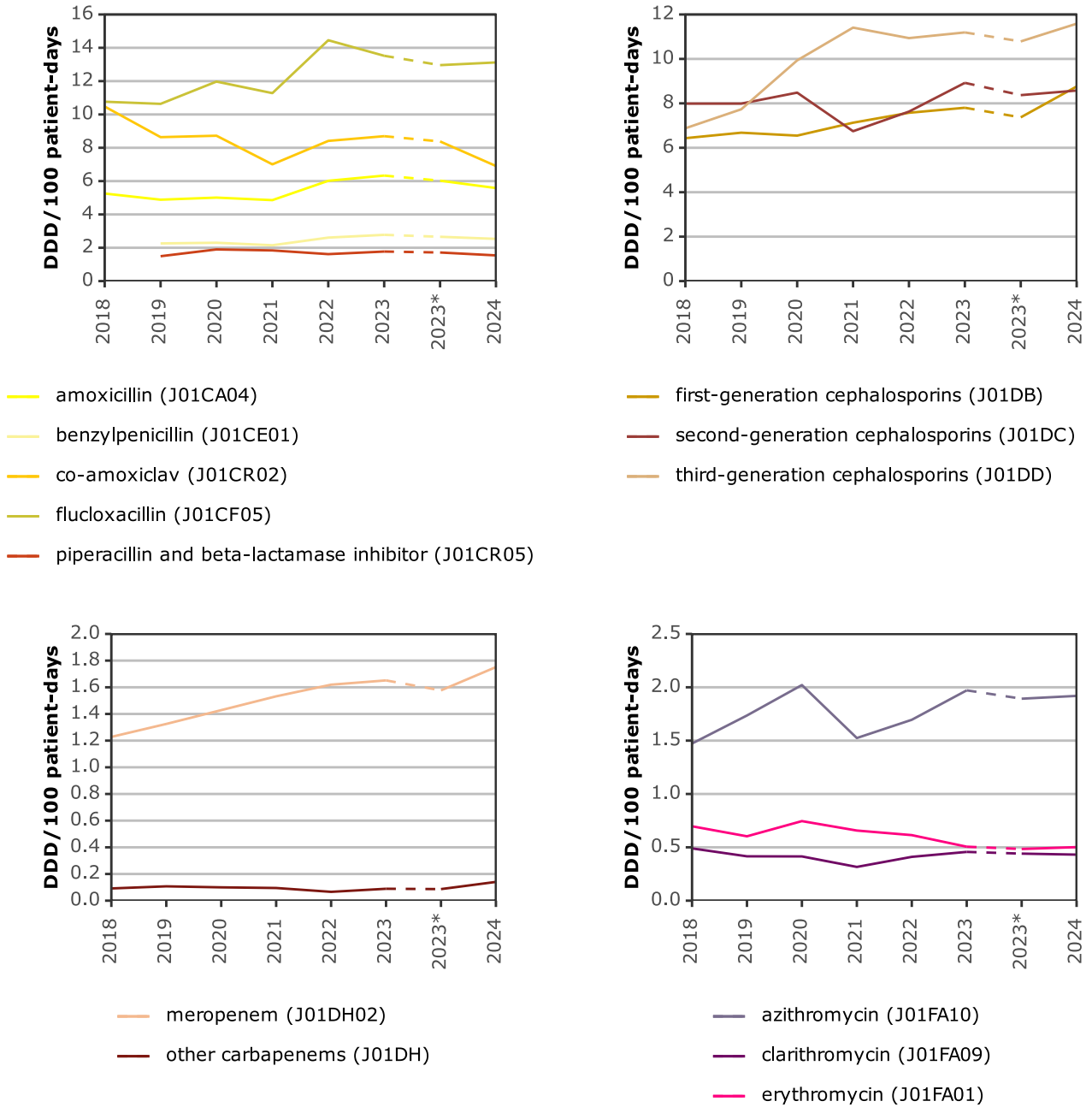
[1] Other antibacterials: fosfomycin, linezolid, daptomycin, methenamine
[2] Others: J01DE, J01DF, J01DI, J01EC
* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and displayed twice.

Figure 3.1.3.1 *Use of antibiotics for systemic use (J01) in hospitals, by therapeutic group, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*



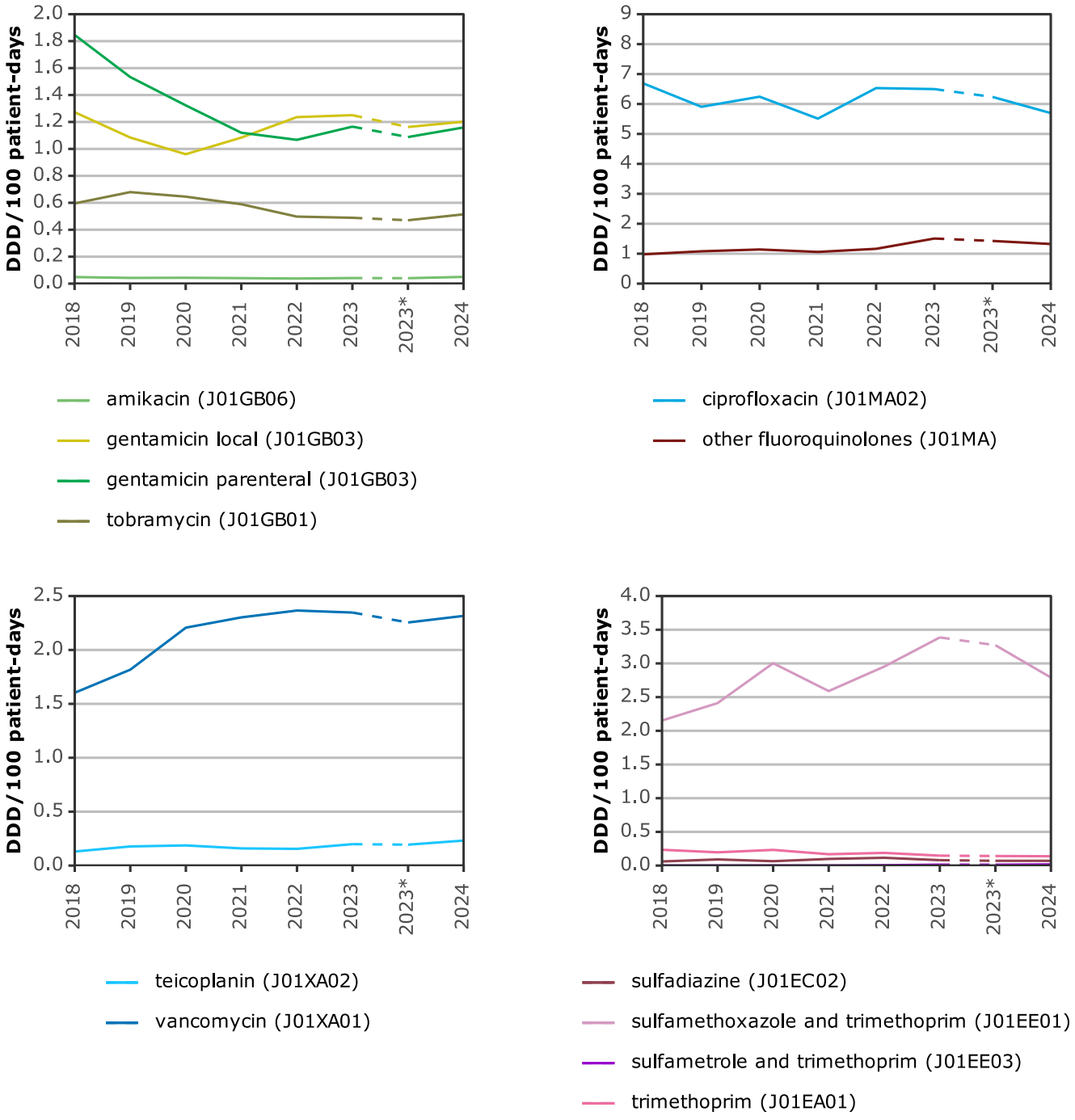
* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and a dashed line indicates the difference in DDD/100 patient−days between both methods. The grey square highlights the years of the COVID−19 pandemic.

Figure 3.1.3.2 *Use of beta-lactams, macrolides, aminoglycosides, fluorquinolones, glycopeptides and other antibiotics in hospitals, by therapeutic agent, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*



* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and a dashed line indicates the difference in DDD/100 patient−days between both methods.

Figure 3.1.3.2 (continued) *Use of beta-lactams, macrolides, aminoglycosides, fluorquinolones, glycopeptides and other antibiotics in hospitals, by therapeutic agent, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*



* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and a dashed line indicates the difference in DDD/100 patient−days between both methods.

Figure 3.1.3.3 *Use of antibiotics for systemic use (J01) in hospitals, by therapeutic group and hospital in 2024, presented as DDD/100 patient-days, and in order of total use by type of hospital (source: SWAB, RIVM)*

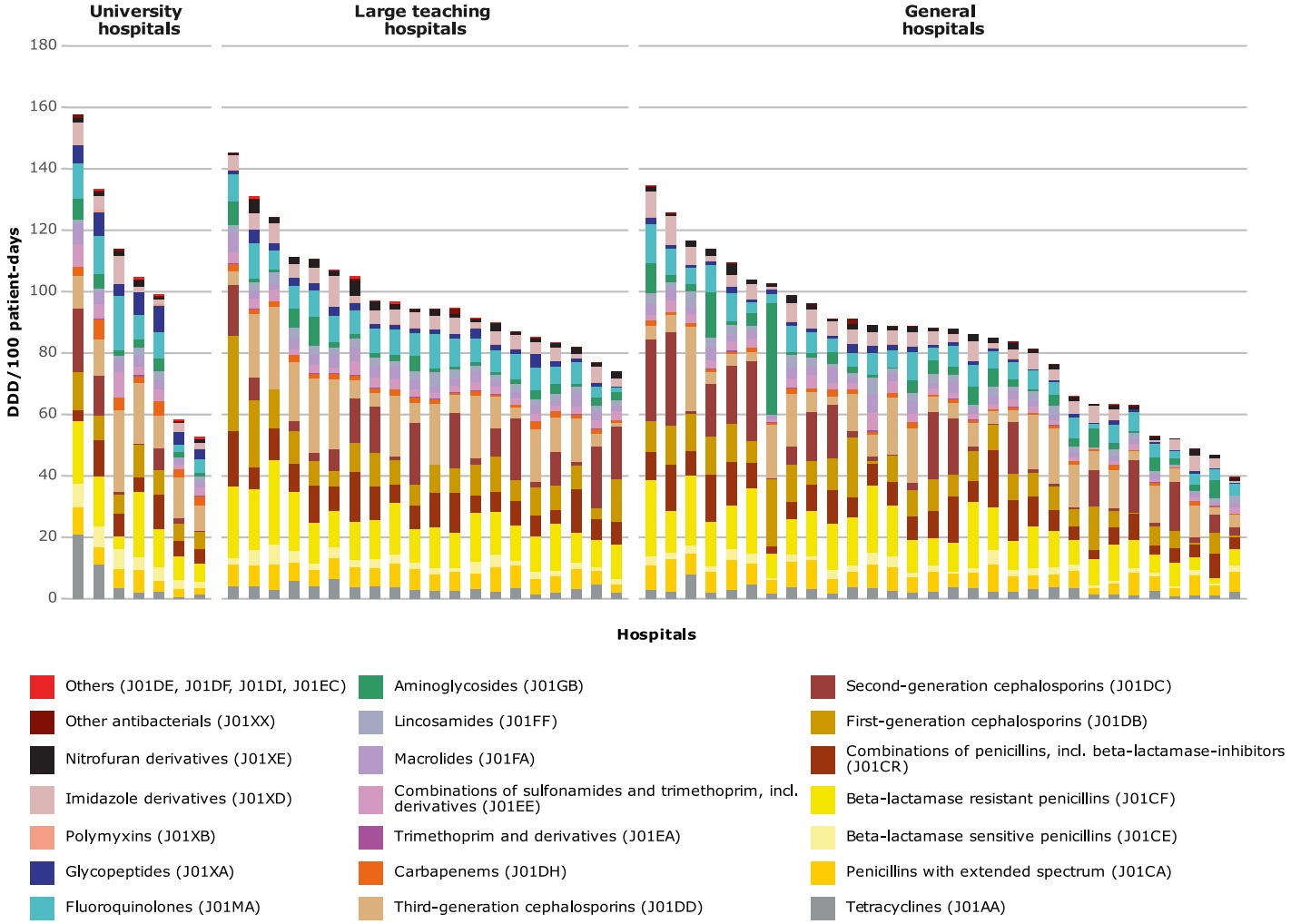
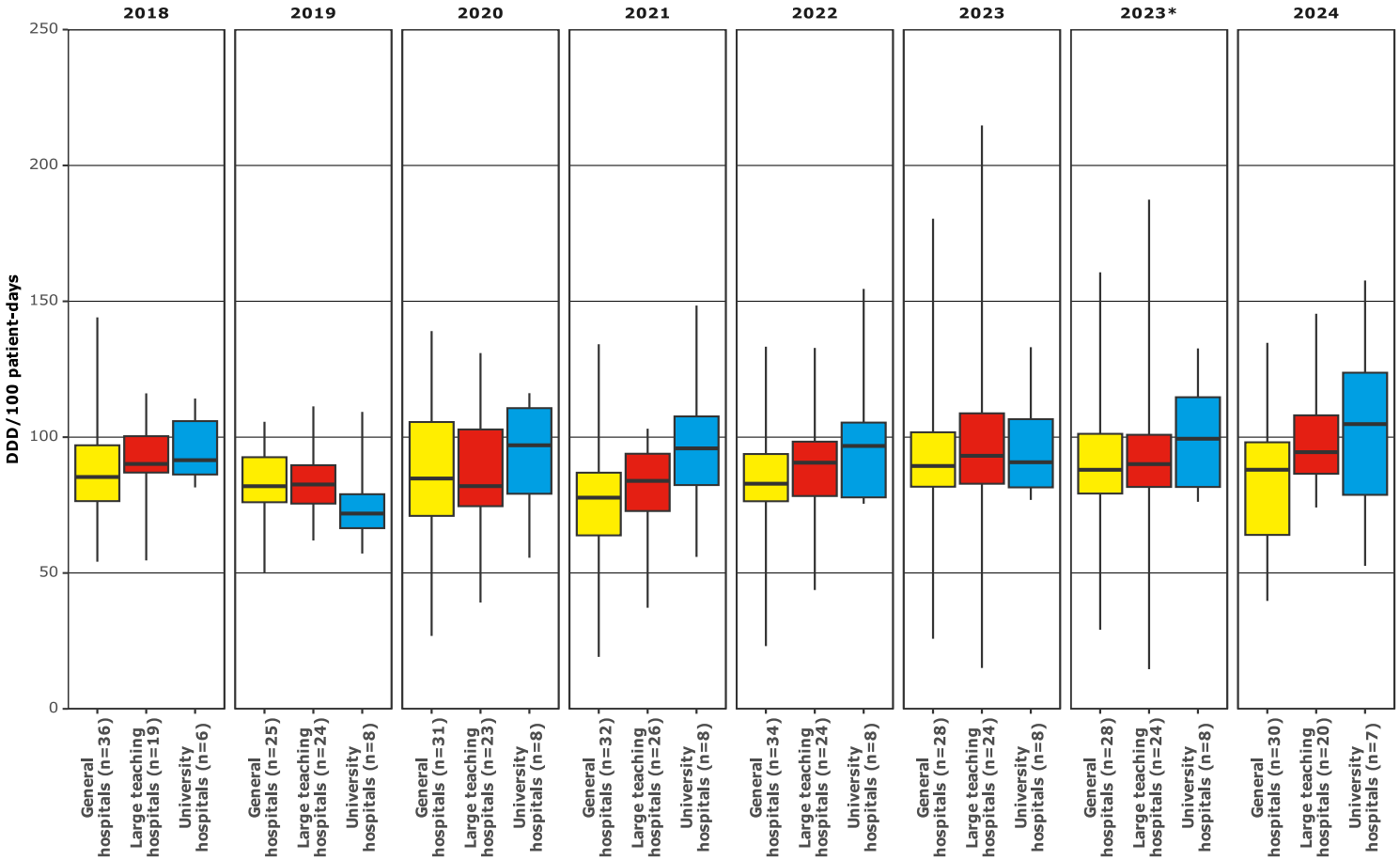


Figure 3.1.3.4 *Distribution of total use of antibiotics for systemic use (J01) in hospitals, by year and type of hospital, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*



Boxplot shows minimum, 25th percentile, median, 75th percentile, maximum.
* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and displayed twice.

Figure 3.1.3.5 *Distribution (%) of therapeutic groups among total use of antibiotics for systemic use (J01) in hospitals, by type of hospital in 2024 (source: SWAB, RIVM)*

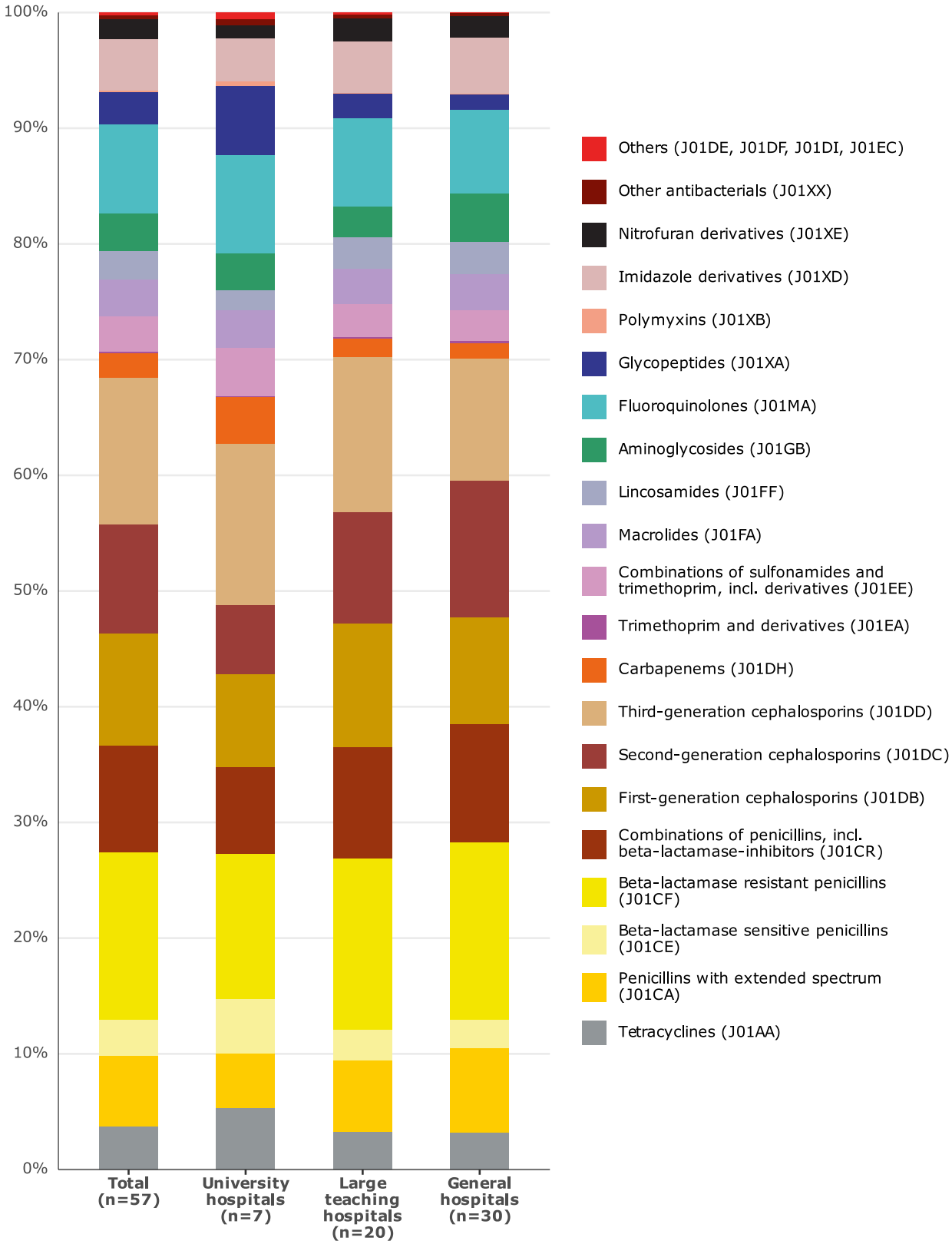
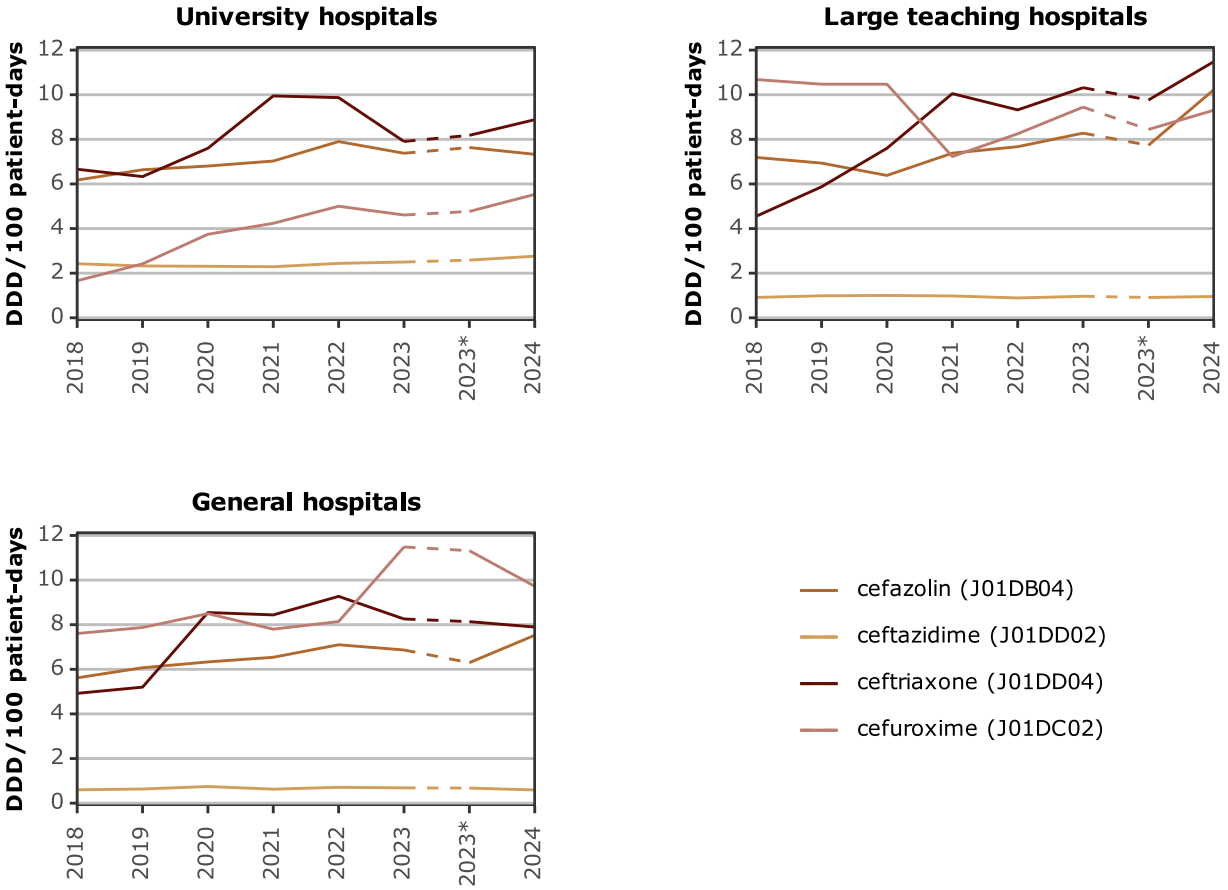
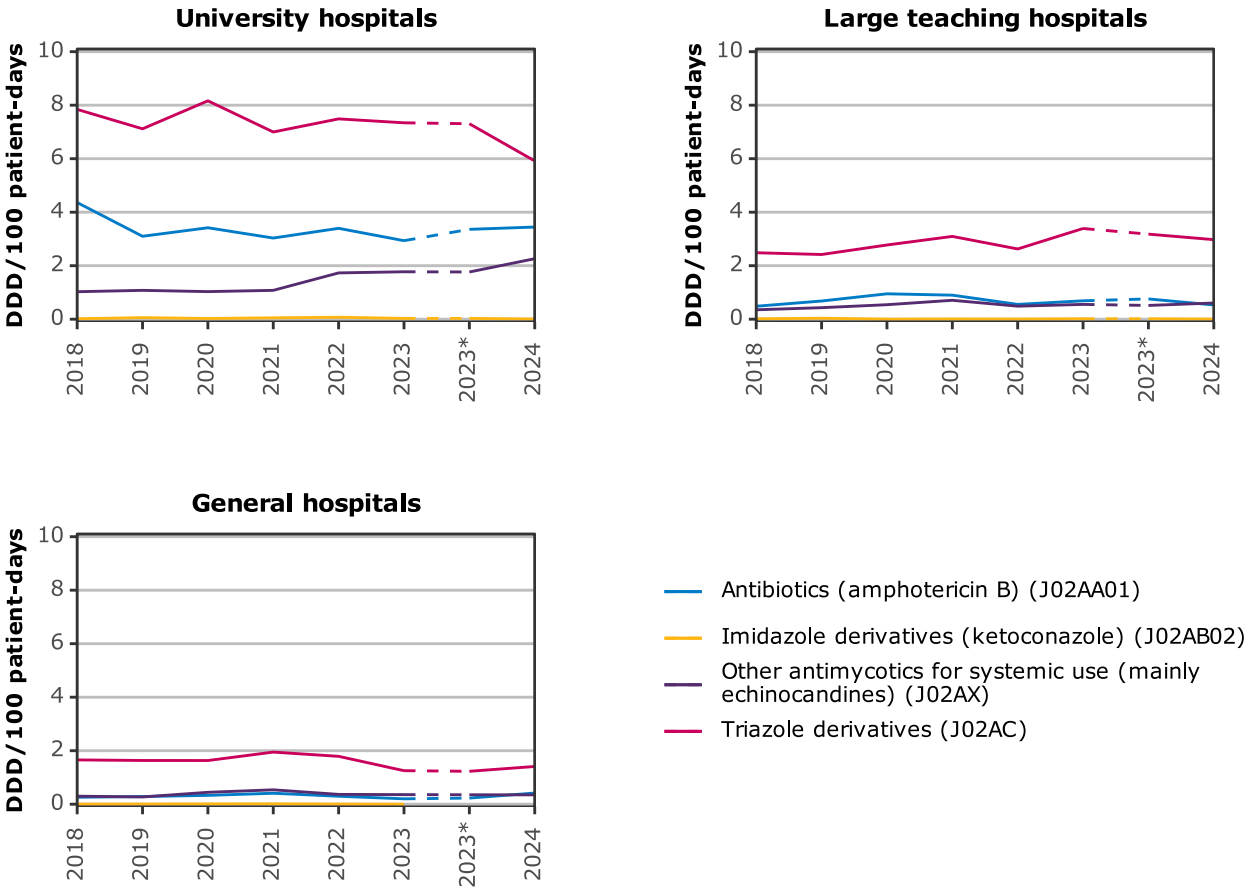


Figure 3.1.3.6 *Use of first, second and third generation cephalosporins in hospitals, by type of hospital, 2018-2024*, presented as DDD/100 patient-days (source: SWAB, RIVM)*



* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and a dashed line indicates the difference in DDD/100 patient−days between both methods.

Figure 3.1.3.7 *Use of antimycotics (J02) in hospitals, by type of hospital and therapeutic group, 2018-2024 *, presented as DDD/100 patient-days (source: SWAB, RIVM)*

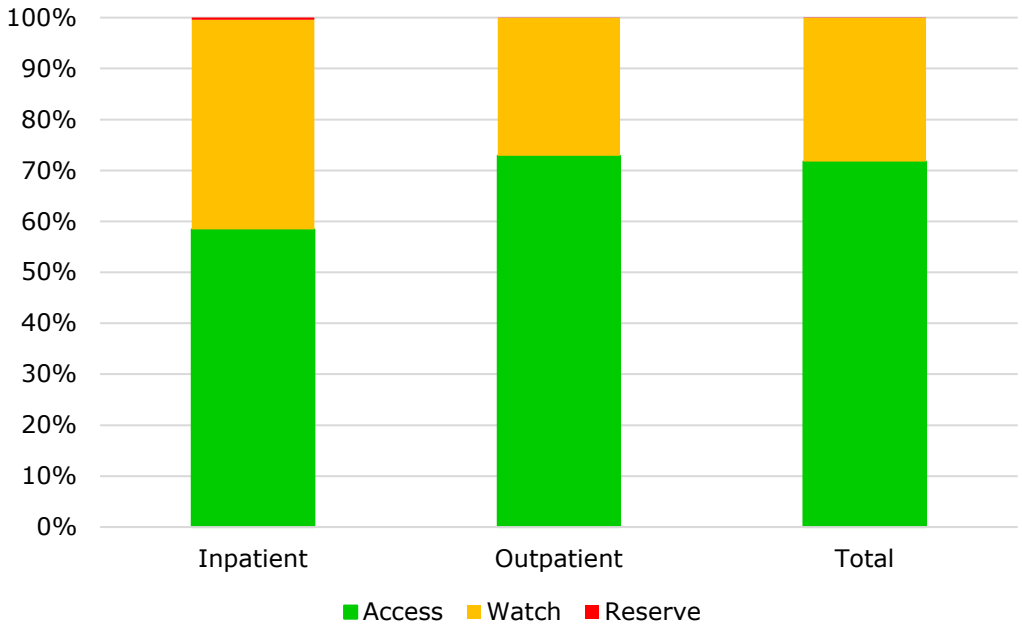


* From 2023 onwards, a new method is used for the analyses (see Methods section). Data from 2023 are analysed according to both methods separately and a dashed line indicates the difference in DDD/100 patient−days between both methods.

3.1.4 AWaRe classification

The AWaRe classification serves as a tool for tracking antibiotic use, setting targets for the correct, least broad antibiotics, and assessing the impact of stewardship policies aimed at optimizing antibiotic use and reducing antimicrobial resistance[3]. The European Union has set a target for 2030 stipulating that at least 65% of total antibiotic consumption should consist of agents classified within the 'Access' group[4].
Applying the WHO AWaRe classification, 72.9% of antibiotics used by outpatients in the Netherlands fall within the Access category, and 27.1% in the Watch category (Figure 3.1.4.1). For inpatient use, these percentages were 58.4% and 41.1%, respectively, and 0.5% in the Reserve category. Consistent with previous years, over 70% of antibiotics consumed in the Netherlands—specifically 71.7% in 2024—are classified within the 'Access' group, indicating that the EU's 2030 target has already been met. The most frequently used reserve antibiotics, only in hospitals, were linezolid, daptomycin, ceftazidime/avibactam and aztreonam.

Figure 3.1.4.1 *Antimicrobial consumption according to AWaRe classification in 2024*



3.1.5 Long-term care facilities

Methods
Data on antibiotic use in long-term care facilities (LTCF) was collected via the Dutch Surveillance Network of Infectious Diseases in Nursing Homes (SNIV). In 2024, data could be provided in two different ways: via the original method (i.e., provided by the pharmacist) or via a custom-designed automatic export from the electronic prescription system. Certain corrections were applied to the data in order to calculate medication dispenses out of the prescription system. To account for the size of the LTCFs, the defined daily dose (DDD) was standardized by calculating the DDD per 1,000 residents per day for each facility.

Results
Data were obtained from 285 LTCFs from 35 organizations serving 18,964 residents (2023: 9,637 residents). The LTCFs had a mean of 67 residents, varying from 3 to 455 per facility. Antibiotic use in LTCFs increased from 47.1 in 2023 to 53.1 DDD/1,000 residents/day in 2024 (Table 3.1.5.1). The most commonly used antibiotics were combinations of penicillins including beta-lactamase-inhibitors, macrolides and nitrofuran derivatives. Among the LTCFs, the DDD/1,000 residents/day ranged from 3.2 to 360.5.

Discussion
In 2024, a marked increase in the use of certain therapeutic groups, including macrolides was observed. This could be partially explained by the differences in methodology. In contrast to previous years, SNIV participants were able to automatically export their data from the prescription system, which has led to a substantial increase in the number of included LTCFs. This may have resulted in slightly different study populations. Furthermore, the method used to calculate medication dispenses may have resulted in an overestimation of the use of certain therapeutic groups, particularly those that are used for maintenance therapy.

Table 3.1.5.1 *Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, (expressed as weighted mean) DDD/1,000 residents/day, 2018-2024 (source: SNIV & SWAB)*

ATC group	Therapeutic group	2018	2019	2020	2021	2022	2023	2024
J01AA	Tetracyclines	5.0	3.7	2.9	2.6	2.3	2.4	3.1
J01CA	Penicillins with extended spectrum	2.4	2.6	4.8	2.4	2.9	3.6	2.1
J01CE	Beta-lactamase sensitive penicillins	0.4	0.5	0.4	0.3	0.3	0.5	0.5
J01CF	Beta-lactamase resistant penicillins	3.3	3.0	2.5	2.4	2.9	3.0	2.6
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	12.1	12.0	10.2	7.7	10.7	10.9	9.9
J01DB	First-generation cephalosporins	0.1	0.0	0.2	0.3	0.1	0.1	0.3
J01DC	Second-generation cephalosporins	0.1	0.2	1.0	0.1	0.1	0.1	0.1
J01DD	Third-generation cephalosporins	0.4	0.4	0.5	0.4	0.6	0.4	0.4
J01DH	Carbapenems	0.1	0.3	0.1	0.0	0.1	0.1	0.1
J01EA	Trimethoprim and derivatives	1.2	0.8	1.2	1.0	0.7	0.9	1.0
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	1.9	3.0	2.6	1.9	1.7	2.0	3.2
J01FA	Macrolides	2.7	2.7	3.0	2.3	3.3	3.7	8.4
J01FF	Lincosamides	3.0	2.9	2.2	1.8	2.2	2.8	3.9
J01GB	Aminoglycosides	0.1	0.0	0.0	0.0	0.1	0.1	0.2
J01MA	Fluoroquinolones	8.7	7.3	9.1	5.5	6.7	7.8	7.0
J01XA	Glycopeptides	0.2	0.4	0.1	0.2	0.1	0.1	0.0
J01XB	Polymyxins	0.1	0.0	0.1	0.0	0.1	0.0	0.1
J01XD	Imidazole derivatives	0.0	0.0	0.0	0.1	0.0	0.0	0.0
J01XE	Nitrofuran derivatives	11.3	9.5	8.2	8.1	8.0	7.6	8.1
J01XX	other antibacterials*	0.7	0.9	1.4	0.8	0.8	0.9	1.9
Others	Others**	0.0	0.0	0.0	0.2	0.0	0.0	0.0
J01	**Antibiotics for systemic use (total)**	**53.9**	**50.4**	**50.4**	**38.1**	**43.9**	**47.1**	**53.1**

References

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3. Web Annex C. WHO AWaRe (access, watch, reserve) classification of antibiotics for evaluation and monitoring of use, 2023. In: The selection and use of essential medicines 2023: Executive summary of the report of the 24th WHO Expert Committee on the Selection and Use of Essential Medicines, 24 – 28 April 2023. Geneva: World Health Organization; 2023 (WHO/MHP/HPS/EML/2023.04).
4. European Centre for Disease Prevention and Control. Antimicrobial consumption in the EU/EEA (ESAC-Net) – Annual Epidemiological Report 2023. Stockholm: ECDC; 2024. Available from: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-consumption-eueea-esac-net-annual-epidemiological-report-2023

3.2 Antimicrobial stewardship in hospitals

Introduction

Antimicrobial stewardship (AMS) has been a recognized practice in the Netherlands since at least 2014, when the Dutch Health Care Inspectorate (IGJ) and the Ministry of Health recommended the establishment of AMS teams (A-teams) in every hospital to optimize antibiotic use and combat antimicrobial resistance. In the same year, the SWAB-Practice guide Antimicrobial Stewardship in the Netherlands was published. The SWAB has played a key role in promoting AMS, providing guidelines, sharing best practices for antibiotic use, and developing the Antimicrobial Stewardship Monitor (AMSM). The AMSM provides insight into the quantity and quality of antibiotic use in Dutch hospitals, benchmarked against national averages.

Method

Participating hospitals and data acquisition

The AMSM extracts information on individual patients' antibiotic prescriptions and hospital admissions from structured data routinely recorded in electronic medical records (EMR). This contrasts with Chapter 3.1 'Antimicrobial consumption in humans', in which either purchase or dispensing data are analyzed. For the AMSM, the following data are extracted:

- antimicrobial prescriptions with ATC codes starting with J01, J02, or J04, prescribed in-hospital and those started at discharge, irrespective of administration route, including start and stop date. ATC codes are part of the Anatomical Therapeutic Chemical Classification System which organizes drugs into five hierarchical levels based on the organ or system on which they act and their therapeutic, pharmacological and chemical properties.
  - J01 include antibacterials for systemic use
  - J02 include antimycotics for systemic use
  - J04 include antimycobacterial for systemic use
- date of admission
- date of discharge
- surgery date(s) (if applicable)

All hospitals also submit 'volume indicators', each year, which are: number of unique patients with a hospital admission, number of clinical admissions and number of patient days.

Data containing antimicrobial prescriptions are considered complete if they include patient information from all patients hospitalized >1 day from an entire year. The collected data are used to calculate days of therapy (DOT) per 100 patient days (see definition below) and number of courses to provide insight into the duration and quantity of antibiotic use on hospital level. Compared to DDD's, DOTs are not influenced by dosage variations and provide a quantity metric for evaluating antimicrobial use across hospitals.

The data required to calculate the DOT/100 patient days consists of two parts: (1) individual patient data, including prescription and admission data and (2) volume indicators. When volume indicators are missing,

DOT/100 patient days cannot be reported despite available individual patient data. Consequently, the number of hospitals reporting DOT/100 patient days will be lower than the number of hospitals with individual patient data. Individual patient data are available for 22 hospitals and the volume indicators are submitted only for 19 hospitals.

In addition, the AMSM reports quality indicators for antimicrobial use which are displayed semi real-time in an interactive dashboard. This dashboard provides feedback to hospital AMS teams (A-teams) and prescribers, comparing local antibiotic use to a national benchmark (NL Benchmark). The NL Benchmark includes data from all hospitals submitting data within the defined reporting period. Participating hospitals upload their data to the interactive dashboard annually, biannually or quarterly. The data presented here are derived from this interactive dashboard.

Quality indicators
With the collected data 'proxy indicators' are calculated. These indicators provide an indication of the appropriateness of several aspects of antibiotic use in the treatment of infections. The following are included: duration of empiric treatment, intravenous-to-oral switch (IV-oral switch), escalation of antimicrobial therapy, use of restricted antimicrobials and duration of surgical prophylaxis.

Definitions
Individual antimicrobial prescriptions include all individual oral and intravenous (IV) prescriptions of antimicrobial therapy. The following definitions are used:

- **Antimicrobial course**: consecutive prescription of antimicrobials with the same ATC code within 24 hours between stop and start of individual prescriptions, irrespective of route of administration. In the interactive dashboard and the current update, prescriptions with missing stop dates are excluded from proxy indicators representing duration of antimicrobial courses.
- **Patient**: unique patient; an individual with a clinical admission lasting longer than 24 hours.
- **Patient day**: 24-hour period during which a patient is admitted to hospital. The total admission period starts on the day of admission and ends on the day of discharge. Patient days are extracted from the above mentioned 'volume data'.
- **Days of therapy (DOT)/100 patient days**: the ratio of the number of days a patient receives an oral or intravenous antimicrobial agent, regardless of the dose or dosing frequency, per 100 patient days.
- **Proxy indicator 'empiric treatment'**: duration of IV antimicrobial course/combination of courses given on the day of admission and not considered as surgical prophylaxis. This indicator is used to assess which (combination of) antimicrobial agents are initiated, how long the initial course is given for and whether it is stopped/discontinued, or followed by targeted intravenous or oral antimicrobial therapy.
- **Proxy indicator 'IV-oral switch'**: A switch from an empiric IV antimicrobial course to an oral antimicrobial course occurring between 24 hours before and 24 hours after stopping the empiric treatment.

- **Proxy indicator 'escalation'**: initiation of empiric treatment with cefuroxime or ceftriaxone courses followed by a new course of aminoglycosides, piperacillin-tazobactam or meropenem within 24 and 96 hours after initial empiric treatment with cefuroxime or ceftriaxone.
- **Proxy indicator 'restricted antimicrobials'**: Proportion of antimicrobial courses of carbapenems, quinolones, glycopeptides, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, cefiderocol and ampicillin-sulbactam compared to the total number of antimicrobial courses used.
- **Proxy indicator 'surgical prophylaxis'**: Antimicrobial course/combination of courses, regardless of ATC code and route of administration, were considered as surgical prophylaxis if it was started on the day of surgery without any antimicrobial prescription being given 1 day prior to surgery. Duration of surgical prophylaxis is derived using this proxy indicator.

Importantly, while the dashboard contains data from all hospitals that submitted data, not all hospitals contribute to all results (DOTs or proxy indicators) due to missing data for specific metrics. For example, hospitals missing prescription data related to surgeries were excluded from the proxy indicator 'surgical prophylaxes'. Hospital type also influences inclusion/exclusion of data for certain indicators; for instance, a specialized orthopedic hospital was excluded from the proxy indicators 'empiric treatment', 'IV-oral switch' and 'escalation'.

The number of hospitals included in the analyses are mentioned in the footnotes of the figures and tables. Lastly, data from two hospital locations have been combined into one hospital for all results except the DOT/100 patient days.

Results

Participating hospitals

Currently, 36 hospitals have joined the AMSM; an additional five hospitals are in the process of joining. For the current edition of NethMap One Health, 22 hospitals (4 academic and 18 non-academic) provided data for the entire year 2024 (Figure 3.2.1). This represents an increase from 18 hospitals in 2023.

Days of Therapy

Figure 3.2.2 shows DOT per 100 patient days for 19 hospitals compared to the NL benchmark for frequently used antimicrobial groups (penicillins, cephalosporins, carbapenems, quinolones and glycopeptides). In this indicator, data for one hospital, from two locations (hospital A and W), are presented as separate hospitals because the patient days were provided separately for both locations and the data were retrieved from the interactive dashboard prior to the fusion of the two hospitals.

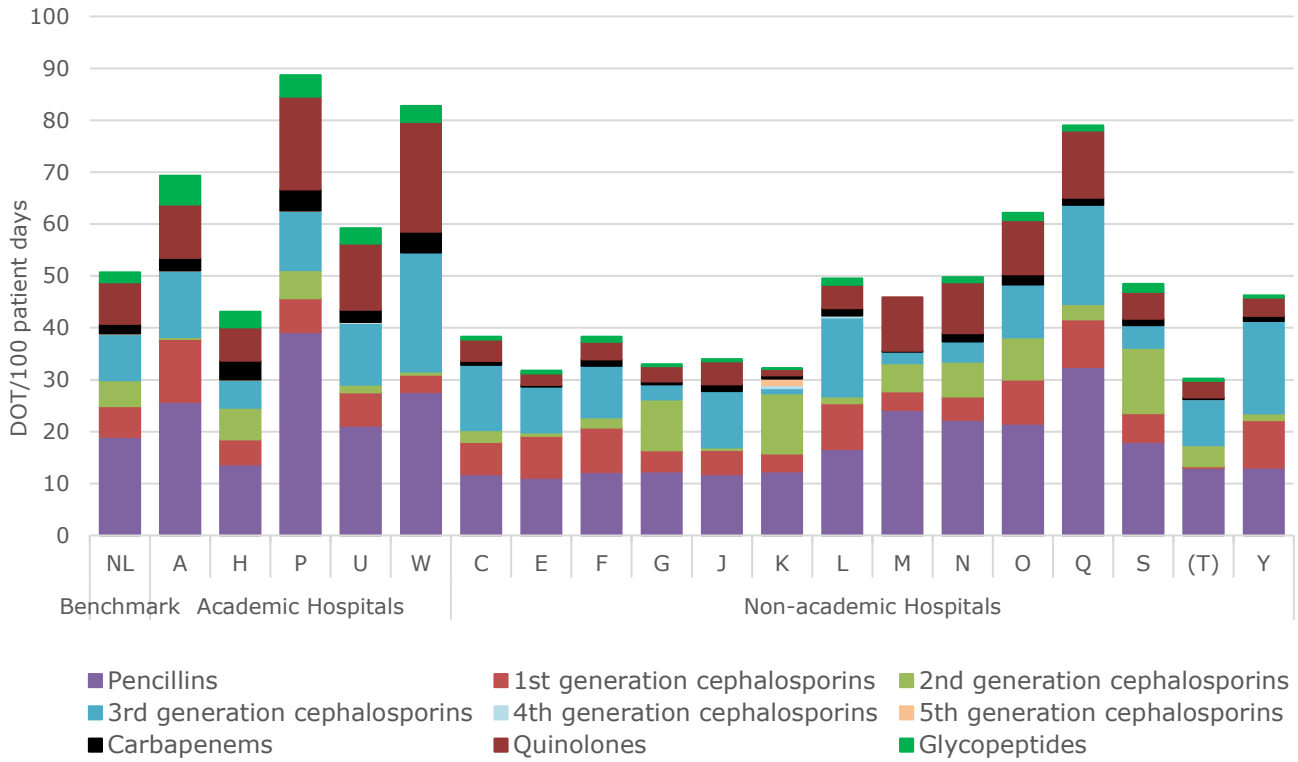
Antibiotic use (both in terms of amount and preferred choice) varies between hospital type (academic versus non-academic) as well as between individual hospitals within their respected hospital type. The academic hospitals generally exhibit higher overall antibiotic use compared to the non-academic hospitals. This difference is particularly

notable for carbapenem, quinolone and glycopeptide use. All academic hospitals use more carbapenems and glycopeptides than non-academic hospitals (academic: median 3.6, IQR 2.4-4.0 and median 3.1, IQR 3.0-4.2 versus non-academic: 1.1, IQR 0.6-1.3 and median 0.5, IQR 0.4-1.0 DOT/100 patient days, respectively). In addition, three out of 5 academic hospitals used median more quinolones than non-academic hospitals (academic: 12.8, IQR 10.3-17.9, versus non-academic: 4.3, IQR 3.3-8.6 DOT/100 patient days).

Figure 3.2.1 *Geographical distribution of 22 hospitals and their affiliated locations providing AMSM data for 2024*

Large green circles represent the primary hospital location selected to represent each of the 22 participating hospitals. Smaller green circles indicate additional affiliated hospital locations submitting data under the same hospital network. For hospitals contributing data from multiple sites, one location was randomly selected as the primary site (large circle) for visualization purposes, while other sites are depicted as smaller circles. Together, these circles illustrate the geographic distribution of hospital networks contributing data.

Figure 3.2.2 *Days of therapy (DOT) per 100 patient days for penicillins, cephalosporins, carbapenems, quinolones and glycopeptides* compared to the NL benchmark in 2024 for 19 hospitals*



( ) hospital without surgery data.
* Hospital M has no data on glycopeptide use (n=18).

Median use of penicillin and third generation cephalosporins was higher in academic hospitals compared to non-academic hospitals (academic: 25.7, IQR 21.2-27.6 and 11.9, IQR 11.4-12.8 versus non-academic: 13.1, IQR 12.2-20.6 and 9.4, IQR 4.0-12.2 DOT/100 patient days, respectively). However, the ranges overlapped substantially, indicating considerable variability between individual hospitals. Overall, first generation cephalosporin use is comparable between hospital types (academic: median 6.4, IQR 4.8-6.5 versus non-academic: median 5.9, IQR 4.2-8.6 DOT/100 patient days), whereas second generation cephalosporin use was lower in academic hospitals than in non-academic hospitals (median 1.5, IQR 0.6-5.5 versus median 3.5, IQR 1.5-7.8 DOT/100 patient days, respectively). Use of fourth and fifth generation cephalosporins was limited across all hospitals.

Duration of empiric treatment
Many of the hospitals in the Netherlands use either cefuroxime or ceftriaxone as empirical treatment for most infections, including sepsis of unknown origin. Empiric treatment ideally lasts between 24 and 72 hours. Figure 3.2.3 shows the duration of these antibiotic courses. An orthopedic hospital was excluded due to a limited number of empiric cefuroxime/ceftriaxone courses (applicable to figures 3.2.3 - 3.2.5). As a result, data from 21 hospitals were used.

On average, more than one-third of the courses given as empiric therapy lasted more than 72 hours for both academic (n=4, mean 35%, range 25-42%) and non-academic hospitals (n=17, mean 37%, range 19-44%), figure 3.2.3. This pattern was observed in 15 of the 21 hospitals, while the remaining hospitals reported lower proportions (range 19-23%). Overall, the academic hospitals displayed a comparable distribution in the duration of empiric therapy for courses lasting until 96 hours (<24 hours, range 30-34%; 24-48 hours, range 14-23%; 48-72 hours, range 9-13%; 72-96 hours, range 5-13%). However, empiric courses lasting longer than 96 hours showed more variation between academic hospitals (range 16-28%). Specifically, hospital P demonstrated the lowest percentage (16%) of courses lasting longer than 96 hours compared to the other academic hospitals.

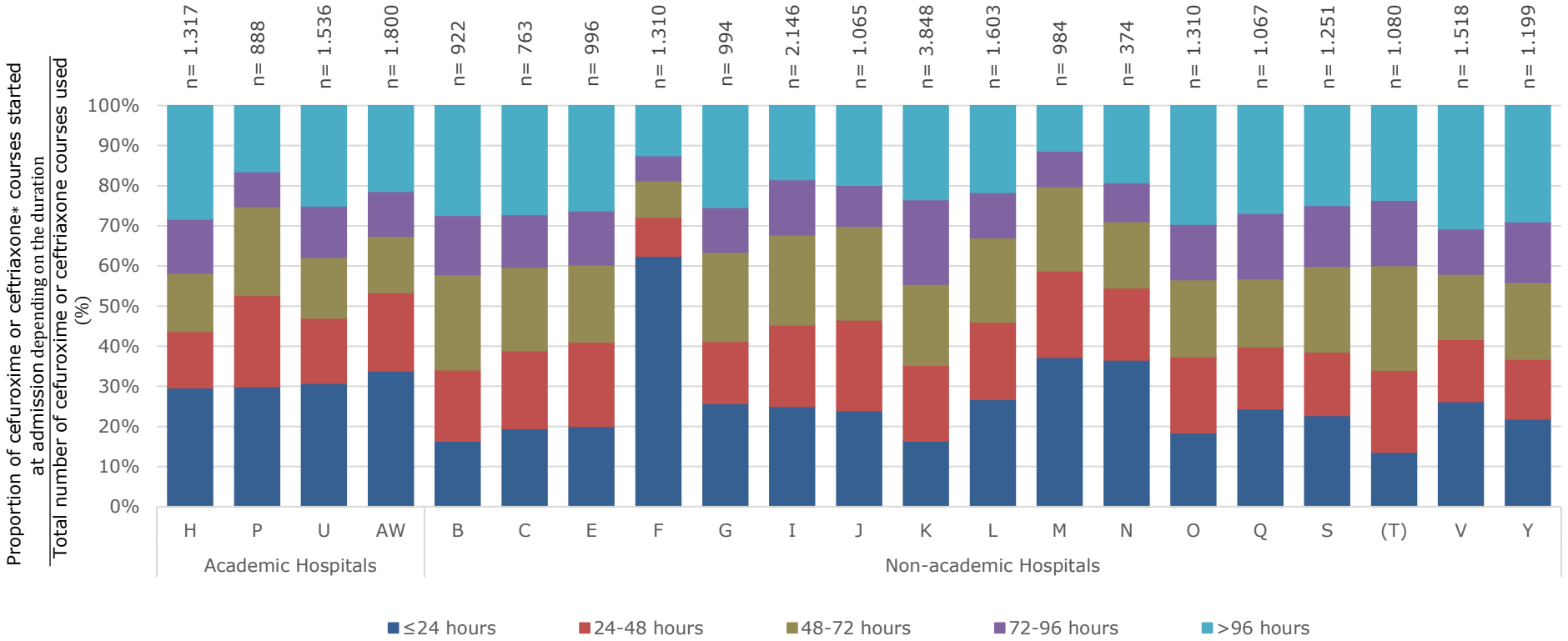
By contrast, non-academic hospitals exhibited greater variability over all empiric therapy durations, particularly evident in hospital F, where 63% of empiric courses lasted 24 hours or less while other hospitals ranged between 14-37%. The greatest variation across all non-academic hospitals was observed in courses lasting 24 hours or less (range 14-63%) and more than 96 hours (range 11-31%).

Intravenous to oral switch
Figure 3.2.4 shows the type of antimicrobial therapy following empiric treatment, irrespective of the duration of initial empiric treatment. On average academic and non-academic hospitals displayed similar proportions of cefuroxime/ceftriaxone empiric treatments that were discontinued without starting a new course, that switched to oral or other IV antibiotics. For academic hospitals (n=4), 57% (mean, range 41-63%) of empiric treatments were discontinued without starting another course, 25% (mean, range 14-40%) were switched to oral antibiotics and 18% (mean, range 14-22%) were switched to other IV antibiotics. For non-academic hospitals (n=17) corresponding proportions were observed: 56% (mean, range 37-67%) discontinued, 31% (mean, range 17-49%) switched to oral antibiotics and 13% (mean, range 9-27%) switched to other IV antibiotics. Non-academic hospitals showed greater inter-hospital variability, particularly in the proportion of courses switched to oral or other IV antibiotics (range 9-27%).

Figure 3.2.5 shows early (≤48 hours) versus later (>48 hours) discontinuation, switch to oral or other intravenous antibiotic following empiric treatment. There was large variation in early IV-to-oral switch (range 13-45%), for both academic (mean 24%) as well as non-academic hospitals (mean 27%). Early switch to other IV antibiotics also varied substantially (range 18-50%), mean 32% versus 27%, respectively.

After more than 48 hours of empiric therapy, antibiotic therapy was discontinued for a larger proportion of patients compared to those patients receiving ≤48 hours of empiric therapy. Specifically, in academic hospitals, 71% (mean, range 55-78%) was discontinued

Figure 3.2.3 *Duration of cefuroxime or ceftriaxone* courses started on the day of admission ('empiric treatment') in 21 hospitals in 2024*

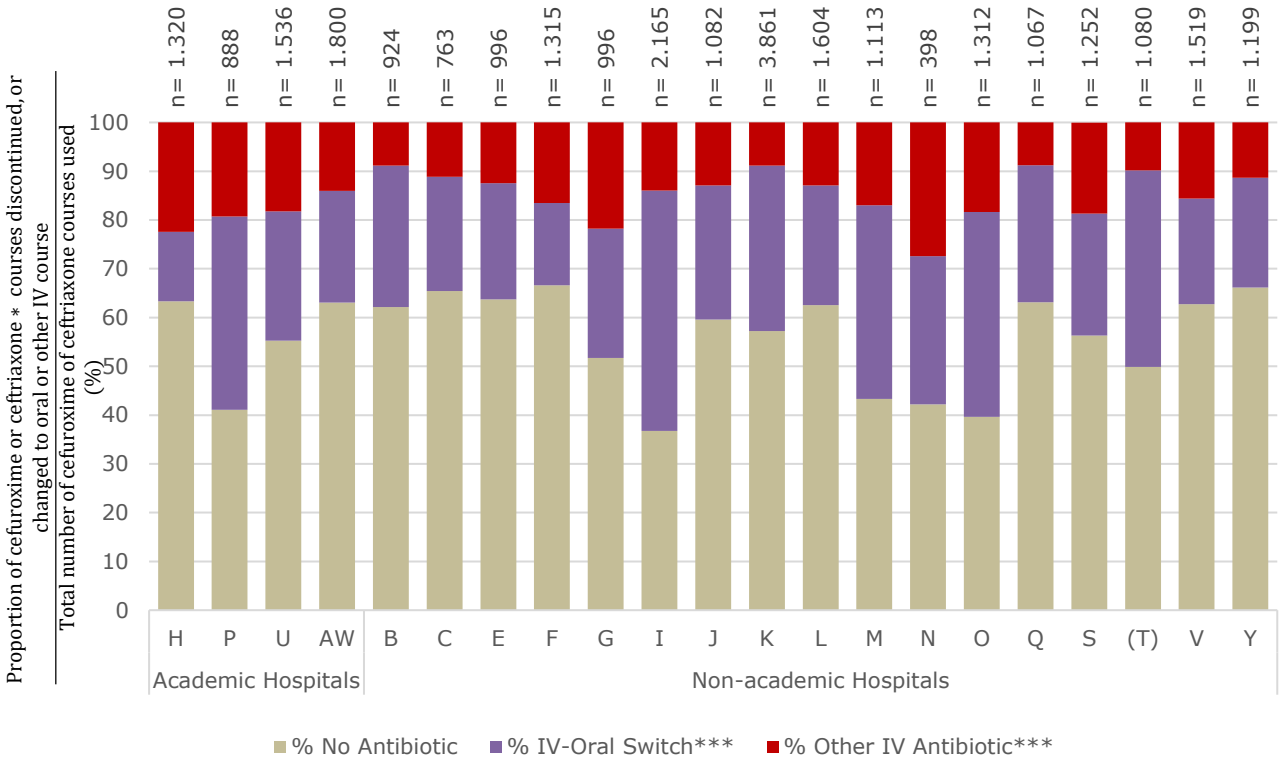


* Cefuroxime or ceftriaxone, depending on the preferred empiric treatment for sepsis of unknown origin.
n = Total number of analyzable** cefuroxime or ceftriaxone courses used in 2024 as empirical treatment is displayed above the columns.
** Analyzable courses include courses with a stop date.
( ) hospital without surgery data
Hospitals I (19%), M (12%) and N (72%) exhibit noteworthy proportions of intravenous (IV) prescriptions missing stop dates.
Hospital R is excluded because of its hospital type; only 5 courses used as empirical treatment in 2024.

versus 44% (mean, range 29-51%) for ≤48 hours. In non-academic hospitals, corresponding proportions are 62% (mean, range 41-77%) versus mean 47% (range 25-63%). Academic hospitals discontinued empiric therapy more frequently than non-academic hospitals when therapy exceeded 48 hours (71% versus 62%).

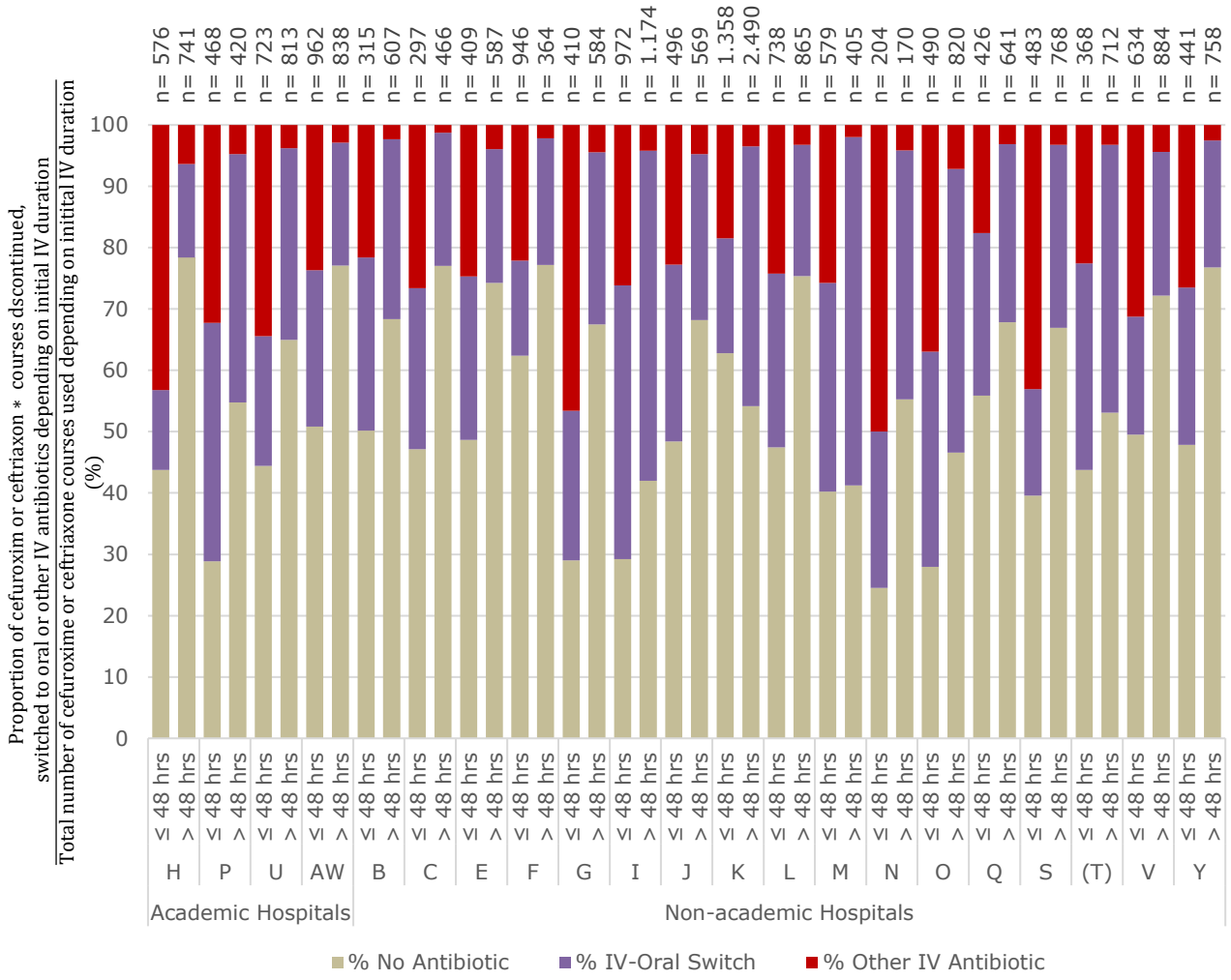
Moreover, when empiric therapy continued beyond 48 hours, switching to oral antibiotics occurred more frequently compared to switching to other IV antibiotics in all hospitals (academic: mean 25% (range 15-40%) versus mean 4% (range 3-6%) and non-academic: mean 35%, (range 21-57%) versus 4% (range 1-7%)).

Figure 3.2.4 *Discontinuation*; change to oral or other intravenous antibiotic treatment of cefuroxime or ceftriaxone** courses started on the day of admission ('empiric treatment') in 21 hospitals in 2024 irrespective of the duration of initial empiric treatment*



This figure shows follow-up therapy among patients who received empiric intravenous treatment irrespective of empiric treatment duration.
* No new antimicrobial course is started after stopping initial empiric treatment.
** Cefuroxime or ceftriaxone, depending on the preferred empiric treatment for sepsis of unknown origin.
n = Total number of cefuroxime or ceftriaxone courses used as empiric treatment in 2024 is displayed above the columns.
*** Switch occurred between 24 hours before and 24 hours after stop.
( ) hospital without surgery data
Hospitals I (19%) and N (70%) exhibit noteworthy proportions of intravenous (IV) prescriptions missing stop dates.
Hospital R is excluded because of its hospital type; only 5 courses used as empirical treatment in 2024.

Figure 3.2.5 *Early (≤ 48 hours) versus later (>48 hours) discontinuation*; change to oral or other intravenous antibiotic of cefuroxime/ceftriaxone** courses started on the day of admission ('empiric treatment') in 21 hospitals therapy in 2024*



This figure shows follow-up therapy among patients who received empiric intravenous treatment ≤ 48 hours and > 48 hours (represented on the x-axis).
* No new antimicrobial course is started after stopping initial empiric treatment.
** Cefuroxime or ceftriaxone, depending on the preferred empiric treatment for sepsis of unknown origin.
n = Total number of analyzable*** cefuroxime or ceftriaxone courses used in 2024 as empirical treatment for ≤ 48 hrs and > 48 hours are displayed above the respective columns.
*** Analyzable courses include courses with a stop date.
( ) hospital without surgery data
Hospital R is excluded because of its hospital type; only 5 courses used as empirical treatment in 2024.

Escalation of antimicrobial therapy
Empiric treatment was escalated to an aminoglycoside-containing regimen, piperacillin-tazobactam or carbapenem, in only a small fraction of the patients ranging from 1.6-4.2% (mean 3.0%) in academic hospitals and 0.4-3.0% (mean 1.9%) in non-academic hospitals (data not shown).

Restricted antimicrobials
Overall, the proportions of restricted antimicrobials relative to total prescribed antimicrobial courses varied across 22 hospitals in 2024 (Table 3.2.1 and Figure 3.2.6). The greatest variability was observed in non-academic hospitals, particularly in the use of amoxicillin-clavulanic courses, with hospital M exhibiting the highest relative usage at 17.9%.

In academic hospitals, carbapenem courses accounted for 1.7-3.5% of total courses, compared to non-academic hospitals with 0.2-2.1% use. Quinolone use ranged from 5.3-10.0% in academic hospitals and 2.3-8.7% in non-academic hospitals. Glycopeptide use varied between 2.8-3.3% in academic hospitals, while non-academic hospitals ranged between 0.2-2.7%. Amoxicillin-clavulanic acid use varied from 6.0-8.1% in academic hospitals and 0.5%-17.9% in non-academic hospitals. Piperacillin-tazobactam use ranged from 0.1-4.8% in academic hospitals and 0.01-5.9% in non-academic hospitals.

The use of three additional antimicrobials, ampicillin-sulbactam, aztreonam and cefiderocol - which became available through a dedicated centralized procedure at the RIVM - were also analyzed. These antimicrobials were used infrequently in both academic and non-academic hospitals. This trend is consistent with the previous two years (data not shown). Nevertheless, when combining data from all 22 participating hospitals in 2024, a general increase relative to the total number of courses was observed, specifically for aztreonam (mean 0.006%, 0.010% and 0.012%) and cefiderocol (mean 0.001%, 0.006% and 0.009%) for 2022, 2023 and 2024, respectively (data not shown).

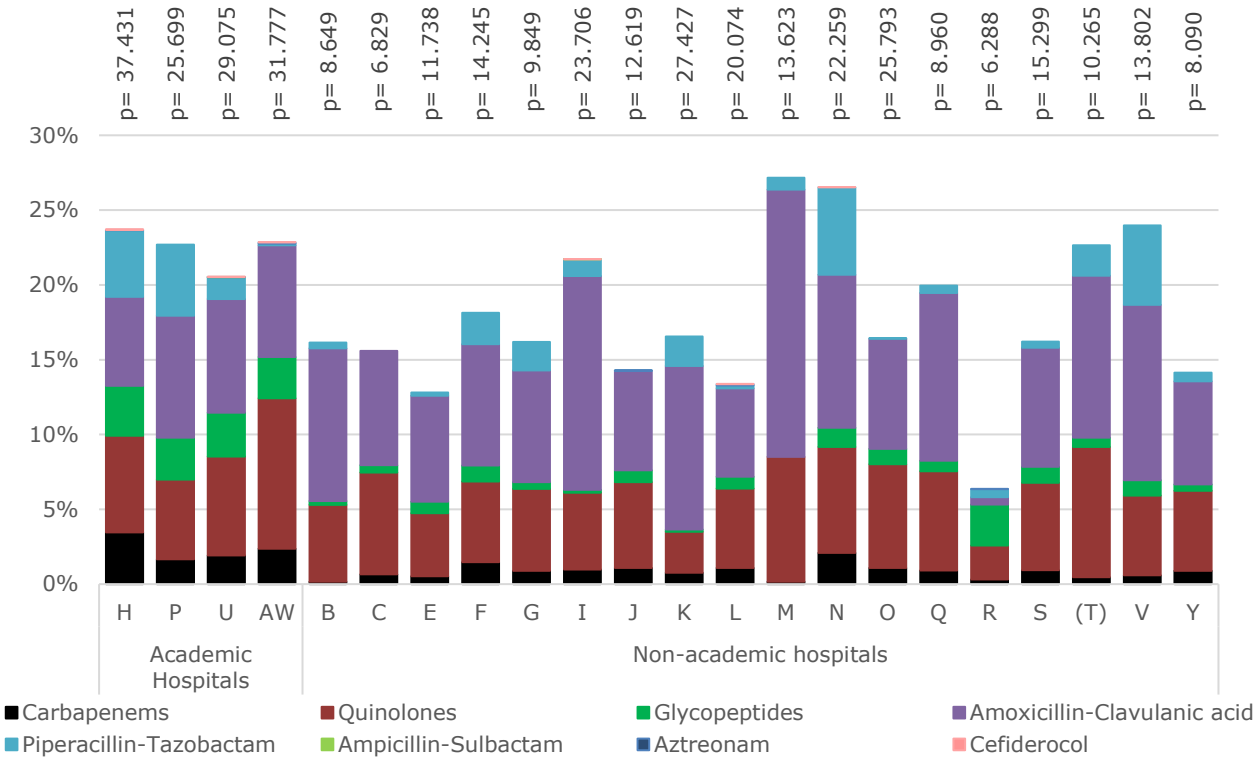
In 2024, a total of 27 courses of aztreonam were used in three out of four academic hospitals and 18 courses in three non-academic hospitals. Cefiderocol was used in three academic (26 courses) and non-academic hospitals (9 courses) while one course of ampicillin-sulbactam was administered in one academic hospital and one non-academic hospital.

Table 3.2.1 *Use of restricted antimicrobials in non-academic and academic hospitals in 22 hospitals in 2024*

Antibiotic group/Antibiotic	Academic hospitals (n=4)	Non-academic hospitals (n=18)
	1.7 - 3.5%	0.2 - 2.1%
Carbapenems	429/25,699 - 1,293/37,431	22/13,623 - 466/22,259
	5.3 - 10.0%	2.3 - 8.7%
Quinolones	1,367/25,699 - 2,420/37,431	142/6,288 - 895/10,265
	2.8 - 3.3%	0.2 - 2.7%
Glycopeptides	1,357/31,777 - 1,246/37,431	41/27,427 - 172/6,288
	6.0 - 8.1%	0.5 - 17.9%
Amoxicillin-clavulanic acid	2,228/37,431- 2,091/25,699	31/6,288 - 2,435/13,623
	0.1 - 4.8%	0.01 - 5.9%
Piperacillin-Tazobactam	18/31,777 - 1,224/25,699	1/1,2619 - 1,304/22,259
	0.01 - 0.09%	0.02 - 0.08%
Aztreonam	4/37,431 - 17/31,777	2/12,619 - 15/20,074
	0.02 - 0.04%	0.004 - 0.025%
Cefiderocol	4/31,777 - 15/37,431	1/2,3706 - 5/20,074
	0.003%	0.004%
Ampicillin-Sulbactam	0 - 1/29,075	0 - 1/2,3706

The total number of hospitals (n) providing data are displayed in the table in the top row.
The percentages in bold show the use of restricted antimicrobial courses compared to the total number of antimicrobial courses as a range from lowest to highest use per hospital in 2024.
The light grey values represent the absolute number of restricted antimicrobial courses used (from lowest to highest) compared to the total number of antimicrobial courses used in 2024.

Figure 3.2.6 *Proportion of restricted antimicrobials relative to the total number of antimicrobial courses prescribed in non-academic and academic hospitals in 2024 (n= 22 hospitals)*

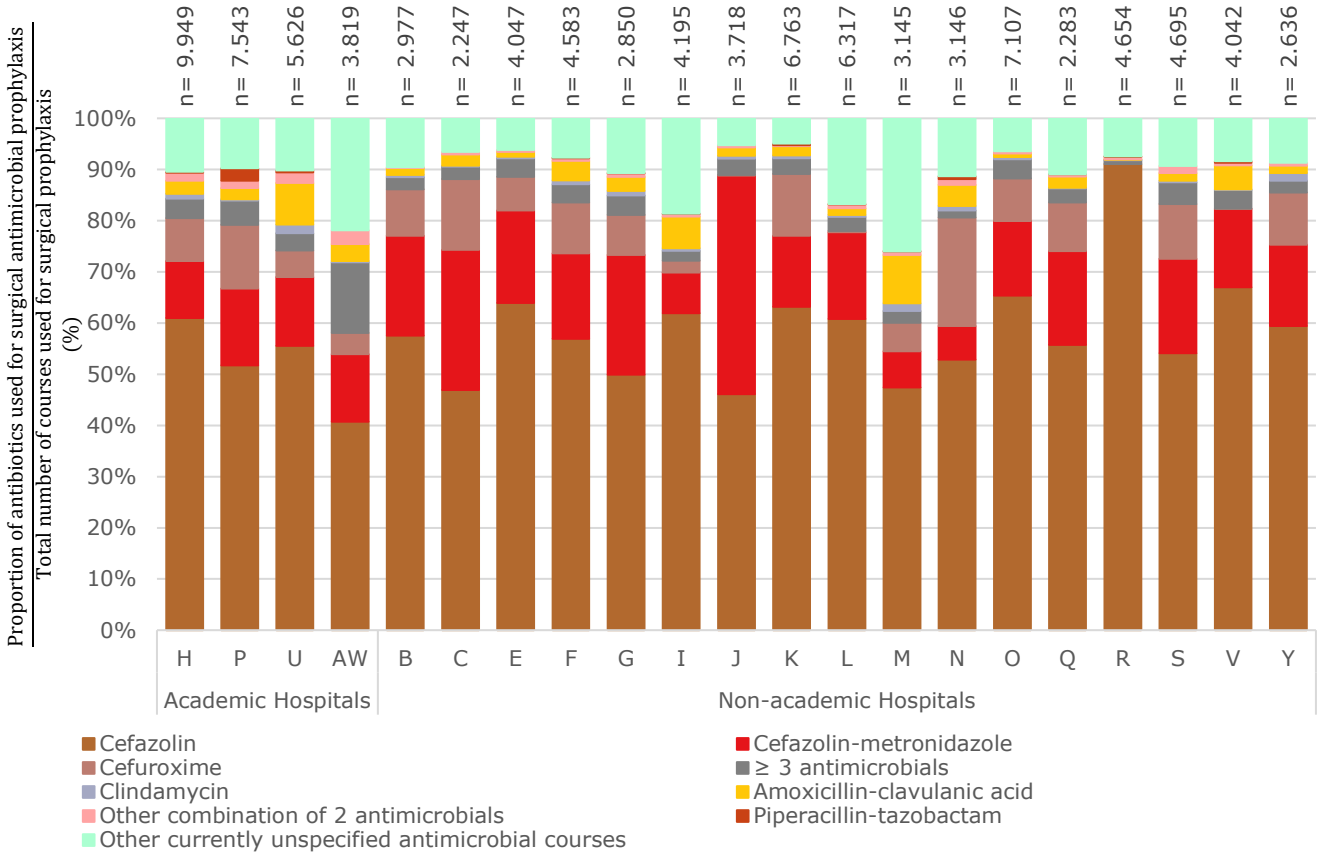


The total number of antimicrobial courses prescribed (p) are shown above the bars which represent the percentage of restricted antimicrobial use within each hospital.
( ) hospital without surgery data
Of note, hospital R is an orthopedic hospital where many operations are conducted.

Surgical prophylaxis
The most commonly prescribed agents as preoperative prophylaxis, according to our proxy definition, are summarized in figure 3.2.7 for 2024. One hospital registering perioperative prescriptions in a parallel system separate from the main EMR was excluded in this analysis because they could not submit these data. Data for the remaining 21 hospitals are shown.

All hospitals used cefazolin as backbone for surgical antimicrobial prophylaxis in 2024, on average it was 59% (range 41-91%), figure 3.2.7 When combining the use of perioperative cefazolin monotherapy with perioperative courses of cefazolin administered alongside metronidazole, the total cefazolin usage increased to an average 74% (range 54-91%). In academic hospitals cefazolin use was 67% (mean, range 54-72%) while in non-academic hospitals it was 76% (mean, range 54-91%). For 2022 this was mean 69% (n=3) and 75% (n=14), and for 2023 it was mean 70% (n=3) and 73% (n=12) in academic and non-academic hospitals, respectively (data not shown).

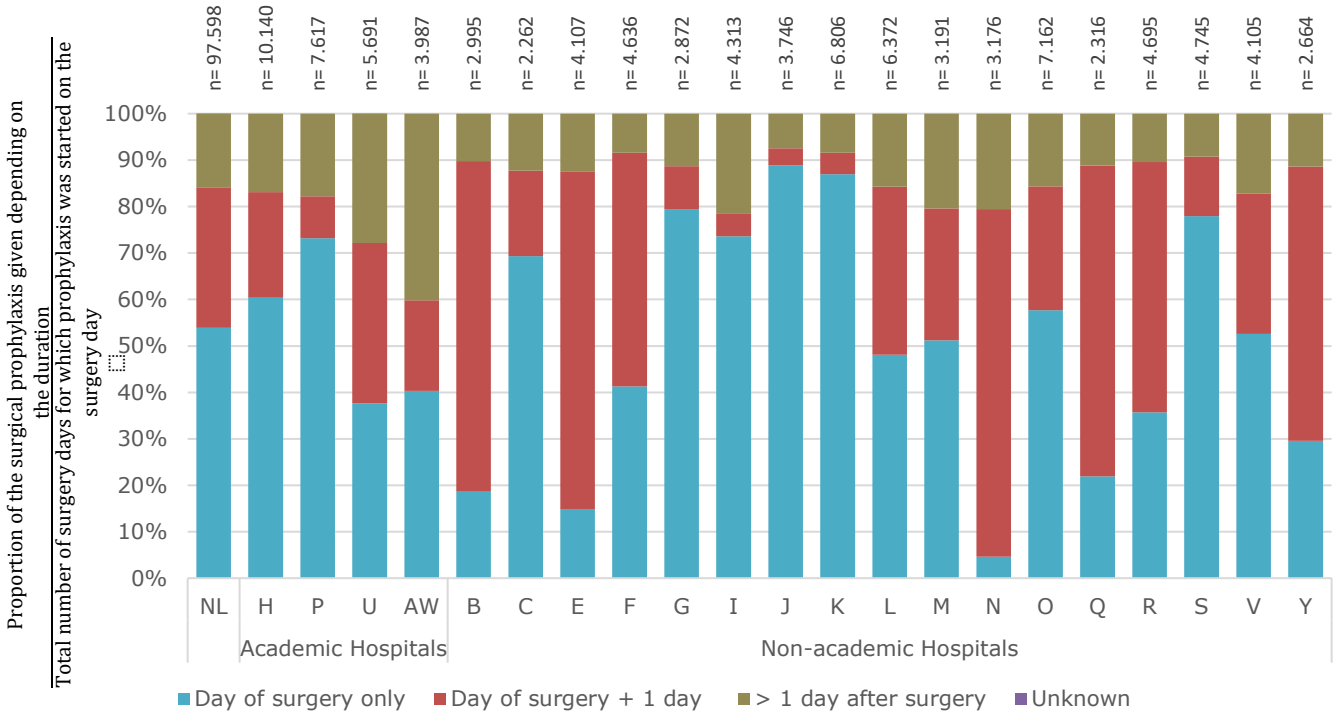
Figure 3.2.7 *Proportion of antibiotics used for surgical antimicrobial prophylaxis compared to the total number of courses used for surgical prophylaxis in 21 hospitals in 2024*



Total number of courses used for surgical prophylaxis (n) are displayed above the columns.
Hospital T was excluded from this analysis because surgery data was missing.

Figure 3.2.8 shows the duration of antimicrobial prophylaxis after surgery. Perioperative antimicrobial prophylaxis should generally be discontinued within 24 hours after surgery. On average 84% (range 60-93%) of surgical antimicrobial prophylaxis was discontinued on the day of surgery or the day after. For academic hospitals (n=4) and non-academic hospitals this was mean 77% (range 60-83%) and mean 87% (range 78-93%), respectively. For 2022 and 2023, this was mean 79%-80% and 86% in academic (n=3) and non-academic (n=14 versus n=12) hospitals, respectively (data not shown).

Figure 3.2.8 *Distribution of the duration of surgical antimicrobial prophylaxis in 21 hospitals relative to the total number of surgery days for which surgical antimicrobial prophylaxis was started on the surgery day in 2024*



Total number of surgery days for which surgical antimicrobial prophylaxis was started on the surgery day (n) is displayed above the columns.
'Unknown' indicates courses with duration > 365 days.
Hospital T was excluded from this analysis because surgery data was missing.

Discussion
Antimicrobial stewardship programs (ASP) have been embedded in Dutch hospitals for several years now. To strengthen stewardship initiatives, the AMSM was developed using structured data routinely recorded in electronic medical records (EMRs). The AMSM provides A-teams and prescribers with benchmarked feedback on both the quantity and quality of antimicrobial use, enabling monitoring, and improvement of guideline adherence and antimicrobial use.

This is the fourth year of data extraction from the interactive dashboard of the AMSM, and the second year of publishing data on DOTs per 100 patient days. For 2024, the dashboard contained data from the entire year (containing antimicrobial prescriptions and linked patient information from all patients hospitalized >1 day) for 22 hospitals, four more than in 2023. Data from two locations (hospital A and W) of the same hospital, reported as two separate hospitals in the previous year, were combined into one for all results except for 'Days of Therapy'.

Highlights 2024
- **Antibiotic use (DOT/100 patient days) and choice**: Overall antibiotic use varied across hospitals and was generally higher in academic hospitals than in non-academic hospitals, particularly for broad-spectrum antibiotics such as carbapenems, quinolones and glycopeptides. This likely reflects differences in patient case-mix, greater prevalence of multidrug resistant organisms and differences in prescribing practices.
- **Beta-lactam prescribing patterns:** Academic hospitals demonstrated a higher use of third-generation cephalosporins (median 11.9 DOT per 100 patient days, IQR 11.4-12.8) compared to non-academic hospitals (9.4, IQR 4.0-12.2), whereas non-academic hospitals showed higher use of second-generation cephalosporins (median 3.5 DOT per 100 patient days, IQR 1.5-7.8) compared to academic hospitals (1.5, IQR 0.6-5.5).
- **Prolonged empiric therapy:** More than one-third of cefuroxime/ceftriaxone courses given as empiric therapy upon admission, lasted longer than 72 hours in both academic (mean 35%, range 25-42%) and non-academic hospitals (mean 37%, range 19-44%). Of the empiric therapy courses lasting longer than 48 hours, 71% (mean, range 55-78%) were discontinued without starting a new course, 25% (mean, range 15-40%) switched to oral and 4% (mean, range 3-6%) switched to other IV antibiotics in academic hospitals. In non-academic hospitals this was 62% (41-77%), 35% (21-57%) and 4% (1-7%), respectively. The substantial inter-hospital variations in the context of the recommended empiric duration (24-72 hrs) highlights opportunities for timely streamlining of empiric therapy.
- **Early (< 48 hrs) IV-to-oral switching:** In both academic and non-academic hospitals, approximately one quarter of empiric therapy courses were switched from intravenous to oral administration within 48 hours. The mean IV-to-oral switch rate was 24% (range 13–39%) in academic hospitals and 27% (range 16–45%) in non-academic hospitals. The range observed across all hospitals (13–45%) suggests there is considerable variability in

practice, indicating clear opportunities for improvement in timely IV-to-oral switching.
- **Escalation:** Escalation of empiric therapy to broad spectrum antimicrobials (e.g., aminoglycosides, piperacillin-tazobactam or carbapenems) was infrequent, occurring in only a small proportion of patients (academic: mean 3.0%, range 1.6-4.2%; non-academic: mean 1.9%, range 0.4-3.0%).
- **Reserve/restricted antibiotics:** Usage of restricted antimicrobials varied notably across the 22 hospitals, with non-academic hospitals showing the greatest variability, particularly amoxicillin-clavulanic acid use (with a maximum relative use up to 17.9%).
- **Last resort antibiotics:** Use of aztreonam, cefiderocol and ampicillin-sulbactam remained low (< 0.1% of all courses) throughout 2022-2024 in both academic and non-academic hospitals.
- **Surgical prophylaxis:** Approximately 74% (mean, range 54-91%) of perioperative antimicrobial prophylaxis courses included cefazolin, either as monotherapy or in combination with metronidazole, which is in line with national guidelines. Eighty-four percent (mean) of surgical antimicrobial prophylaxis courses (range 60–93%) were discontinued on the day of surgery or the following day. This rate was slightly lower in academic hospitals, with a mean of 77% (range 60–83%), compared to non-academic hospitals, which showed a higher mean of 87% (range 78–93%). No substantial changes were observed in comparison to 2022 and 2023 for both prophylactic cefazolin use and surgical prophylaxis duration.

Strengths and limitations
The AMSM coordinator assists hospitals and monitors data quality, collaborating with individual A-teams to enhance the completeness and validity of the data provided by the hospitals. Hospitals extract their data from the EMR using a script based on the AMSM data dictionary, which occasionally requires updating, and upload the data to the interactive dashboard. Each time the data dictionary is improved, hospitals are requested not only to update the version of their script for future data uploads, but also to update previously uploaded data in the dashboard using the new script. This iterative process of script updates reflects an ongoing, collaborative effort between the coordinator and participating hospitals illustrating the importance of updating the dashboard when hospitals are interested in temporal trends.

Although the dashboard contained complete data from participating hospitals, data could still be missing. For example, low numbers of courses for ‘duration of surgical prophylaxis’ or ‘duration of empiric therapy’ for a hospital can indicate missing surgery dates or missing antimicrobial prescription stop dates since the first proxy indicator couples course start dates with surgery dates and the latter proxy indicator, as well as the first, needs stop dates to calculate a duration. As a result, not all hospitals submitting data could contribute to all ‘proxy indicators’.

As we used a proxy indicator to calculate cefazolin use specifically for surgical prophylaxis, it is possible that some therapeutic antimicrobial prescriptions were misclassified as prophylactic. This misclassification

may have led to an overestimation of the total number of courses considered as surgical prophylaxis, thereby underestimating the actual proportion of cefazolin prophylaxis used. Refinement of the proxy indicator, or the use of antimicrobial filters to better include/exclude specific prophylactic courses, may be necessary to address this uncertainty

Further validation of the AMSM including linking indications to antibiotic use will enhance interpretability. Nonetheless, these findings identify a relevant focus area for A-teams with respect to antimicrobial stewardship interventions within their respective hospitals.

Conclusions

The AMSM uses proxy indicators and DOTS per 100 patient days to monitor and gain insight into antibiotic use in Dutch hospitals. The observed differences between academic and non-academic hospitals, as well as substantial inter-hospital variability, highlight the importance of benchmarking against hospitals of the same type to support A-teams and prescribers in improving the rational use of antimicrobial drugs. Additional benchmarks tailored specifically to hospital types are currently under development.

Practice variation is one of the most informative findings in this annual AMSM report for NethMap One Health. For instance, the rate of early IV-to-oral switching within 48 hours varies widely between hospitals, ranging from 13% to 45%, indicating substantial room for improvement. Likewise, the proportion of surgical prophylaxis courses discontinued on the day of surgery or the following day, ranges from 60% to 93%, suggesting that hospitals at the lower end should evaluate their practices. These are just two examples of how AMSM data can highlight areas for targeted intervention. In the coming years, AMSM will provide increasingly active feedback to support and encourage improvement initiatives at the hospital level.

A-teams should also continually and critically analyze and interpret their own data within the framework of local antibiotic guidelines to accurately assess appropriate antimicrobial use within their hospital. Ongoing data validation, combined with future efforts to link clinical indications to antibiotic use, will further improve the AMSM providing deeper insights into the quality of antibiotic use in The Netherlands.

4 Usage of antibiotics in animal husbandry in the Netherlands

Sales and use of antimicrobial veterinary medicinal product (AVMPs) are monitored by the Netherlands Veterinary Medicines Institute (SDa, Diergeneesmiddelenautoriteit). The information described in this part of MARAN is presented in more detail in the annual reports of the SDa.[1]

4.1 Total sales of veterinary antibiotics in the Netherlands 2024

4.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all Antimicrobial Veterinary Medicinal Products (AVMPs) on package level sold in 2024 in the Netherlands, as extracted from the Vetindex and supplemented with AVMPs data of non-FIDIN members. These data are estimated to cover approximately 98% of all sales in the Netherlands, according to FIDIN. 3.9% (in mass) of the sold AVMPs (including all administration forms like tablets and injectables) is exclusively authorized for companion animals or horses. AVMPs that are marketed in accordance with legal exemptions such as products that are imported from other EU member states in accordance with cascade legislation, are not included in sales figures, but are revealed in usage. For 2024 2.3 % of use was cascade other EU products due to increasing Dutch veterinary medicinal products shortages particularly in veal calves. In 2023 this cascade use comprised 1%, mostly in sectors for which Dutch authorized products aren't available, like in rabbits, goats and other poultry sectors.

Actual use in animal husbandries can be somewhat different from the quantities sold due to stock piling and cross border use. Monitored mass used in the major livestock farming sectors (pigs, broilers, turkey, other poultry, veal calves, dairy- and other cattle, meat rabbits) covered 89.2% of total sales in 2024. This coverage fluctuates over the years, due to not yet monitored sectors (e.g. sheep, horses, companion animals) and stockage differences between the years.
AVMPs are reported as active base substance mass (excluding mass of salts and esters), including oral products, injectables, intramammary injectors and topical applications like ointments, eye drops and sprays. The sales data in this report involves total sales for all animals, not stratified by animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported in chapter 4.2.

4.1.2 Trends in total sales

Table 4.1.1 shows the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. In 2024 in total 121 tonnes of AVMPs were sold, representing an increase of 4.0% in comparison with 2023. A decrease in sales by 75.5 % over the years 2009-2024 is attained (with 2009 considered the reference year by the Dutch Government).

Figure 4.1.1 shows the trends in sales (mass, black line) in relation to the dynamics of liveweight of Dutch livestock (dashed line) and the total use on farms (mass, bars) in the livestock sectors monitored, from 2009

to 2024. Antimicrobial use (in kg) in livestock sectors is presented as bars in which the use in different animal species can be distinguished. Figure 4.1.1 shows a slightly decreasing trend in liveweight of Dutch livestock. Compared to 2009 the liveweight of Dutch livestock has decreased by 16%. The decrease in antimicrobial use is much greater, demonstrating that trends in total mass sold and used cannot be explained by a drop in the liveweight of Dutch livestock. Veal calves (light blue) and pigs (green) used 80% of the total mass of all antibiotics used for therapy. Animals treated in these two sectors are large and therefore need more antibiotics per administration than small animals like broiler chickens. This illustrates that sales data provide limited information about exposure of animals at risk. Use data based on mass may result in the suggestion that exposure of broiler chickens to antibiotics is limited based on the small proportion of total mass used in these animals.

The discrepancy in mass in 2024 between sales and usage in monitored sectors was 10.8% as illustrated in Figure 4.1.1. The difference between sales and use data fluctuates as described by the difference between the solid black line (mass sold) and bars (mass used in monitored sectors).

As demonstrated in Figure 4.1.2, antimicrobial sales by antibiotic class show a fluctuating pattern over the years, with an overall decreasing tendency in most antibiotic classes, and some variation from year to year.

Tetracyclines
Tetracyclines represent the first place when expressed in mass; the sales have increased with 12.4% compared to 2023. The fraction of doxycycline (not specified in Figure 4.1.2) was in 2024 65.9% of the total sales of tetracyclines (63.7% in 2023, fluctuations between 31% and 69% in the years 2011-2024).

Penicillins
Second place in mass, also sales of penicillins (including aminopenicillins) increased in 2024 compared to 2023, with 3.7%. The distribution of broad and narrow spectrum penicillins (in mass sold) is comparable to previous years with 69.4% aminopenicillins.

(Fluoro)quinolones
The sales of fluoroquinolones increased with 8kg (+7.4% compared to 2023). An overall reduction of 91.5% was realized since 2011. In 2024, 52% of the sold fluoroquinolones were applied in the monitored sectors. Extending monitoring to other animal species (as is regulated with EU 2019/6) is warranted. The sales of quinolones (flumequine) decreased with 21.2% in 2024 when compared to 2023; these AVMPs are exclusively applied in food producing animals, and partly substitute the use of colistin. Although the EMA Antimicrobial Advice ad hoc Expert Group (AMEG) decided not to differentiate between quinolones and fluoroquinolones (both category B), in the Netherlands quinolones are still classified at a level of lower importance (2nd line, comparable with category C) than fluoroquinolones (3rd line, comparable with category B). This discrepancy is under evaluation in 2025 by the revised Netherlands working party for veterinary antibiotic policy (NWVAB).

Cephalosporins 3rd/4th generation
Sales of these AVMPs were relatively stable at a low level since 2016, fluctuating in kg range. In 2024 only two products were sold, representing 3 kg active substance.

A reduction of 99.97% of cephalosporins 3rd/4th generation sales has been achieved since 2011.

Polymyxins
Colistin sales decreased in 2024 again, with 16.8%. The reduction since 2011 is 86.2%. Based on the classification of polymyxins as *Highest Priority Critically Important Antimicrobials* (CIAs) in the 6th revision of the WHO CIA list (2019), the *Expert Panel of the* Netherlands Veterinary Medicines Institute considers polymyxins as third choice antibiotics, and this antibiotic class is reported as such. This implies that similar as for fluoroquinolones and 3rd/4th generation cephalosporins the Dutch target for use since 2020 is 0 $DDDA_F$. The ESVAC group introduced in 2016 the colistin desirable-level-benchmark for EU member states. This benchmark is below 1 mg/PCU for sales data, irrespective of the sectors in which colistin is used. Netherlands is below that unified benchmark. However, in weaned piglets and laying hens room for improvement can still be recognized. Use in weaned piglets did drop for the fourth consecutive year. Use in laying hens was on a comparable, but relatively high level, level compared to 2023. In the other livestock sectors virtually no colistin is used.

4.2 Usage in pigs, veal calves, cattle, broilers, turkeys and rabbits in the Netherlands

In figure 4.2.1, antimicrobial use (AMU) based on annual prescription data is presented for each livestock sector. Important reductions in AMU have been achieved in all sectors (Figure 4.2.1) since monitoring of AMU was established.

Figure 4.2.2 shows that in most sectors first choice antimicrobials (green and blue bars) are dominant. In most sectors, except for pigs, broilers and turkeys, this proportion of first choice AVMP's has attained a stable level, at over 80%. Figure 4.2.2 also illustrates that use of fluoroquinolones (red bar) is low in all livestock sectors, use is the highest in turkeys. Fluctuations from year to year in turkeys and rabbits can be attributed to the small number of farms (30-40). In turkeys an important overall use reduction since 2016 (first year of monitoring) has been realized of 67% in 2024. In veal calves, a large sector using 85% first choice AVMP's, the steady decrease in total use was halted in 2020 (55%), and in 2024 the remaining reduction was 53%.

In rabbits, the use of colistin was abandoned in 2020, at the cost of introducing flumequine. Total AMU in this sector has attained a huge reduction in 2022, stabilizing since then, still using flumequine though. Since flumequine is categorized in the same class as other fluoroquinolones by the AMEG categorization of EMA its use needs to be revised. In mass the impact of the rabbit sector is limited in comparison to other sectors with comparable use in number of DDDA (poultry and veal calves).

Expressing antibiotic use in number of Defined-Daily Dosage Animal like in figures 4.2.1 and 4.2.2 shows that AMU in broilers, turkeys and in pigs in 2024 is comparable in number of DDDA, although distinct differences in applied antibiotic classes are notable.

For more details about all animal sectors, annual reports of the SDa should be consulted.[1]

EU regulation 2019/6 (VMP-reg)
EU Regulations about, amongst others, monitoring of veterinary antimicrobial *use,* started in 2023 (collecting data 2024 and planning to report in 2025) are implemented in national legislation for all EU member states. Sales data are reported to EMA, as has been done by most EU member states in the ESVAC project until 2022. Additionally, use data have been reported for pigs, cattle, turkeys and broilers in 2024 concerning 2023 use data. Monitoring of sales and use data may be expanded from anti*bacterial* substances to anti*microbial* substances including antimycotic, antifungal, antiviral and anticoccidial substances. Cascade use of products imported from other EU countries will have to be incorporated in sales (and use) data.

In 2026, monitoring of use of indicated products will be extended to rabbits, sheep, goats, ducks, geese, finfish and horses. Most of these sectors are already preparing the implementation of a monitoring system, rabbits are already included in the Dutch AMU monitoring. In 2029 the use of these products will also be monitored in cats and dogs. For horses and companion animals cascade use of antimicrobial medicinal products for human use will have to be included as well in the *use* monitoring.

Conclusions

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. A small increase in sales of AVMPs in the Netherlands in 2024 is not reflected by an overall increase in used mass as observed in the use monitoring data. The calculation of consumption, based on national conversion factors (DDDAs) of authorized veterinary medicinal products shows that use has stabilized in most sectors.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is low, even in the unmonitored sectors. Use and sales of polymyxins decreased again in 2024, overall decrease since 2011 is 86% in sales. Of the fluoroquinolones, 48% is applied is sectors not yet monitored; an overall decrease of 92.1% since 2011 is observed.

Table 4.1.1 *Antimicrobial veterinary medicinal product sales from 2004-2024 in kg (thousands) (FIDIN, 2025)*

Year	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20	'21	'22	'23	'24
beta lactam antibiotics	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43	36	40	34	29	30	31
tetracyclines	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51	49	47	31	32	36
macrolides & lincosamides	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25	23	24	21	19	21	20
aminoglycosides	9	11	11	12	11	10	8.6	7.3	5.8	3.4	1.8	2.7	2.1	1.9	2.0	1.8	1.7	1.8	1.5	1.5	2.0
(fluoro) quinolones	7	8	7	9	8	8	6.6	5.1	3.1	2.8	3.8	4.2	3.4	3.4	3.9	2.7	2.6	2.1	2.4	2.3	1.9
trimethoprim/ sulfonamides	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33	29	30	32	22	24	24
other antibacterials	6	6	8	8	7	15	13	10	10	8.1	7.8	7.5	7.4	7.2	7.5	7.4	7.2	6.9	6.3	5.6	6.1
total sales	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179	150	154	145	112	117	121

Figure 4.1.1 *Mass balance of AVMPs sales data (black line, left y-axis) and use data (colored bars, left x-axis) (kg x 1000), combined with total live weight of the food animal population (dotted line, right y-axis, kg x 10^6) from 2009-2024*

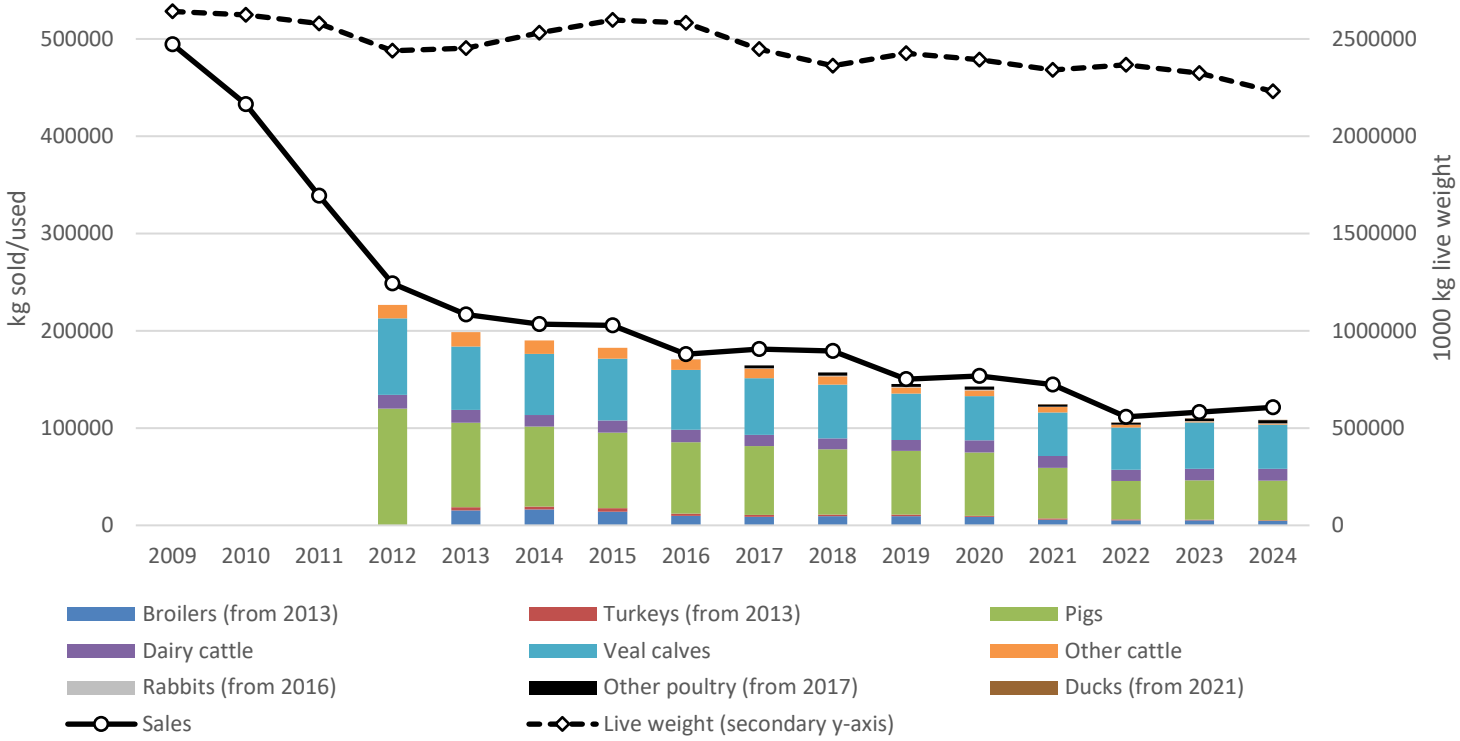


Figure 4.1.2 *Antimicrobial Veterinary Medicinal Product sales by antibiotic class from 2011-2024 in kg (thousands); antibiotic class "other" comprises bacitracin, fusidic acid and nitro-imidazole's*

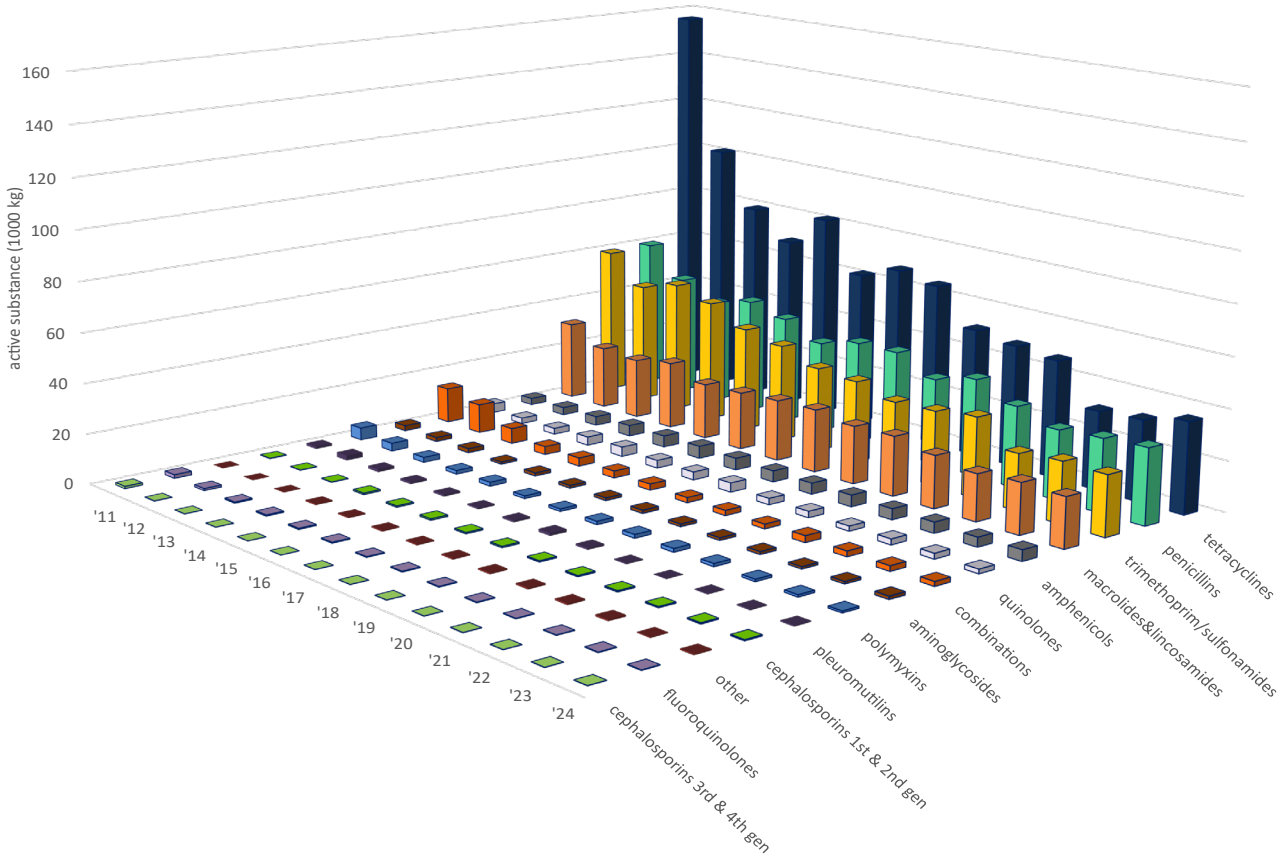


Figure 4.2.1 *Number of animal-defined daily dosages per animal-year for rabbits (grey), turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2024 as $DDDA_{NAT}$) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area)*

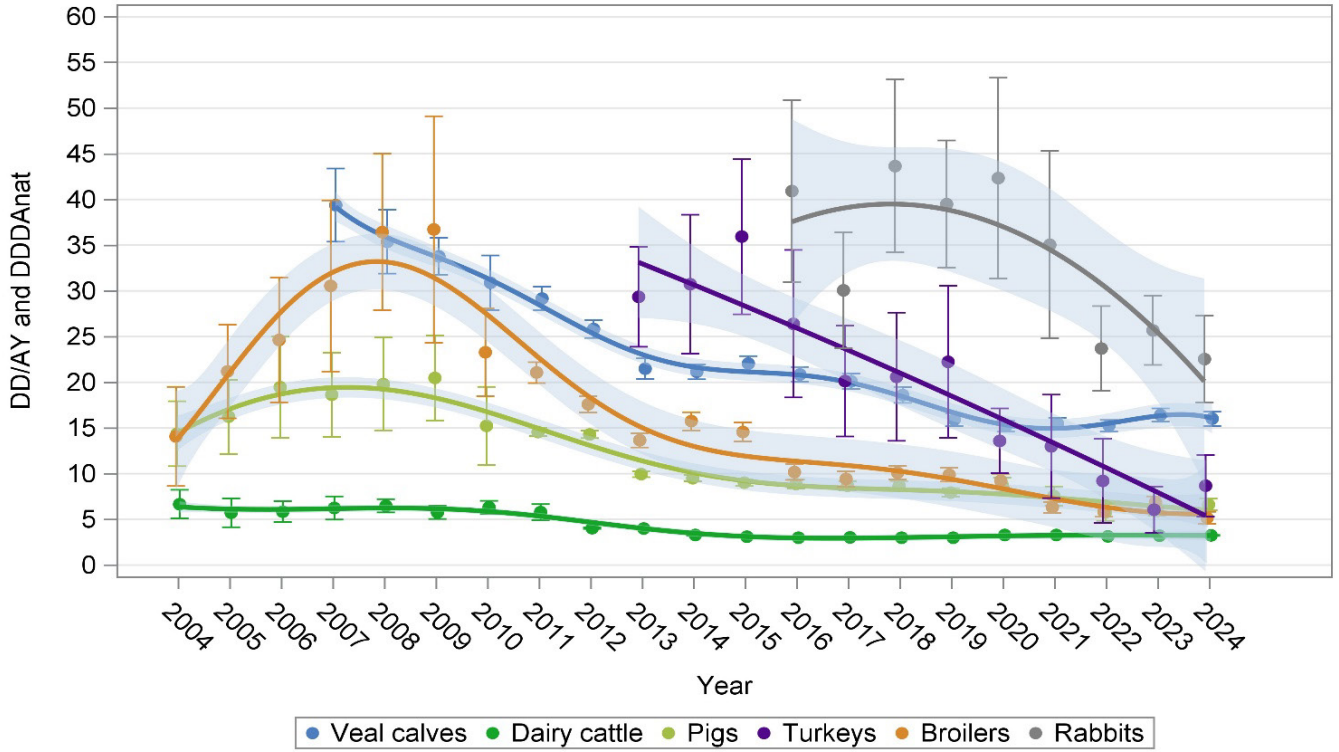
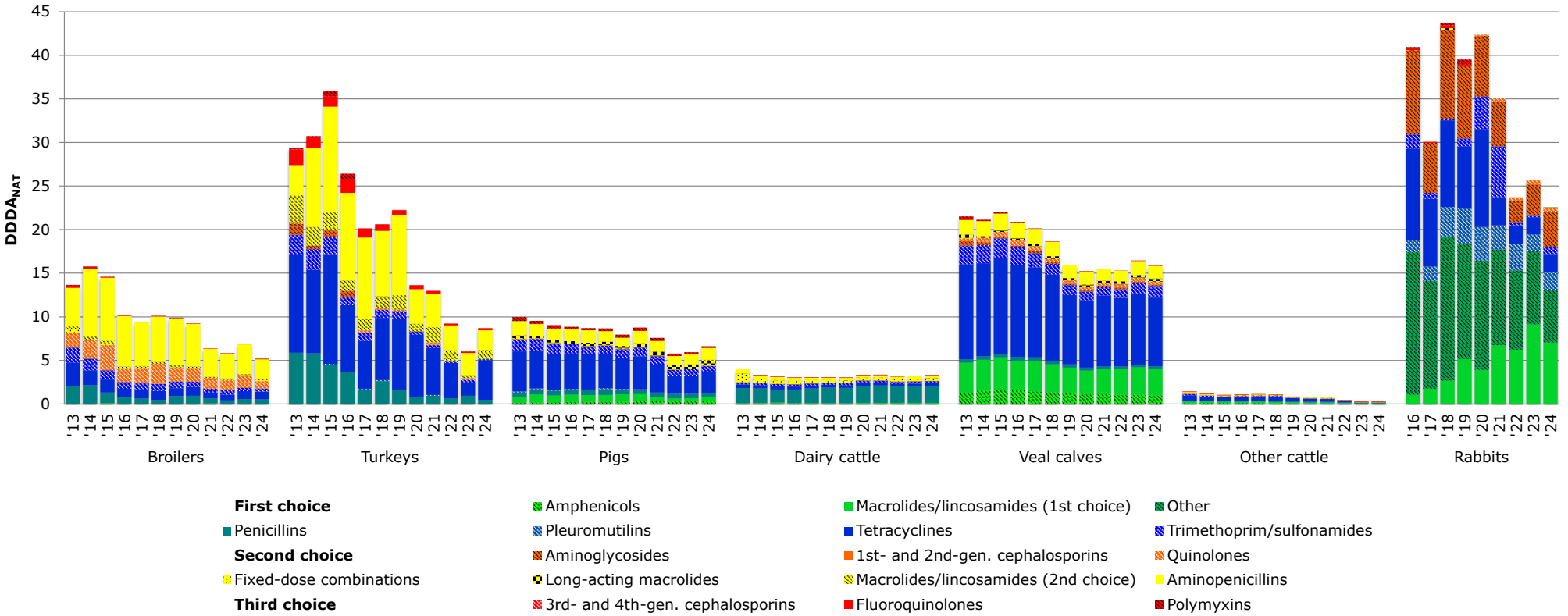


Figure 4.2.2 *Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by antibiotic class per animal sector over the years 2013-2024*



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1. SDa annual reports: https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports.

5 AMR in human pathogens

5.1 General AMR surveillance

5.1.1 Methods and description of data from the Infectious Diseases Surveillance Information System for Antimicrobial resistance (ISIS-AR)

5.1.1.1 Methods

Since 2008, routinely available antimicrobial susceptibility data of all isolates from medical microbiology laboratories in the Netherlands, including minimal inhibitory concentration (MIC) values and disk zone diameters, are collected in the Infectious Diseases Surveillance Information System for Antimicrobial Resistance (ISIS-AR). This surveillance system is a combined initiative of the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM), and is coordinated by the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) in Bilthoven.

In 2024, 47 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 47 laboratories, 39 provided complete data on the last five years (2020 to 2024). Seven of these 39 laboratories exclusively served university hospitals; 29 laboratories served non-university hospitals, general practices, and long-term care facilities; and three laboratories exclusively served general practices and long-term care facilities. We selected only data from these 39 laboratories to avoid bias in time trends due to incomplete data. Due to laboratory merges in 2024, merged entities are counted as single laboratories. Additionally, the set of laboratories that provides complete data over the last 5 years fluctuates per edition of NethMap, due to technical issues, or because of differences in starting date. As a result, the number of participating laboratories is not directly comparable between different editions of NethMap. Therefore, time trends were calculated based on the current selection of included laboratories.

All data provided to ISIS-AR are carefully validated[1]. Data with confirmed or probable technical errors are, after consultation with the laboratory that provided the data, corrected or excluded from the analyses in this report.

Selection of isolates

We calculated resistance levels and, if applicable, time trends by setting of care, i.e., general practices, outpatient departments, inpatient departments (excl. intensive care units, incl. emergency departments), intensive care units, urology departments (inpatient and outpatient separately), and long-term care facilities. For general practices (section 5.1.2) and long-term care facilities (section 5.1.4), we selected urine isolates for analysis of resistance in Enterobacterales and *Pseudomonas aeruginosa*; wound or pus isolates for analysis of resistance in the *Staphylococcus aureus* complex; wound or pus, respiratory, and genital isolates for analysis of resistance in β-haemolytic *Streptococcus* group

A; and urinary and genital isolates for analysis of resistance in β-haemolytic *Streptococcus* group B. In accordance with age categories used in the guidelines of the Dutch College of General Practitioners (NHG) for urinary tract infections, resistance levels and five-year trends for Enterobacterales and *P. aeruginosa* isolates from urine of general practice patients were calculated separately for patients aged ≤12 years and patients aged >12 years. For analyses on data from outpatient departments (section 5.1.3.1), inpatient departments (excl. intensive care units, section 5.1.3.2), and intensive care units (section 5.1.3.3), we selected isolates from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus. Additionally, we conducted a separate analysis for blood isolates from inpatients (incl. patients from intensive care units, section 5.1.3.4). For urology departments (section 5.1.3.5), we selected only urine isolates. In section 5.1.5, we show resistance levels for respiratory pathogens (S*treptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) in general practitioners' patients and hospital patients. For the analysis on general practitioners' patients, we selected isolates from the upper and lower respiratory tract. For the analysis on hospital patients, we additionally selected isolates from blood and cerebrospinal fluid.

The category 'wound or pus isolates' comprises isolates from deep and superficial wounds, pus (including pus from abscesses), but also skin (excluding perineal swabs), normally sterile sites or cultures taken using a sterile procedure (i.e., biopsy, aspiration), synovial fluid, peritoneal cavity fluid and fluid for continuous ambulatory peritoneal dialysis (CAPD), eyes (both normally sterile and non-sterile sites), amniotic fluid, and samples from- or related to medical implants. The category 'lower respiratory isolates' comprises respiratory isolates from below the glottis, whereas 'upper respiratory tract isolates' originate from respiratory samples that were taken above the glottis.

Since the number of *Staphylococcus argenteus* and *Staphylococcus schweitzeri* isolates was too small for separate analyses, the data for *S. aureus*, *S. argenteus* and *S. schweitzeri,* all belonging to the *S. aureus* complex, were analysed together and further referred to as *S. aureus*. In all sections 5.1.2 through 5.1.4, *S. argenteus* comprised 0 to 0.07% of the isolates from this complex. *S. schweitzeri*, the third member of the *S. aureus* complex, was found only once in a patient from an outpatient department, and once in a patient from a non-ICU hospital department.

For each analysis, we selected the first isolate per species per patient per year to avoid repeated sampling causing bias in the calculation of resistance levels and time trends. We included only data on diagnostic (=infection related) samples, and only calculated resistance levels for pathogens for which at least 100 isolates in each year were available for analysis. Furthermore, to avoid bias due to selective testing of agents, for each pathogen-agent combination, we included only data from laboratories that tested at least 50% of isolates for that specific agent in each year. Finally, for sufficient representativeness of the results, we only calculated the resistance level and time trend of a pathogen-agent combination if the data from at least 50% of the selected laboratories could be included, with a minimum of 15 laboratories.

Calculation of resistance levels
We calculated the percentage of resistant isolates ('R'). To avoid bias due to differences in (versions of) breakpoint guidelines and expert rules used in the participating laboratories, we first reinterpreted all crude test values (minimum inhibitory concentrations (MICs) and diameters) according to EUCAST breakpoints version 14.0 (2024).
Since 2019, EUCAST has defined an area of technical uncertainty (ATU) for several pathogen-agent combinations. These ATUs are warnings to laboratory staff that there is an uncertainty that needs to be addressed before reporting the susceptibility results to clinical colleagues. EUCAST specifically states that "the ATU is not a susceptibility category and does not prevent the laboratory from interpreting the susceptibility results". Laboratories are encouraged (but not obliged) by EUCAST to perform an alternative test (e.g., an MIC-test instead of disk diffusion) when the test value is within the ATU. Therefore, we reinterpreted all test values according to the EUCAST breakpoints version 14.0, including the test values that were within the ATU, trusting that laboratories conducted and reported re-tests if indicated. However, this policy might have resulted in some misclassification if laboratories did not perform an alternative test, resulting in an interpretation of the test value that lies within the ATU to 'R', whereas the isolate is in reality susceptible or vice versa. Nevertheless, we do not expect that this misclassification has strongly influenced resistance percentages, since the proportion of isolates with test values in the ATU is low.
Also in 2019, EUCAST has redefined the category 'I' from a lumped definition of 1) uncertain therapeutic effect, 2) susceptible only for treatment in specific body sites or with high dosing regime, and 3) a buffer zone for technical laboratory uncertainties, to the definition 'Susceptible, increased exposure'. From then onwards, the technical uncertainty was covered by the ATU, as described before, and the number of pathogen-agent combinations for which an I-category was defined in the breakpoints decreased. Nevertheless, because we calculated the percentage of resistant isolates ('R'), and reinterpreted all test-values, including those from previous years, according to EUCAST breakpoints version 14.0 this did not influence resistance percentages or trends.
We included data from all laboratories for which at least 80% of test values could be reinterpreted each year. Where reinterpretation was not possible, this was due to missing crude data or test values that were not compatible with EUCAST breakpoints.

For several pathogen-agent combinations EUCAST has specified breakpoints that apply only to a specific diagnosis or treatment strategy, for example, separate breakpoints for meningitis and other indications than meningitis. For each of those pathogen-agent combinations resistance percentages are shown for the diagnosis or treatment strategy that is most common. However, for Enterobacterales, the co-amoxiclav MIC breakpoint for uncomplicated urinary tract infection could not be used to reinterpret MIC values because the maximum test value of >16 mg/L that can be measured by the VITEK2 system does not reach the breakpoint of 32 mg/L. Therefore, in sections 5.1.2 through 5.1.4, for Enterobacterales*,* we only present resistance to co-amoxiclav and all combinations of agents that include co-amoxiclav according to the breakpoint for oral administration in infections originating from the

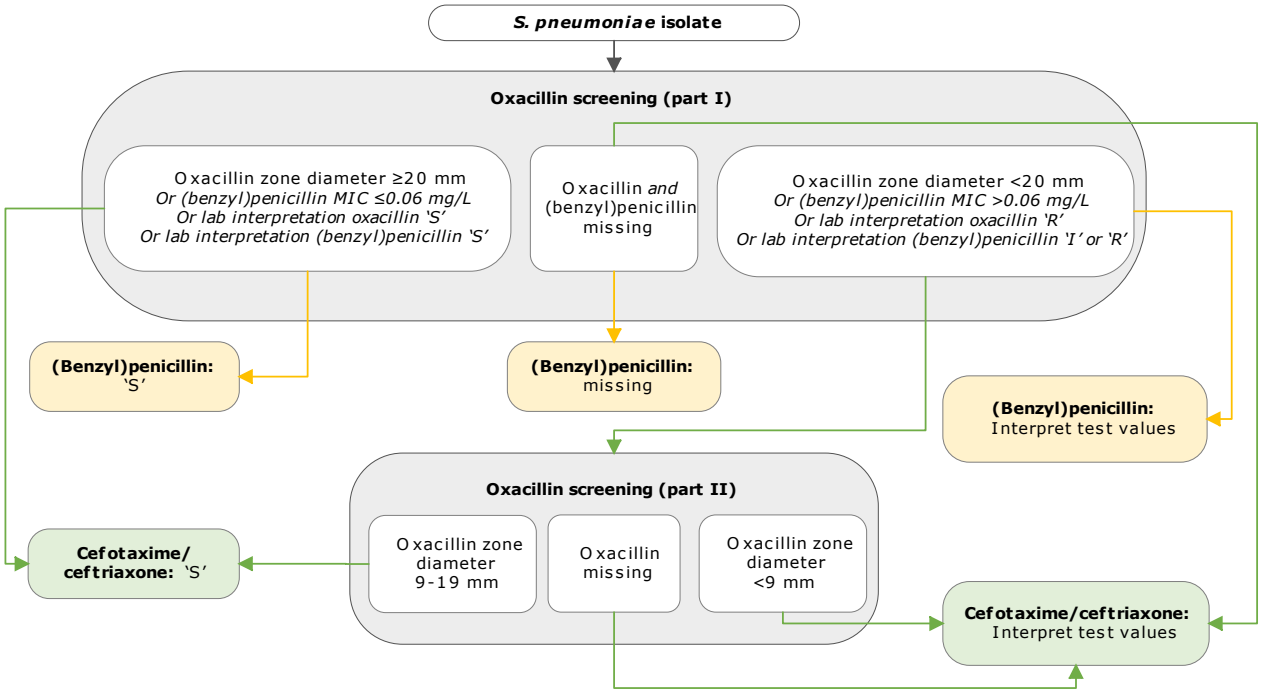
urinary tract, which is equal to the breakpoint for intravenous administration.
Likewise, in *Escherichia coli*, the fosfomycin MIC breakpoint for oral administration in uncomplicated urinary tract infection could not be used to reinterpret MIC values, because the minimum test value of ≤16 as measured by both the VITEK2 system and the Phoenix system do not reach the breakpoint of 8 mg/L. To approximate resistance percentages for oral administration as close as possible, we reinterpreted MIC-values according to the lowest cut-off that was possible; which was 16 mg/L, whereas we reinterpreted diameters according to the EUCAST breakpoint for oral administration (24 mm).

For *Staphylococcus* spp. and *Streptococcus* spp. we present both resistance to clindamycin as measured, as well as the percentage of isolates resistant to clindamycin including inducible resistance. Because data on tests for inducible clindamycin were often not available in ISIS-AR, we calculated resistance levels for clindamycin including inducible resistance based on re-interpretation of raw test values for clindamycin resistance, unless there was a positive test on inducible clindamycin resistance. In that case we counted the isolate as resistant to clindamycin. If no data on a test for inducible clindamycin test were available, but the laboratory reported the isolate being resistant to clindamycin we assumed that results of the test for inducible resistance was taken into account and counted the isolate as resistant.

We approximated the percentage MRSA among *S. aureus* based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2), whereas if these tests were lacking, prevalence was based on reinterpretation of crude test values for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin, according to EUCAST breakpoints version 14.0.

To test resistance of *S. pneumoniae* to β-lactam antibiotics EUCAST has specified a flowchart with testing steps based on testvalues for oxacillin, (benzyl)penicillin, and the β-lactam antibiotic of interest. To resemble this flowchart we used an algorithm to estimate resistance to (benzyl)penicillin and cefotaxime/ceftriaxone as depicted in figure 5.1.1.1.1. However, available gradient tests (ETEST™ and MTS™) for (benzyl)penicillin systematically underestimate MIC-values in *S. pneumoniae*[2]. Therefore, resistance percentages for (benzyl)penicillin in *S. pneumoniae* may be biased towards a lower level.
Similarly, for *H. influenzae* EUCAST has specified a flowchart to estimate resistance for β-lactam antibiotics, with testing steps based on testvalues for (benzyl)penicillin, β-lactamase production, co-amoxiclav, and the β-lactam antibiotic of interest. To resemble this flowchart we used an algorithm to estimate resistance to amoxicillin/ampicillin, co-amoxiclav, and cefuroxime as depicted in figure 5.1.1.1.2.

Figure 5.1.1.1.1 *Flowchart depicting the algorithm used to calculate resistance to β-lactam antibiotics in S. pneumoniae*

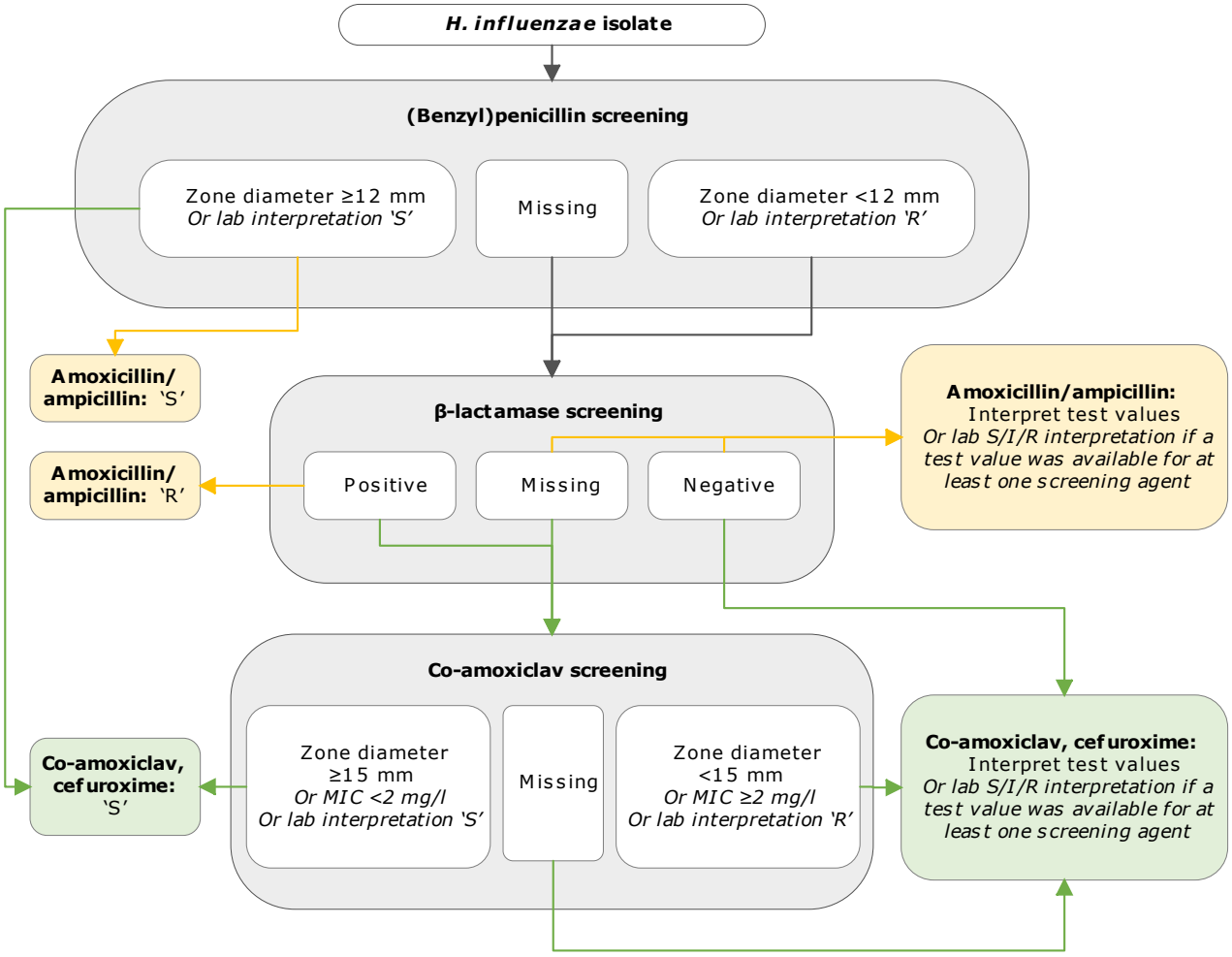


Note: The non-meningitis breakpoint was applied for (benzyl)penicillin and cefotaxime/ceftriaxone.
'Or' indicates that if the primary option was not available, the concurrent option was used.
Color Legend: (1) Yellow arrows and boxes: Specific to (benzyl)penicillin. (2) Green arrows and boxes: Specific to cefotaxime/ceftriaxone.

We considered *S. pneumoniae* susceptible to levofloxacin/moxifloxacin if the isolate was susceptible to norfloxacin according to the screening breakpoint. Otherwise susceptibility was based on reinterpretation of testvalues of levofloxacin/moxifloxacin, or, if no testvalues were available and the isolate was resistant to norfloxacin, on laboratory interpretation of susceptibility. Likewise, we considered the viridans streptococci as being susceptible to amoxicillin/ampicillin if the isolate was susceptible to (benzyl)penicillin according to the screening breakpoint. Otherwise susceptibility was based on reinterpretation of testvalues of amoxicillin/ampicillin, or, if no testvalues were available and the isolate was resistant to (benzyl)penicillin, on laboratory interpretation of susceptibility.

For some antimicrobial agents presented in this report, mutual comparable resistance mechanisms exist, namely benzylpenicillin/penicillin, amoxicillin/ampicillin, cefotaxime/ceftriaxone, meropenem/imipenem (except for *P. aeruginosa* and *Proteus mirabilis*), and doxycycline/tetracycline (except for *H. pylori*), and often the laboratories report results for either one. For these combinations, we calculated the percentage of isolates that was resistant to at least one of both agents. However, it should be mentioned that *S. aureus* can be susceptible for doxycycline while being resistant to tetracycline. Therefore, the resistance to doxycycline/tetracycline cannot be used as a proxy for the resistance to doxycycline, since that might be an overestimation.

Figure 5.1.1.1.2 *Flowchart depicting the algorithm used to calculate resistance to β-lactam antibiotics in H. influenzae*



Note: The IV and non-meningitis breakpoint was applied for amoxicillin/ampicillin, co-amoxiclav and cefuroxime.
'Or' indicates that if the primary option was not available, the concurrent option was used.
Color Legend: (1) Yellow arrows and boxes: Specific to amoxicillin/ampicillin. (2) Green arrows and boxes: Specific to co-amoxiclav and cefuroxime.

For *H. pylori* and all Gram-negative bacteria except *Enterobacter cloacae* complex and *Acinetobacter baumannii/calcoaceticus* complex, we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (for Enterobacterales: cefuroxime + gentamicin, cefuroxime + ciprofloxacin, cefotaxime/ceftriaxone + gentamicin, and cefotaxime/ceftriaxone + ciprofloxacin; for *P. aeruginosa*: ceftazidime + tobramycin; for *H. pylori* amoxicillin/ampicillin + clarithromycin, and amoxicillin/ampicillin + metronidazole, clarithromycin + metronidazole, and tetracycline + metronidazole). For these combinations, we defined resistance as resistance to both agents.

For *S. aureus*, no data on levofloxacin were available for a large part of laboratories. Therefore, we also calculated resistance to ciprofloxacin as a class indicator for resistance to fluoroquinolones.

For Enterobacterales isolates, we calculated the percentage of isolates that was multidrug resistant to oral therapy (MDOT), which we defined as resistance to the oral agents co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole combined.

Calculation of time trends
In addition to resistance levels in 2024, for sections 5.1.2 to 5.1.5 we calculated time trends over the last five years (2020 to 2024) using logistic regression models, except when data in one or more years before 2024 did not meet criteria for calculation of resistance levels. Because adoption of new guidelines or changes in breakpoints can have a substantial effect on resistance levels, we only analysed trends for resistance levels that were based on reinterpretation of crude test values from all five years according to EUCAST breakpoint guidelines version 14.0. We made an exception for trends in resistance to clindamycin including inducible resistance in *S. aureus* and resistance to β-lactam antibiotics in *S. pneumoniae* and *H. influenzae* (figures 5.1.1.1.1 and 5.1.1.1.2), which we partly based on laboratory S/I/R interpretation. However, we do not expect spurious time trends in resistance for these pathogen-agent combinations because EUCAST breakpoints for these combinations were not changed between 2020 and 2024.

We considered two-sided p-values for trend <0.05 to be statistically significant. When the absolute difference in predicted resistance from the logistic regression model between 2020 and 2024 was larger than the square root of the predicted resistance in 2020, we considered the trend to be microbiologically relevant. In the tables, statistically significant increasing trends that were considered to be microbiologically relevant are indicated in a red font, together with an up arrow, whereas decreasing trends that meet the same criteria are indicated in green, together with a down arrow. In addition, for each pathogen-agent combination, the resistance levels from 2020 to 2024 are shown in bar charts. Trends that meet the criteria for significant and microbiologically relevant are indicated with an asterisk.

References

1. Altorf-van der Kuil W, Schoffelen AF, de Greeff SC, et al. (2017) National laboratory-based surveillance system for antimicrobial resistance: a successful tool to support the control of antimicrobial resistance in the Netherlands. Euro Surveill 22(46).
2. EUCAST 2019, *Warning against the use of gradient tests for benzylpenicillin MIC in Streptococcus pneumoniae*, accessed August 6. 2025, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_-_gradient_for_benzyl_and_pnc_21nov2019.pdf

5.1.1.2 Descriptive characteristics of ISIS-AR data

In this section, several descriptive characteristics of the data from the ISIS-AR antimicrobial resistance surveillance system are presented. In figure 5.1.1.2.1, the smoothed distribution of isolates over the country, based on the percentage of inhabitants for whom at least one isolate was included in the analyses in sections 5.1.2 through 5.1.5, is shown by 4-digit postal code area. Furthermore, in the same figure the geographical distribution of laboratories is presented by status of connection to ISIS-AR and inclusion in the analyses in sections 5.1.2 through 5.1.5 (see section 5.1.1.1 for inclusion criteria). In table 5.1.1.2.1, characteristics of included isolates are listed by pathogen.

Each year all laboratories are included that send data on at least the last five years to ISIS-AR. This results in variation in the mixture of included laboratories through the years, and data from this chapter cannot be compared to data from NethMap 2024.

Key results

Descriptive characteristics of ISIS-AR data

- For the 2024 analyses, data of 39 laboratories could be used, resulting in inclusion of data on 542,318 isolates.
- The distribution of laboratories that have provided antimicrobial resistance data over the past five years was considerably more balanced across the Netherlands compared to earlier editions of NethMap
- Laboratories from which data could be included in the analyses was relatively low in the regions ‘Noord-Holland Oost’ and ‘Limburg’. The distribution of included laboratories was reflected in the geographical distribution of isolates.

Figure 5.1.1.2.1 *Geographical distribution of laboratories, by status of connection to ISIS-AR and inclusion in the analyses in sections 5.1.2 through 5.1.5, together with smoothed geographical distribution of isolates, based on the percentage of inhabitants for whom at least one isolate was included in those analyses, by 4-digit postal code area and with regional cooperative network borders, ISIS-AR 2024*

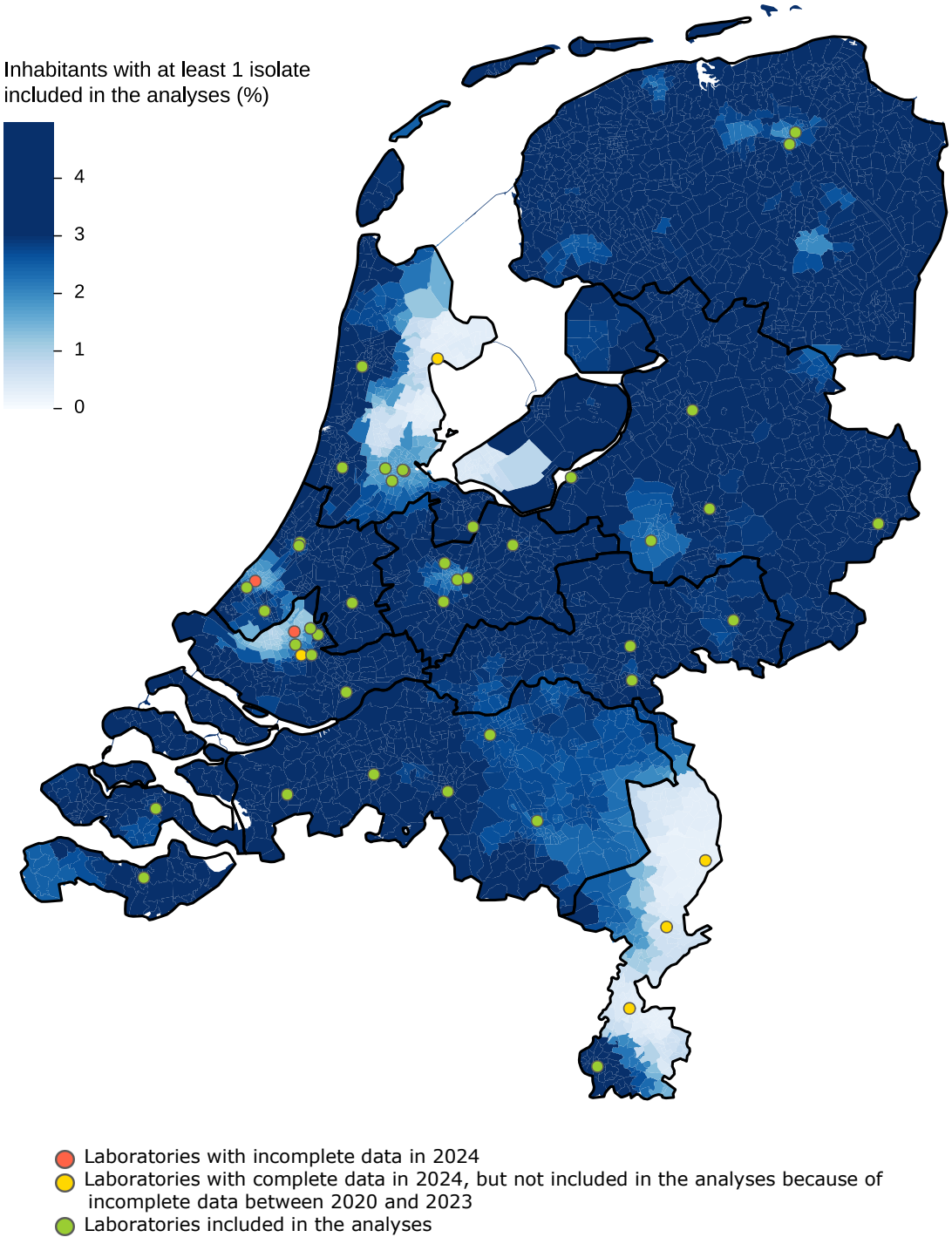


Table 5.1.1.2.1 *Characteristics of 542,318 isolates, sampled in 2024, included in the analyses in sections 5.1.2 through 5.1.5, by pathogen*

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*A. baumannii / calcoaceticus* complex	*E. faecalis*	*E. faecium*	*S. aureus*
Total number of isolates	240,155	42,346	27,999	14,382	31,080	2,088	34,769	7,011	78,198
Sex of patient (%)									
Male	27	34	42	55	53	61	56	54	54
Female	73	66	58	45	47	39	44	46	46
Setting of care (%)									
General practices	65	54	52	37	37	38	46	10	35
Outpatient departments	13	18	17	25	29	26	21	13	36
Inpatient departments (excl. Intensive Care Units)	17	20	19	31	26	27	26	59	24
Intensive Care Units	1	1	1	3	2	4	2	15	3
Long-term care facilities	5	7	11	5	6	5	5	3	3
Age category of patient in years (%)									
0-4	3	1	3	3	2	5	3	1	5
5-18	4	1	2	2	5	4	2	1	9
19-64	33	26	22	29	30	34	27	32	44
>65	59	72	73	66	63	58	67	67	42
Isolate source (%)									
Blood	3	3	1	3	2	3	3	14	4
Respiratory tract	1	3	2	7	14	8	0	1	13
Urine	91	87	83	60	43	55	85	49	13
Wound or Pus	3	4	10	23	34	25	10	30	54
Genital	1	1	1	0	1	0	0	0	3
Other	1	2	3	6	7	9	2	6	13
Type of hospital (hospital isolates only, %)									
General	36	34	37	32	32	30	32	24	34
Top clinical	44	44	44	43	41	44	49	45	43
University hospital	20	22	19	25	27	26	19	31	23

The first isolate per patient, per pathogen, per setting of care was selected.

Table 5.1.1.2.1 (continued) *Characteristics of 542,318 isolates, sampled in 2024, included in the analyses in sections 5.1.2 through 5.1.5, by pathogen*

	ß-haemolytic *Streptococcus* spp. group A	ß-haemolytic *Streptococcus* spp. group B	ß-haemolytic *Streptococcus* spp. groups C and G	*S anginosus* group	*S. mitis* group	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
Total number of isolates	8'536	25'185	3'220	5'448	2'083	1'559	428	0	14'111	3'646
Sex of patient (%)										
Male	42	24	52	54	58	56	59	0	50	50
Female	58	76	48	46	42	44	41	0	50	50
Setting of care (%)										
General practices	47	56	34	7	8	3	4	0	12	12
Outpatient departments	27	23	28	30	21	25	21	0	46	46
Inpatient departments (excl. Intensive Care Units)	23	19	35	58	68	65	70	0	38	36
Intensive Care Units	2	1	1	4	4	5	4	0	3	4
Long-term care facilities	0	2	2	1	0	2	1	0	1	2
Age category of patient in years (%)										
0-4	12	1	1	1	4	1	0	0	9	9
5-18	16	2	3	5	4	4	1	0	4	3
19-64	52	65	51	53	41	42	34	0	35	31
>65	20	32	45	41	52	53	65	0	53	58
Isolate source (%)										
Blood	7	2	11	12	43	25	30	0	2	1
Respiratory tract	11	1	3	4	1	0	0	0	86	90
Urine	6	56	13	9	14	1	3	0	0	0
Wound or Pus	48	10	51	66	38	65	59	0	10	9
Genital	20	26	14	3	0	2	1	0	2	0
Other	8	4	9	6	4	7	6	0	0	0
Type of hospital (hospital isolates only, %)										
General	35	37	41	30	30	34	24	0	32	33
Top clinical	46	47	41	46	35	45	48	0	47	47
University hospital	19	16	18	24	35	20	28	0	20	20

The first isolate per patient, per pathogen, per setting of care was selected.

5.1.2 Primary care

The distribution of pathogens in diagnostic urine, wound or pus, respiratory, and genital samples from general practitioners' (GP) patients in 2024 is presented in table 5.1.2.1. The resistance levels in 2024 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 5.1.2.2. In accordance with age categories used in the Dutch College of General Practitioners (NHG) guidelines for urinary tract infections, resistance levels and five-year trends for urine isolates are calculated separately for patients aged ≤12 years and patients aged >12 years. For *S. aureus* isolates from wound or pus samples resistance levels in 2024 are presented in table 5.1.2.3. For β-haemolytic *Streptococcus* spp. group A isolates from wound/pus, respiratory, or genital samples as well as for β-haemolytic *Streptococcus* spp. group B isolates from urine or genital samples resistance levels in 2024 are shown in table 5.1.2.4. Five-year trends in resistance are shown in figure 5.1.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), figure 5.1.2.2 (*S. aureus*) and figure 5.1.2.3 (β-haemolytic *Streptococcus* spp. group A and group B).

In accordance with the NHG guidelines, GPs typically initiate empirical antibiotic treatment before sending urine, wound, or pus samples for culture and susceptibility testing. Diagnostic samples are usually taken only in cases of therapy failure or, in the case of urine samples, suspected complicated urinary tract infections. Therefore, many of the pathogens isolated in primary care are likely to have been exposed to prior antibiotic treatment, introducing antibiotic selective pressure. As a result, the presented resistance levels are likely to be higher than those for all patients with urinary tract infections caused by Enterobacterales or *P. aeruginosa,* or wound infections or pus caused by *S. aureus* or β-haemolytic *Streptococcus* spp. group A presenting at the GP. Bias due to selective sampling of patients is expected to be limited for β-haemolytic *Streptococcus* spp. group B, because initial therapy of urinary tract infections is not expected to affect resistance to most antibiotics presented for *Streptococcus* spp. in this report and genital samples are taken as part of routine diagnostics.

Because of the potential bias in results for Enterobacterales, *P. aeruginosa*, *S. aureus* and β-haemolytic *Streptococcus* spp. group A, the patients from whom samples were taken are hereafter referred to as 'selected general practitioners' patients'.

Table 5.1.2.1 *Distribution of isolated pathogens in diagnostic urine samples (by patient age category) and diagnostic wound or pus, respiratory, and genital samples from selected general practitioners' patients, ISIS-AR 2024*

	Urine		Wound or pus	Respiratory tract	Genital
	Age≤12	Age>12			
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	10,516 (71)	140,918 (55)	960 (3)	101 (2)	751 (9)
K. pneumoniae	302 (2)	21,682 (8)	316 (1)	68 (1)	109 (1)
P. mirabilis	831 (6)	12,807 (5)	726 (2)	35 (1)	74 (1)
Other Enterobacterales[1]	788 (5)	28,555 (11)	3,219 (9)	396 (7)	182 (2)
P. aeruginosa	230 (2)	5,963 (2)	4,689 (13)	278 (5)	94 (1)
Other non-fermenters[2]	160 (1)	2,824 (1)	939 (3)	440 (8)	16 (0)
Other Gram-negatives[3]	8 (0)	38 (0)	482 (1)	1,205 (23)	125 (2)
S. aureus	173 (1)	4,759 (2)	18,702 (52)	2,137 (40)	1,287 (16)
ß-haemolytic *Streptococcus* spp. group A	171 (1)	228 (0)	1,949 (5)	273 (5)	1,390 (17)
ß-haemolytic *Streptococcus* spp. group B	128 (1)	9,812 (4)	782 (2)	48 (1)	3,217 (40)
Other Gram-positives[4]	1,580 (11)	30,905 (12)	2,913 (8)	315 (6)	720 (9)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Citrobacter* spp., *Enterobacter* spp., *Morganella* spp., *Serratia* spp., *Proteus* spp. (non-*mirabilis*), *Raoultella* spp., *Providencia* spp., *Pantoea* spp., *Escherichia* spp. (non-*coli*), *Salmonella* spp., *Hafnia* spp., *Mixta* spp., *Shigella* spp., *Cronobacter* spp., *Yersinia* spp.
[2] In order of frequency: *Acinetobacter* spp., *Pseudomonas* spp. (non-*aeruginosa*), *S. maltophilia*, *M. catarrhalis*, *B. cepacia*.
[3] In order of frequency: *H. parainfluenzae*, *H. influenzae*, *B. fragilis complex*, *B. fragilis*, *N. meningitidis*, *C. jejuni*, *H. pylori*.
[4] In order of frequency: *Enterococcus* spp., *Staphylococcus* spp. (non-*aureus*), *A. urinae*, *S. infantis*, *S. dysgalactiae* subsp. *equisimilis*, *S. equi*, *ß-haemolytische Streptokokken groep C n.n.g.*, *S. canis*, *Streptococcus sanguinis groep n.n.g.*, *S. gordonii*, *Streptococcus anginosus groep n.n.g.*, *S. intermedius*, *S. pneumoniae*, *S. anginosus*, *Streptococcus mitis/oralis n.n.g.*, *S. dysgalactiae* n.n.g., *streptococcus dysgalactiae subsp. dysgalactiae*, *S. constellatus*, *ß-haemolytische Streptokokken groep G n.n.g.*, *S. oralis*, *Streptococcus mitis groep n.n.g.*, *S. mitis*, *C. perfringens*.

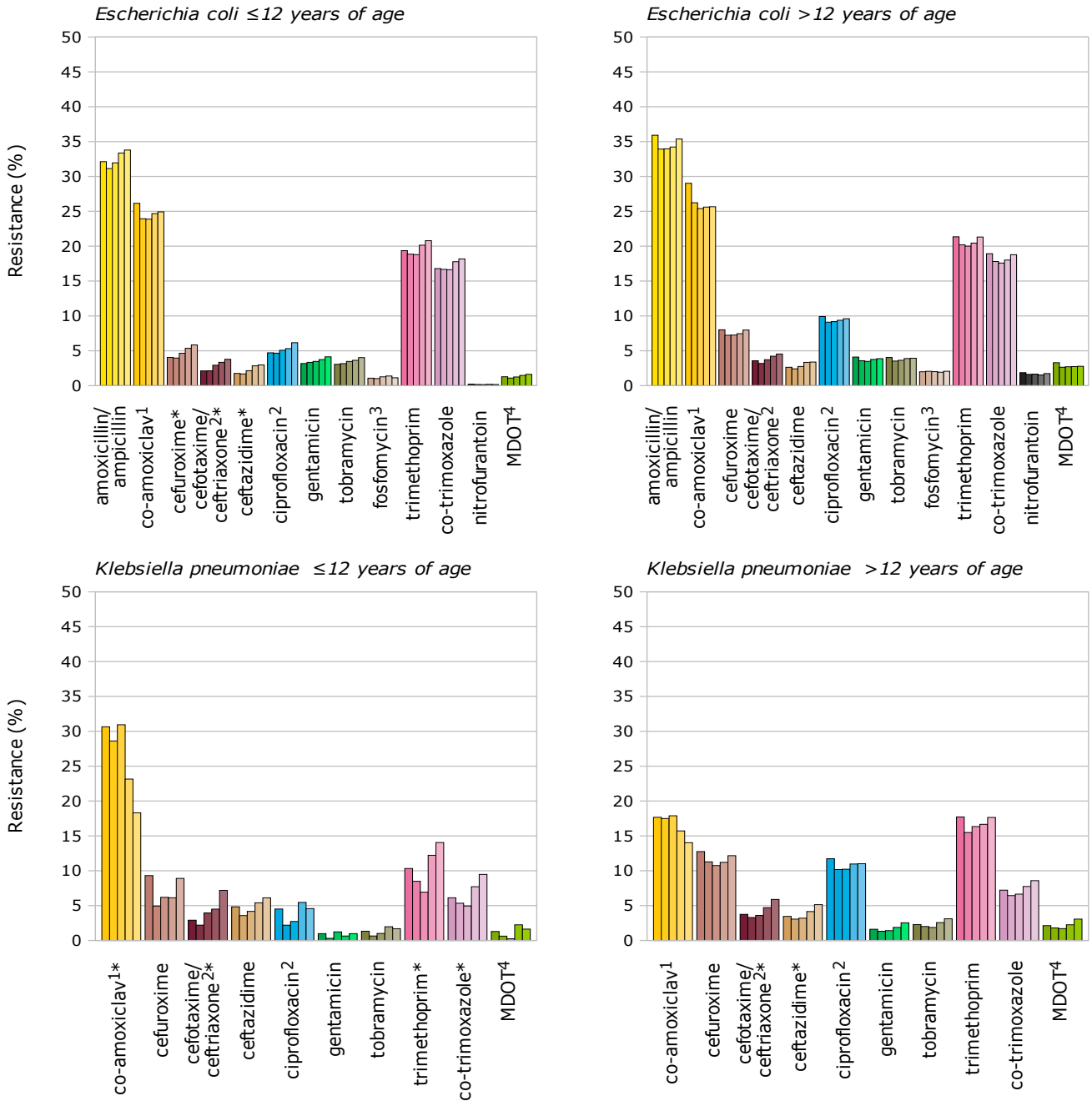
Table 5.1.2.2 *Resistance levels (%) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected general practitioners' patients, by age category, ISIS-AR 2024*

	E. coli		***K. pneumoniae***		***P. mirabilis***		***P. aeruginosa***	
	Age ≤12	**Age >12**	**Age ≤12**	**Age >12**	**Age ≤12**	**Age >12**	**Age ≤12**	**Age >12**
median age	5	69	5	74	3	76	3	79
Antibiotic								
amoxicillin/ampicillin	34	35	-	-	16	17	-	-
co-amoxiclav[a]	25	26	18 ↓	14	3	5	-	-
piperacillin-tazobactam	-	-	-	-	-	-	1.8	4
cefuroxime	6 ↑	8	9	12	0.7	1.0	-	-
cefotaxime/ceftriaxone[b]	4 ↑	5	7 ↑	6 ↑	0.8	0.5	-	-
ceftazidime	3 ↑	3	6	5 ↑	0.5	0.2	0.4	1.5
meropenem[b]	-	-	-	-	-	-	0.0	0.6
imipenem	-	-	-	-	-	-	1.5	4
ciprofloxacin[b]	6	10	5	11	5	8	0.9	8
gentamicin	4	4	1.0	3	4	4	-	-
tobramycin	4	4	1.7	3	2	3	0.4	0.6
fosfomycin[1]	1.1	2	-	-	-	-	-	-
trimethoprim	21	21	14 ↑	18	22	27	-	-
co-trimoxazole	18	19	9 ↑	9	18	21	-	-
nitrofurantoin	0.2	1.7	-	-	-	-	-	-
Multidrug resistance								
MDOT[2]	1.6	3	1.6	3	0.4	1.0	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

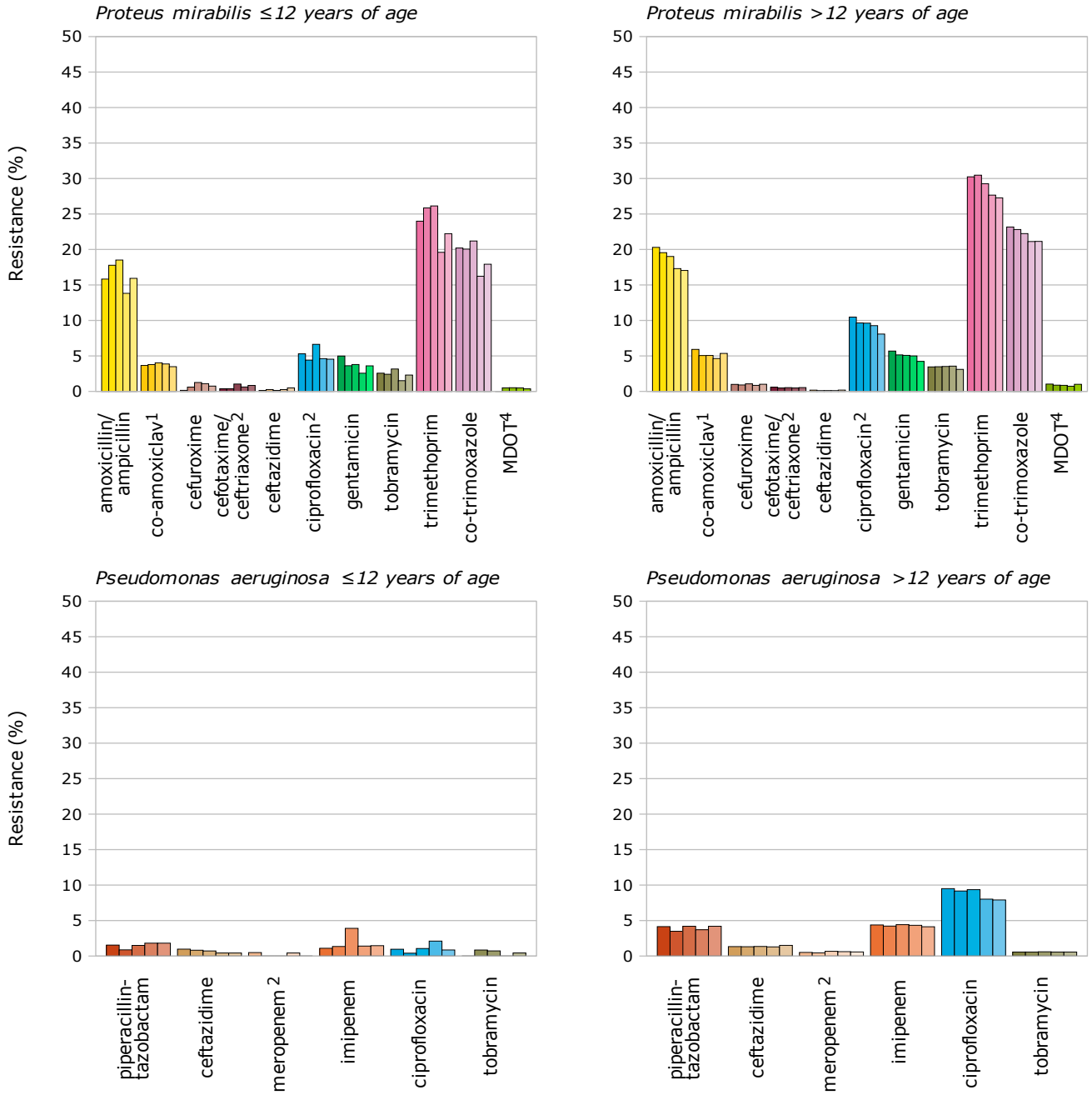
[1] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[2] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, and *P. mirabilis*). For more details see section 5.1.1.1.

Figure 5.1.2.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected general practitioners' patients in ISIS-AR, by age category*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Figure 5.1.2.1 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected general practitioners' patients in ISIS-AR, by age category*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

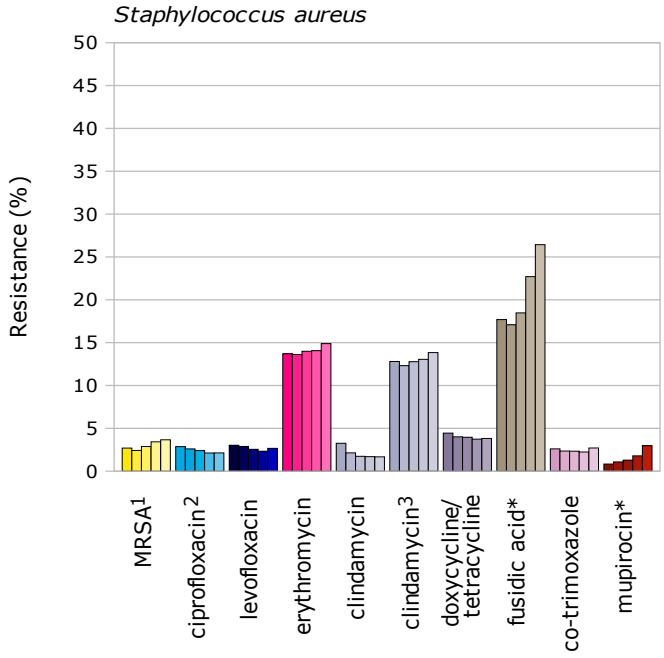
Table 5.1.2.3 *Resistance levels (%) among diagnostic wound or pus isolates of S. aureus from selected general practitioners' patients, ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	4
ciprofloxacin[2]	2
levofloxacin	3
erythromycin	15
clindamycin	1.7
clindamycin (including inducible resistance)[3]	14
doxycycline/tetracycline	4
fusidic acid	26 ↑
co-trimoxazole	3
mupirocine	3 ↑

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 1 out of 7 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.2.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic wound or pus isolates of S. aureus from selected general practitioners' patients in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.

[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.

[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

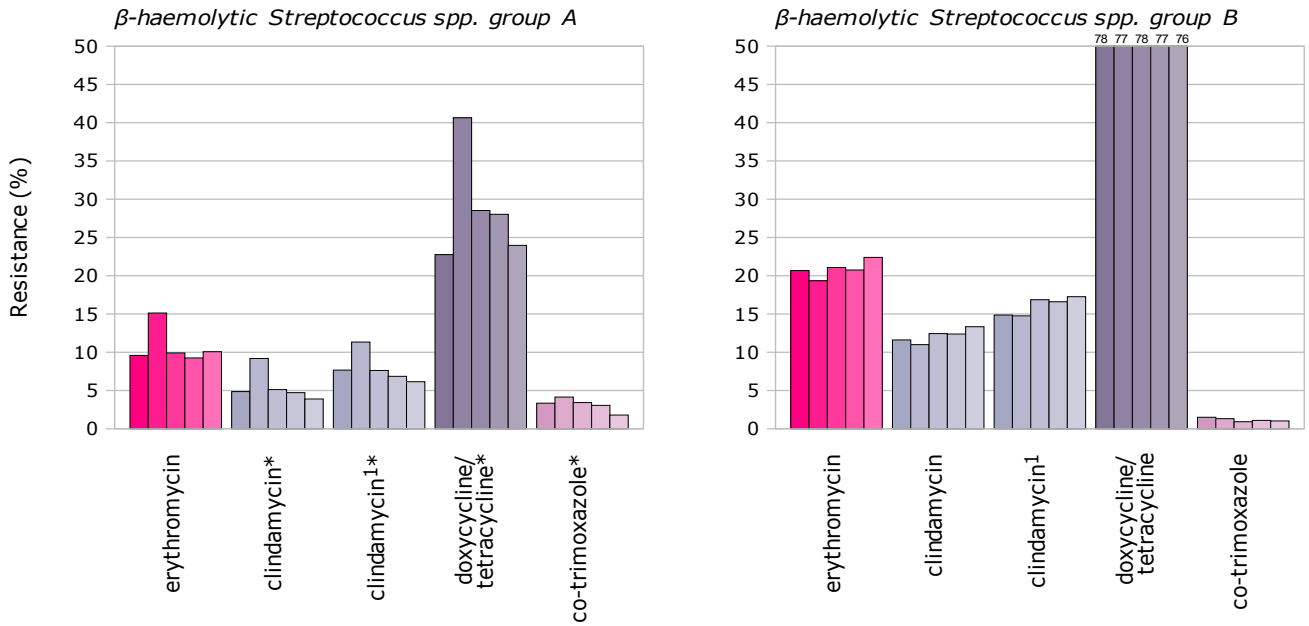
Table 5.1.2.4 *Resistance levels (%) among diagnostic wound/pus, respiratory, or genital isolates of β-haemolytic Streptococcus spp. group A and diagnostic urine or genital isolates of β-haemolytic Streptococcus spp. group B from selected general practitioners' patients, ISIS-AR 2024*

	ß-haemolytic *Streptococcus* spp. group A	**ß-haemolytic *Streptococcus* spp. group B**
Antibiotic		
erythromycin	10	22
clindamycin	4 ↓	13
clindamycin (including inducible resistance)[1]	6 ↓	17
doxycycline/tetracycline	24 ↓	76
co-trimoxazole	1.8 ↓	1.0

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.2.3 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic Streptococcus spp. group A and diagnostic urine or genital isolates of β-haemolytic Streptococcus spp. group B from selected general practitioners' patients in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Key results
Primary care

Urine: Enterobacterales and P. aeruginosa

Uncomplicated urinary tract infections (UTI)

- In *E. coli*, resistance levels for **nitrofurantoin** and **fosfomycin**, first and second choice antibiotics for the treatment of uncomplicated UTI in adults in primary care, were stable and low (≤2%). For **trimethoprim**, third choice antibiotic for the treatment of uncomplicated UTI in adults, resistance was 21%. For other Enterobacterales, no EUCAST breakpoints are available for **nitrofurantoin** and **fosfomycin** for the treatment of urinary tract infections.

Complicated UTI

- Resistance levels for **ciprofloxacin**, first choice antibiotic for the empirical treatment of complicated UTI in adults in primary care, was stable at 11% or lower for all Enterobacterales and *P. aeruginosa*. Resistance levels for **co-amoxiclav**, second empirical choice antibiotic for the empirical treatment of complicated UTI in primary care, was 26% in *E. coli* and 14% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third empirical choice antibiotic for this indication, was 19% in *E. coli* and 9% in *K. pneumoniae* and remained stable over the last five years.
- Combined resistance for **co-amoxiclav, ciprofloxacin, and co-trimoxazole** in all Enterobacterales was low (≤3%).

Wound or pus: S. aureus

- Antibiotic resistance levels in *S. aureus* were relatively low except for **erythromycin** (15%), clindamycin (including inducible resistance, 14%) and fusidic acid (26%).
- We also report **clindamycin** resistance without considering inducible resistance (1.7%). According to EUCAST, clindamycin may still be used for short-term therapy in less severe skin and soft tissue infections if tested susceptible, even in the presence of an inducible resistance mechanism.
- A worrisome increase in resistance for the two topically used agents **fusidic acid** (26%) and **mupirocine** (3%) was found in *S. aureus* over the last 5 years. This likely reflects a real rise in resistance; however, the relatively high percentages may also be influenced by a selective sampling population, as cultures are often obtained from patients who have failed fusidic acid or mupirocine therapy. In addition, for primary care we selected only *S. aureus* isolates from skin infections, whereas in hospital populations isolates from a wider range of clinical specimens were included.
- **MRSA** was found in 4% of isolates of primary care patients which remained stable over the previous 5 years.

Wound/pus, respiratory or genital: β-haemolytic Streptococcus spp. groups A and B

- Resistance to **doxycycline/tetracycline** (24%), **clindamycin** (including inducible resistance, 6%) and **co-trimoxazole** (1.8%) in β-haemolytic *Streptococcus* spp. group A decreased over the last five years.
- Resistance levels for **doxycycline/tetracycline** (76%), **clindamycin** (including inducible resistance, 17%) and **erythromycin** (22%) in β-haemolytic *Streptococcus* spp. group B were high, which might complicate treatment in case of beta-lactam allergy.

5.1.3 *Hospital departments*

In this section, resistance levels among isolates from patients in outpatient departments (section 5.1.3), inpatient departments (excluding intensive care units, section 5.1.3), and intensive care units (section 5.1.3) are presented. Additionally, resistance levels are shown separately for blood isolates from patients admitted to inpatient hospital departments (including intensive care units) in section 5.1.3 and for urine isolates from patients in urology departments (outpatient and inpatient departments) in section 5.1.3.

5.1.3.1 Outpatient departments

The distribution of pathogens isolated from diagnostic samples (lower respiratory tract, urine, and wound or pus) from patients attending outpatient departments in 2024 is presented in table 5.1.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2024 are presented in tables 5.1.3.1.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 5.1.3.1.3 (*S. aureus)*. Five-year trends in resistance are shown in figures 5.1.3.1.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 5.1.3.1.2 (*S. aureus*).

In outpatient departments in the Netherlands, a sample is taken from the majority of patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Therefore, bias due to selective sampling will be lower than in GP patients and resistance percentages in this section are considered representative of resistance in outpatient departments.

Table 5.1.3.1.1 *Distribution of isolated pathogens in diagnostic samples from patients attending outpatient departments, ISIS-AR 2024*

	Lower respiratory tract	**Urine**	**Wound or pus**
Pathogen	**N (%)**	**N (%)**	**N (%)**
E. coli	424 (3)	24,963 (41)	2,486 (5)
K. pneumoniae	260 (2)	5,733 (9)	567 (1)
P. mirabilis	104 (1)	2,738 (4)	1,345 (3)
Other Enterobacterales[1]	1,058 (8)	9,553 (16)	4,679 (10)
P. aeruginosa	1,694 (13)	2,309 (4)	4,103 (9)
Other non-fermenters[2]	1,934 (14)	907 (1)	1,006 (2)
Other Gram-negatives[3]	5,034 (37)	29 (0)	1,275 (3)
S. aureus	1,806 (13)	2,062 (3)	18,730 (41)
Other Gram-positives[4]	1,200 (9)	12,959 (21)	11,314 (25)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella* spp., *Proteus* spp. (non-*mirabilis*), *Providencia* spp., *Raoultella* spp., *Pantoea* spp., *Escherichia* spp. (non-*coli*), *Hafnia* spp., *Salmonella* spp., *Mixta* spp., *Cronobacter* spp., *Yersinia* spp.
[2] In order of frequency: *M. catarrhalis*, *Acinetobacter* spp., *S. maltophilia*, *Pseudomonas* spp. (non-*aeruginosa*).
[3] In order of frequency: *H. influenzae*, *H. parainfluenzae*, *B. fragilis complex*, *B. fragilis*, *N. meningitidis*, *H. pylori*.
[4] In order of frequency: *Streptococcus sanguinis groep n.n.g.*, *S. peroris*, *S. canis*, *ß-haemolytische Streptokokken groep G n.n.g.*, *S. mitis*, *S. anginosus*, *S. pyogenes*, *S. dysgalactiae* subsp. *equisimilis*, *ß-haemolytische Streptokokken groep A n.n.g.*, *S. cristatus*, *S. equi*, *S. intermedius*, *S. agalactiae*, *S. oralis*, *S. dysgalactiae* n.n.g., *S. pneumoniae*, *S. australis*, *Streptococcus mitis/oralis n.n.g.*, *S. constellatus*, *streptococcus dysgalactiae subsp. dysgalactiae*, *ß-haemolytische Streptokokken groep C n.n.g.*, *Streptococcus anginosus groep n.n.g.*, *Streptococcus mitis groep n.n.g.*, *S. gordonii*, *Enterococcus* spp., *Staphylococcus* spp. (non-*aureus*), *A. urinae*, *C. perfringens*.

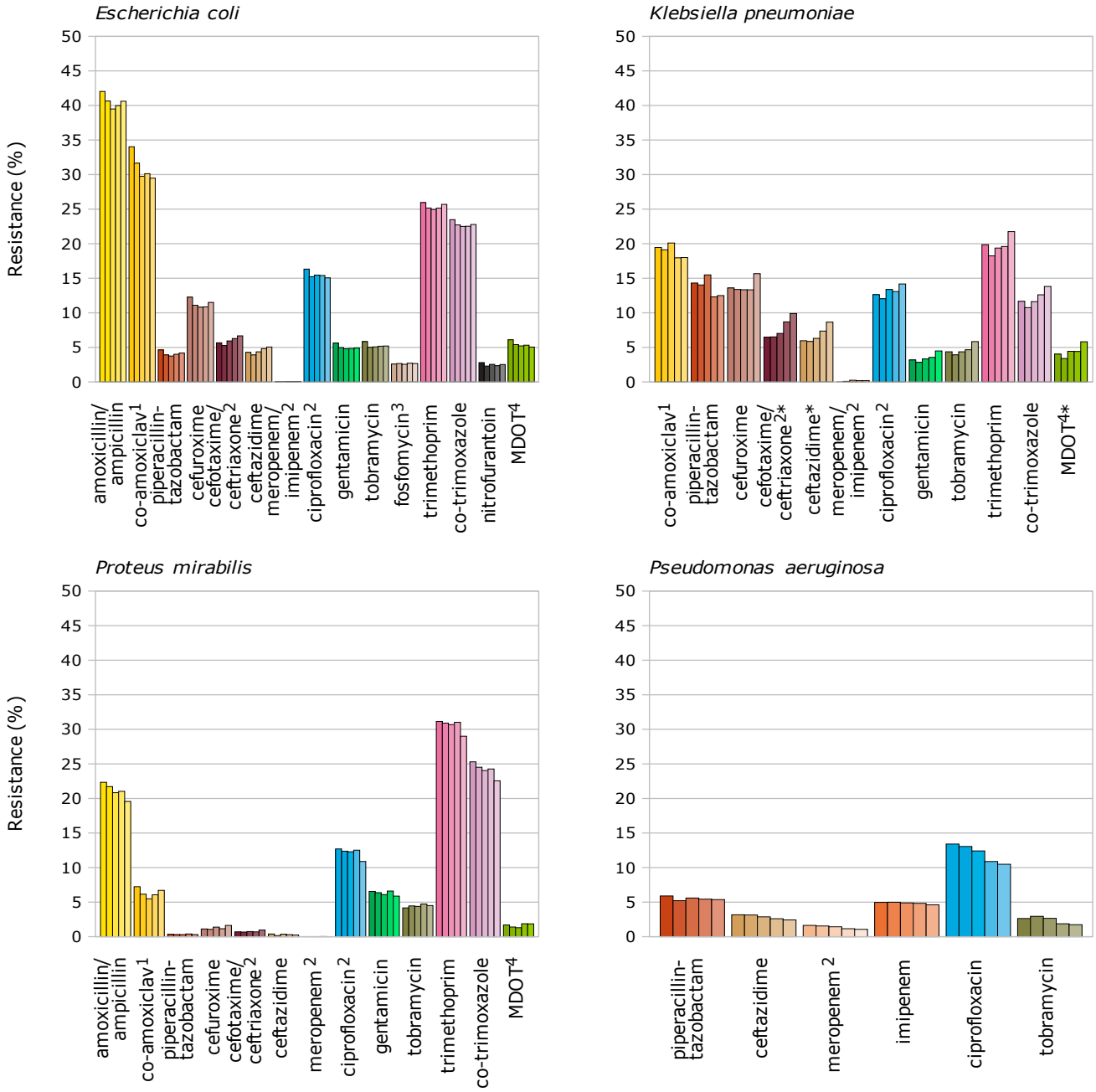
Table 5.1.3.1.2 *Resistance levels (%) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending outpatient departments, ISIS-AR 2024*

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	41	-	20	-
co-amoxiclav[a]	29	18	7	-
piperacillin-tazobactam	4	12	0.3	5
cefuroxime	12	16	1.6	-
cefotaxime/ceftriaxone[b]	7	10 ↑	1.0	-
ceftazidime	5	9 ↑	0.3	2
meropenem/imipenem[b]	0.0	0.2	-	-
meropenem[b]	-	-	0.0	1.1
imipenem	-	-	-	5
ciprofloxacin[b]	15	14	11	10
gentamicin	5	4	6	-
tobramycin	5	6	5	1.7
fosfomycin[1]	3	-	-	-
trimethoprim	26	22	29	-
co-trimoxazole	23	14	23	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
cefuroxime + gentamicin	1.7	4	0.6	-
cefuroxime + ciprofloxacin[b]	6	9	0.7	-
cefotaxime/ceftriaxone + gentamicin[b]	1.4	4 ↑	0.5	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	4	7 ↑	0.6	-
Multidrug resistance				
MDOT[2]	5	6 ↑	1.9	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[2] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, and *P. mirabilis*). For more details see section 5.1.1.1.

Figure 5.1.3.1.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending outpatient departments in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

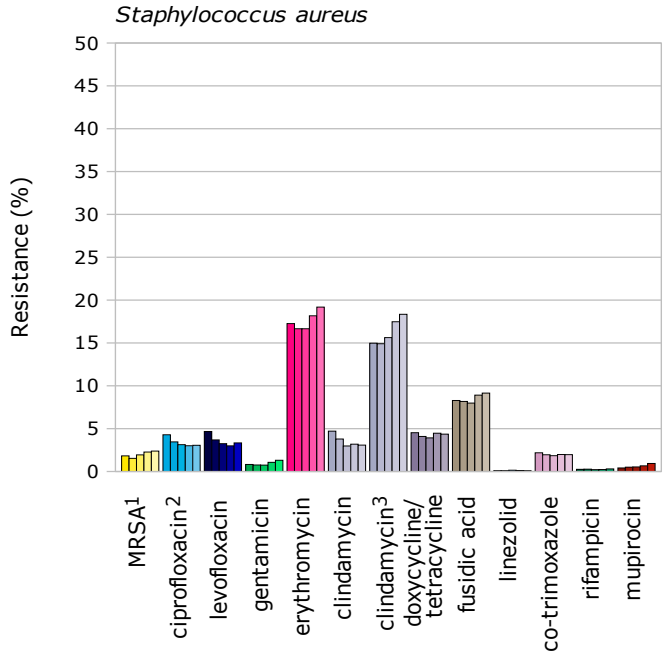
Table 5.1.3.1.3 *Resistance levels (%) among diagnostic isolates of S. aureus from patients attending outpatient departments, ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	2
ciprofloxacin[2]	3
levofloxacin	3
gentamicin	1.3
erythromycin	19
clindamycin	3
clindamycin (including inducible resistance)[3]	18
doxycycline/tetracycline	4
fusidic acid	9
linezolid	0.1
co-trimoxazole	2.0
rifampicin	0.3
mupirocine	0.9

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 1 out of 12 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.1.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of S. aureus from patients attending outpatient departments in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).
[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Key results

Outpatient departments

Urine, wound/pus and respiratory:
E. coli, K. pneumoniae, and P. aeruginosa

- For both Enterobacterales and *P. aeruginosa*, resistance levels for **all tested antibiotics** were higher in isolates of OPD patients compared to resistance levels in isolates from primary care patients.
- For the three most important oral antibiotics used in OPD setting, **co-amoxiclav**, **co-trimoxazole** and **ciprofloxacin**, no significant increase in resistance levels were found. Resistance to **co-amoxiclav** was 29% in *E. coli* and 18% in *K. pneumoniae*. Resistance to **co-trimoxazole** was 23% in *E. coli* and 14% in *K. pneumoniae*. Resistance levels for **ciprofloxacin** were 15% in *E. coli*, 14% in *K. pneumoniae* and 10% in *P. aeruginosa.* In the majority of cases, at least one oral treatment option remained with these three antibiotics, given the relatively low (≤6%) combined resistance rates.
- In *K. pneumoniae*, resistance to **third generation cephalosporins** increased significantly over the last 5 years (10%).
- Although **meropenem/imipenem** resistance in *Klebsiella pneumoniae* was less than 1%, there was an increase over the last five years from 0.05% to 0.2%.

Urine, wound/pus and respiratory: S. aureus

- In *S. aureus* of OPD patients, resistance to **levofloxacin**, **doxycycline**, and **co-trimoxazole** was less than 4%.
- Resistance to **erythromycin** was 19% and **clindamycin** (including inducible resistance) 18%. We also report **clindamycin** resistance without considering inducible resistance (3%). According to EUCAST, clindamycin may still be used for short-term therapy in less severe skin and soft tissue infections if tested susceptible, even in the presence of an inducible resistance mechanism.
- Resistance to **fusidic acid** (9%) and **mupirocine** (0.9%) was lower in *S. aureus* isolates of OPD patients than in *S. aureus* isolates of GP patients (26% and 3%). This difference might be attributed to two factors: first, isolates in OPD patients are obtained not only from skin infections but also from a variety of other clinical specimens; and second, in primary care a relatively larger proportion of cultures are derived from patients in whom fusidic acid or mupirocine therapy has failed, which increases the likelihood of detecting resistant strains.
- **MRSA** was found in 2% of isolates of OPD patients which was stable over the previous 5 years.

5.1.3.2 Inpatient hospital departments (excl. ICU)

The distribution of pathogens from diagnostic samples (blood, cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to inpatient hospital departments (excl. ICU) in 2024 is presented in table 5.1.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2024 are presented in tables 5.1.3.2.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *A. baumannii/calcoaceticus* complex), 5.1.3.2.3 (*E. faecalis* and *E. faecium*), 5.1.3.2.4 (*S. aureus*), 5.1.3.2.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus* group*,* and *S. mitis* group), and 5.1.3.2.6 (*B. fragilis* complex and *C. perfringens*). Five-year trends in resistance are shown in figures 5.1.3.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *A. baumannii/calcoaceticus* complex) 5.1.3.2.2 (*E. faecalis* and *E. faecium*), 5.1.3.2.3 (*S. aureus*), 5.1.3.2.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus* group and *S. mitis* group), and 5.1.3.2.5 (*B. fragilis* complex and *C. perfringens*).

In inpatient hospital departments in the Netherlands, a sample is taken from the majority of patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Therefore, bias due to selective sampling of patients is expected to be limited.

Table 5.1.3.2.1 *Distribution of isolated pathogens in diagnostic samples from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	Blood	Cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	6,439 (20)	8 (2)	1,115 (6)	24,735 (42)	4,188 (10)
K. pneumoniae	1,247 (4)	2 (0)	616 (3)	4,963 (8)	912 (2)
P. mirabilis	367 (1)	1 (0)	224 (1)	3,436 (6)	990 (2)
E. cloacae complex	461 (1)	6 (1)	627 (3)	1,563 (3)	1,725 (4)
Other Enterobacterales[1]	1,883 (6)	8 (2)	2,133 (12)	6,658 (11)	4,021 (10)
P. aeruginosa	637 (2)	2 (0)	1,836 (10)	3,006 (5)	2,228 (5)
A. baumannii-calcoaceticus complex	58 (0)	1 (0)	91 (0)	210 (0)	225 (1)
Other non-fermenters[2]	321 (1)	14 (3)	1,985 (11)	409 (1)	708 (2)
B. fragilis complex	364 (1)	0 (0)	0 (0)	13 (0)	620 (1)
Other Gram-negatives[3]	398 (1)	29 (6)	5,274 (29)	12 (0)	277 (1)
E. faecalis	983 (3)	13 (3)	27 (0)	5,689 (10)	2,395 (6)
E. faecium	839 (3)	9 (2)	28 (0)	1,738 (3)	1,537 (4)
S. aureus	3,163 (10)	37 (8)	2,601 (14)	1,716 (3)	10,601 (25)
ß-haemolytic *Streptococcus* spp. group A	557 (2)	3 (1)	174 (1)	56 (0)	950 (2)
ß-haemolytic *Streptococcus* spp. group B	425 (1)	5 (1)	121 (1)	1,408 (2)	814 (2)
ß-haemolytic *Streptococcus* spp. groups C and G	340 (1)	1 (0)	22 (0)	66 (0)	548 (1)
S. anginosus group	596 (2)	4 (1)	51 (0)	106 (0)	2,285 (5)
S. mitis group	841 (3)	19 (4)	8 (0)	47 (0)	493 (1)
C. perfringens	125 (0)	0 (0)	1 (0)	4 (0)	164 (0)
Other Gram-positives[4]	12,927 (39)	327 (67)	1,544 (8)	2,859 (5)	6,488 (15)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Citrobacter* spp., *Serratia* spp., *Morganella* spp., *Proteus* spp. (non-*mirabilis*), *Raoultella* spp., *Providencia* spp., *Salmonella* spp., *Hafnia* spp., *Pantoea* spp., *Enterobacter* spp. (non-*cloacae* complex), *Escherichia* spp. (non-*coli*), *Mixta* spp., *Cronobacter* spp., *Yersinia* spp., *Shigella* spp.
[2] In order of frequency: *M. catarrhalis*, *S. maltophilia*, *Acinetobacter* spp., *Pseudomonas* spp. (non-*aeruginosa*), *B. cepacia*.
[3] In order of frequency: *H. parainfluenzae*, *H. influenzae*, *N. meningitidis*, *C. coli*, *C. lari*, *C. jejuni*, *H. pylori*.
[4] In order of frequency: *Staphylococcus* spp. (non-*aureus*), *S. dysgalactiae* subsp. *equisimilis*, *S. pneumoniae*, *S. dysgalactiae* n.n.g., *A. urinae*, *Enterococcus* spp. (non-*faecalis*, non-*faecium*), *L. monocytogenes*.

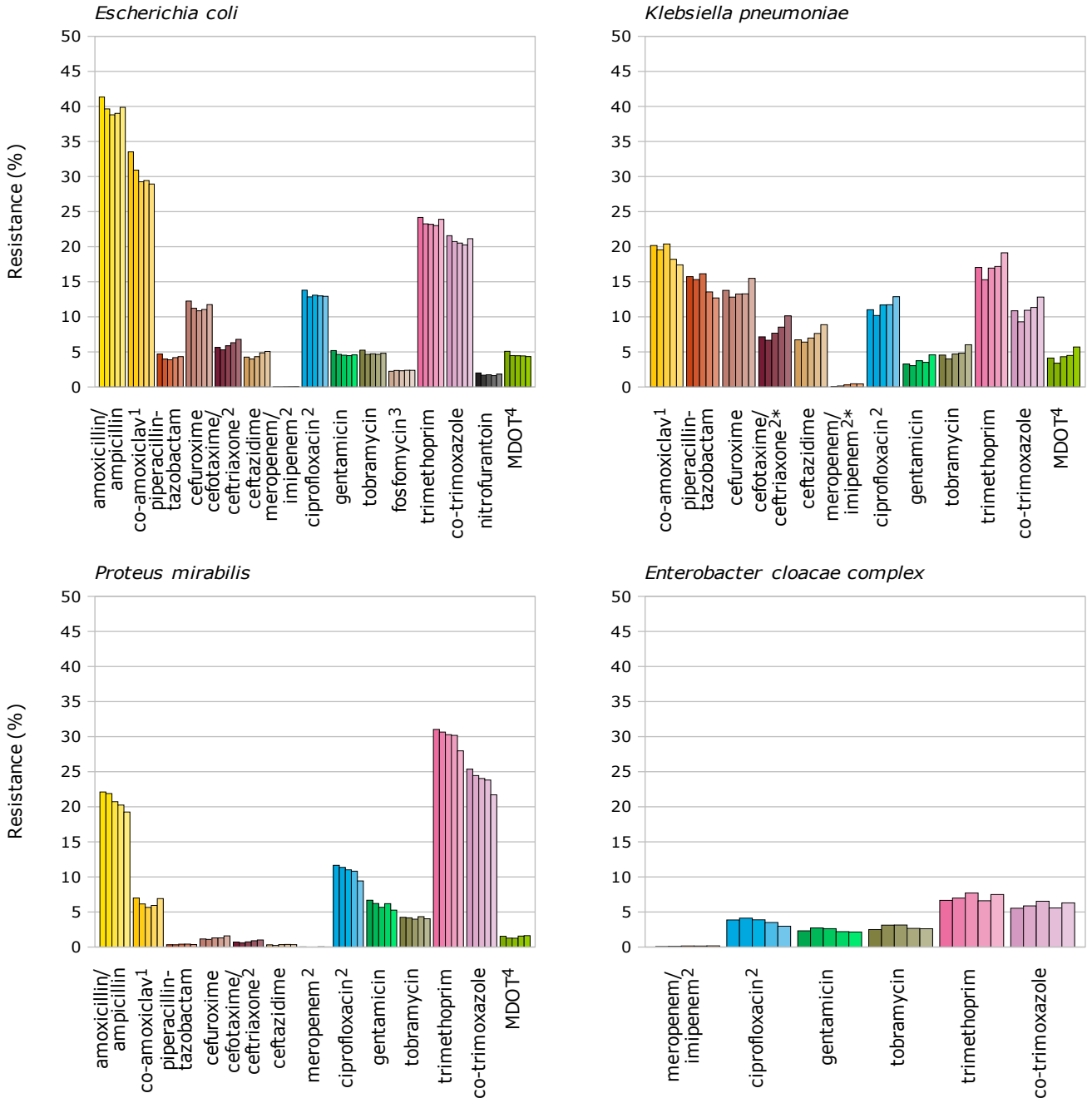
Table 5.1.3.2.2 *Resistance levels (%) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, P. aeruginosa, and A. baumannii/calcoaceticus complex from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*A. baumannii/calcoaceticus* complex
Antibiotic						
amoxicillin/ampicillin	40	-	19	-	-	-
co-amoxiclav[a]	29	17	7	-	-	-
piperacillin-tazobactam	4	13	0.4	-	6	-
cefuroxime	12	15	1.6	-	-	-
cefotaxime/ceftriaxone[b]	7	10 ↑	1.0	-	-	-
ceftazidime	5	9	0.3	-	3	-
meropenem/imipenem[b]	0.0	0.4 ↑	-	0.2	-	2 ↑
meropenem[b]	-	-	0.0	-	1.2	-
imipenem	-	-	-	-	5	-
ciprofloxacin[b]	13	13	9	3	9	5 ↑
gentamicin	5	5	5	2	-	3
tobramycin	5	6	4	3	1.5	3 ↑
fosfomycin[1]	2	-	-	-	-	-
trimethoprim	24	19	28	7	-	-
co-trimoxazole	21	13	22	6	-	4
nitrofurantoin	1.8	-	-	-	-	-
Empiric therapy combinations						
cefuroxime + gentamicin	1.7	4	0.6	-	-	-
cefuroxime + ciprofloxacin[b]	6	9	0.7	-	-	-
cefotaxime/ceftriaxone + gentamicin[b]	1.4	4	0.5	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	4	7 ↑	0.6	-	-	-
ceftazidime + tobramycin	-	-	-	-	0.5	-
Multidrug resistance						
MDOT[2]	4	6	1.6	-	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[2] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae complex*). For more details see section 5.1.1.1.

Figure 5.1.3.2.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, P. aeruginosa, and A. baumannii/calcoaceticus complex from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

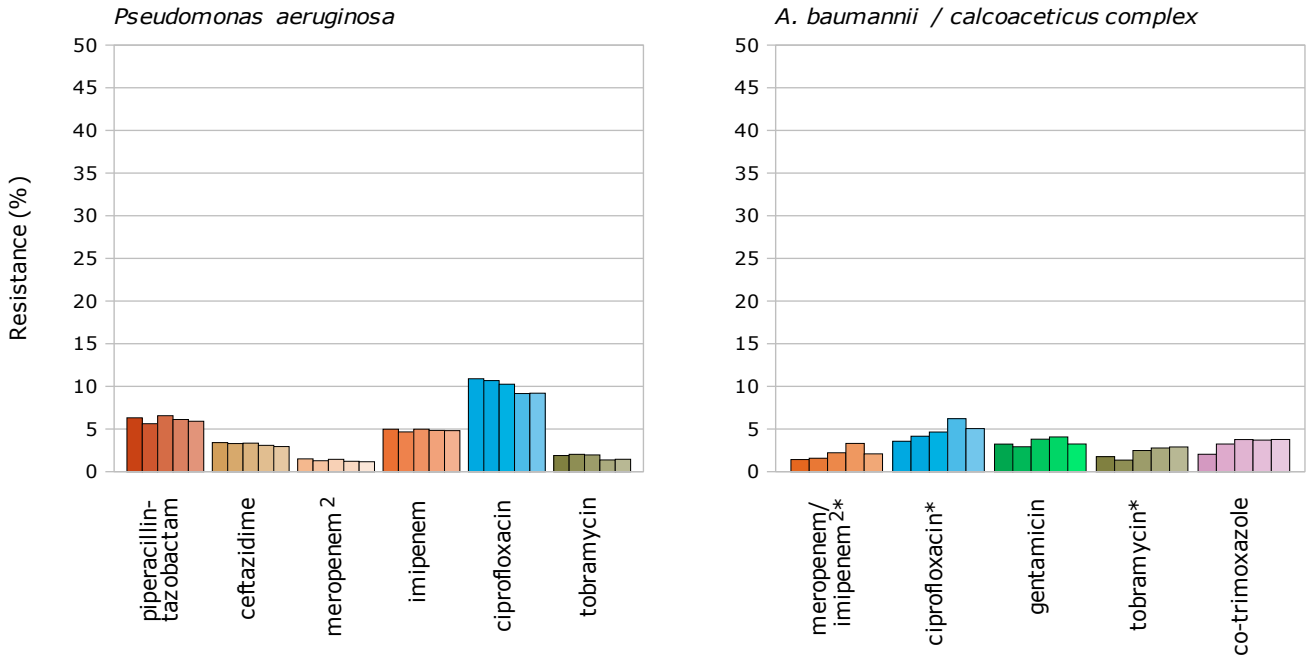
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.

[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.

[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.

[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Figure 5.1.3.2.1 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, P. aeruginosa, and A. baumannii/calcoaceticus complex from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



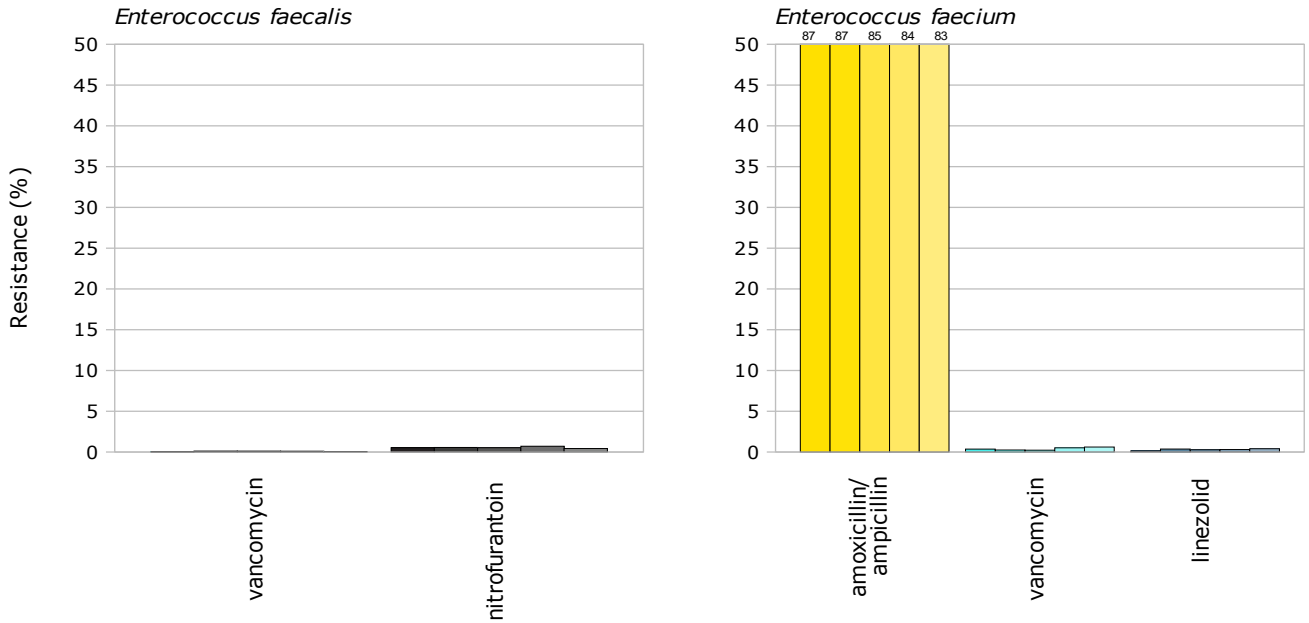
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Table 5.1.3.2.3 *Resistance levels (%) among diagnostic isolates of E. faecalis and E. faecium from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	E. faecalis	***E. faecium***
Antibiotic		
amoxicillin/ampicillin	-	83
vancomycin	0.1	0.6
linezolid	-	0.4
nitrofurantoin	0.4	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.2.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. faecalis and E. faecium from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

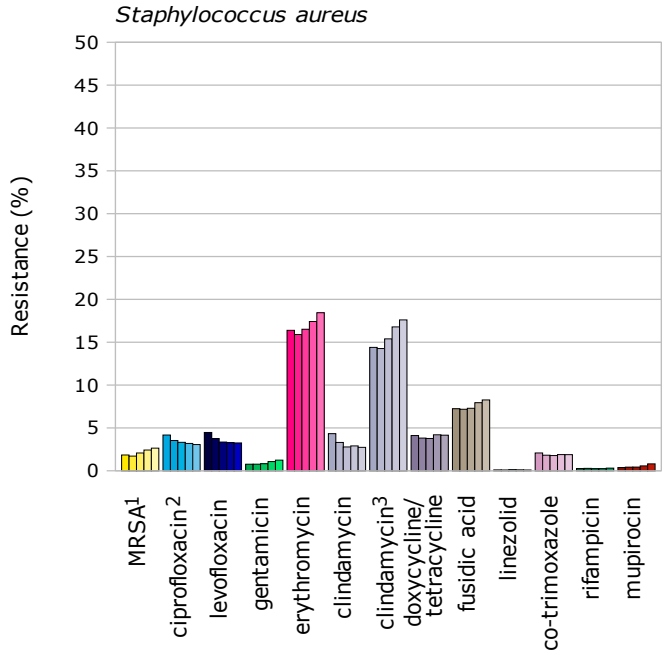
Table 5.1.3.2.4 *Resistance levels (%) among diagnostic isolates of S. aureus from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	3
ciprofloxacin[2]	3
levofloxacin	3
gentamicin	1.2
erythromycin	18
clindamycin	3
clindamycin (including inducible resistance)[3]	18
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0.1
co-trimoxazole	1.9
rifampicin	0.3
mupirocine	0.8

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 3 out of 26 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.2.3 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of S. aureus from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).
[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

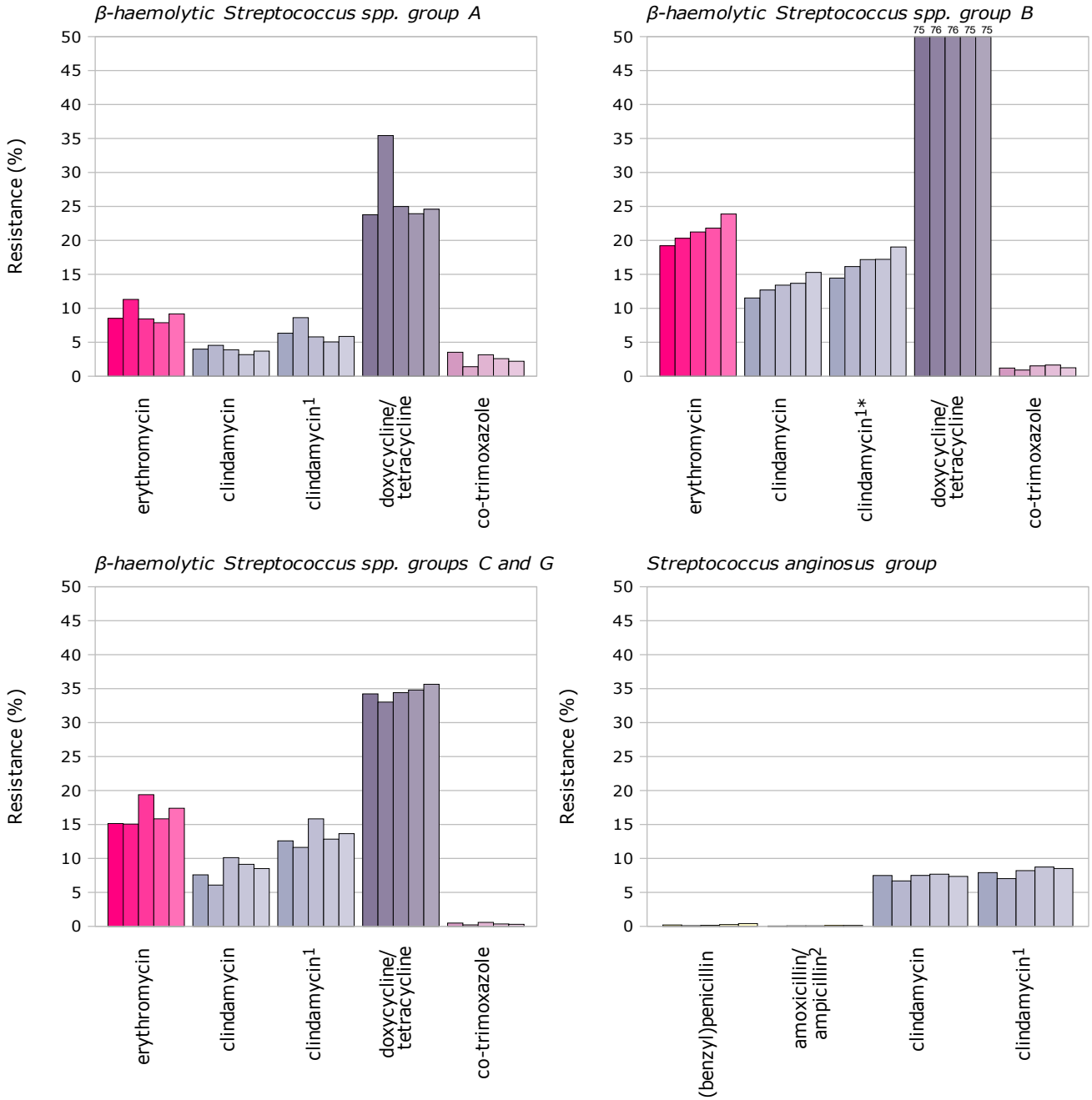
Table 5.1.3.2.5 *Resistance levels (%) among diagnostic isolates of β-haemolytic Streptococcus spp. groups A,B,C and G, S. anginosus group, and S. mitis group from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	ß-haemolytic *Streptococcus* spp. group A	**ß-haemolytic *Streptococcus* spp. group B**	**ß-haemolytic *Streptococcus* spp. groups C and G**	***S. anginosus* group**	***S. mitis* group**
Antibiotic					
(benzyl)penicillin	-	-	-	0.4	4
amoxicillin/ampicillin[1]	-	-	-	0.1	4 ↑
erythromycin	9	24	17	-	-
clindamycin	4	15	9	7	8
clindamycin (including inducible resistance)[2]	6	19 ↑	14	9	8
doxycycline/tetracycline	25	75	36	-	-
co-trimoxazole	2	1.2	0.3	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

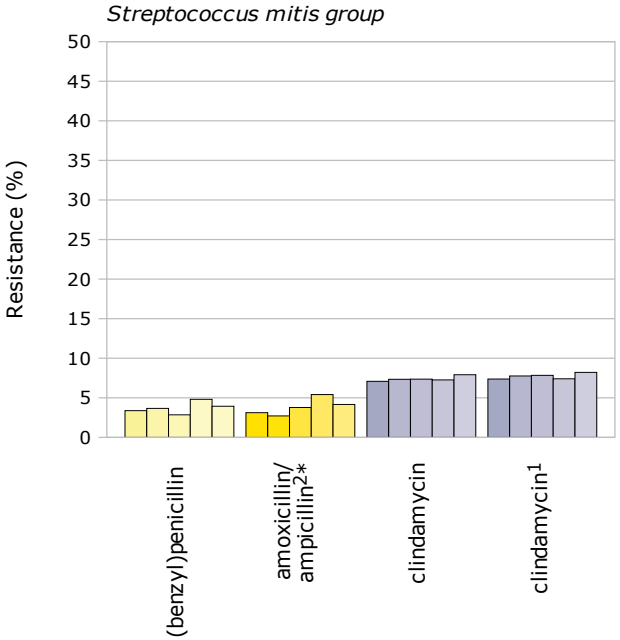
[1] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.
[2] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.2.4 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of β-haemolytic Streptococcus spp. groups A,B,C and G, S. anginosus group and S. mitis group from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
[2] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

Figure 5.1.3.2.4 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of β-haemolytic Streptococcus spp. groups A,B,C and G, S. anginosus group and S. mitis group from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



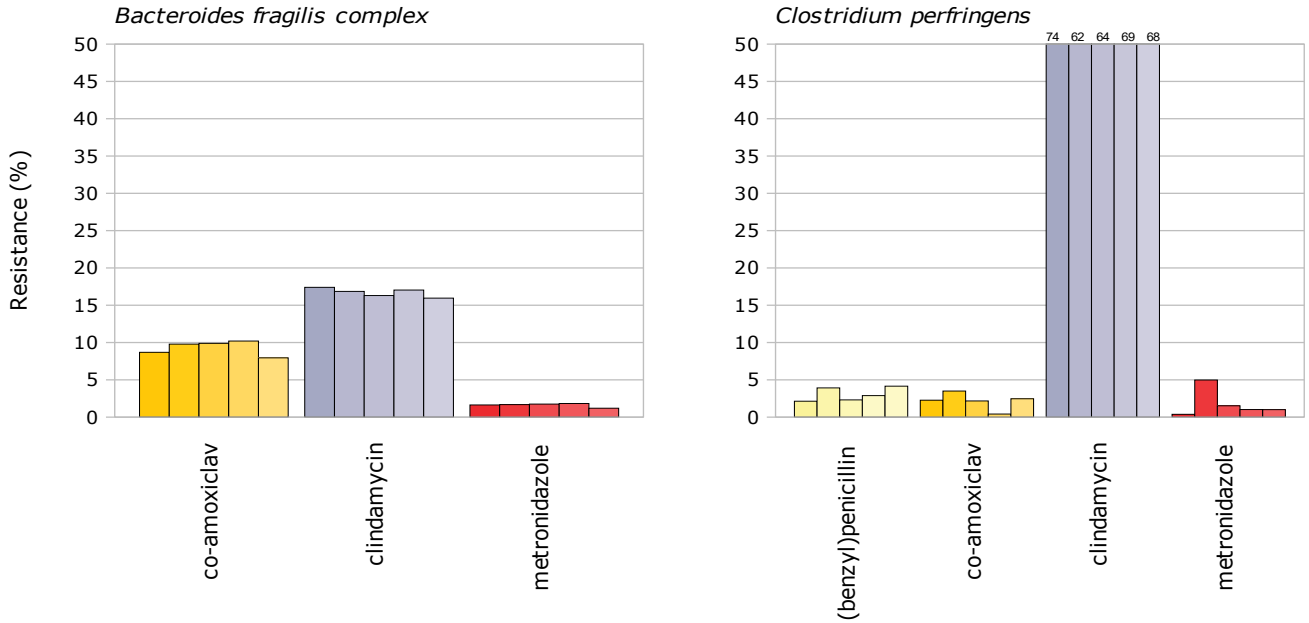
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
[2] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

Table 5.1.3.2.6 *Resistance levels (%) among diagnostic isolates of B. fragilis complex and C. perfringens from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2024*

	***B. fragilis* complex**	***C. perfringens***
Antibiotic		
(benzyl)penicillin	-	4
co-amoxiclav	8	2
clindamycin	16	68
metronidazole	1.2	1.0

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.2.5 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of B. fragilis complex and C. perfringens from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

Key results

Inpatient hospital departments (excl. ICU)

Blood, urine, wound/pus and respiratory: E. coli, K. pneumoniae, P. aeruginosa, and Acinetobacter baumannii/calcoaceticus complex

- For both *Enterobacterales* and *P. aeruginosa*, resistance levels for **all tested antibiotics** were comparable to resistance levels in isolates of OPD patients.
- For *E. coli* resistance to **second and third generation cephalosporins** seemed to have plateaued over the past five years. Resistance to **cefuroxime** was 12% and to **cefotaxime/ceftriaxone** 7%.
- For *K. pneumoniae*, there was a significant increase in resistance to **cefotaxime/ceftriaxone** (10%). Resistance to **cefuroxime** was 15%. This means that patients that are infected with *K. pneumoniae* have a considerable risk of non-adequate empiric treatment with a **second** or (to a lesser extent) **third generation cephalosporins**. In case of severe infection, **empiric combination therapy with aminoglycosides**, reduced likelihood of resistance to 4%.
- Although **meropenem/imipenem** resistance in *Klebsiella pneumoniae* was less than 1%, there was a significant increase over the last five years from 0,1% to 0.4%.
- For the three most important oral antibiotics, **co-amoxiclav**, **co-trimoxazole** and **ciprofloxacin**, resistance levels of *E. coli, K. pneumoniae* and *P. aeruginosa* were comparable to resistance levels in OPD patients.
- For *P. aeruginosa*, resistance was relatively low and stable for **all antibiotics** over the last five years. Empirical treatment with ceftazidime when infections are potentially caused by *P. aeruginosa* remains therefore adequate.
- For this NethMap edition, we only selected *Acinetobacter baumannii/calcoaceticus* complex isolates for analyses. We found a significant increase in resistance to **meropenem/imipenem** (2%), **ciprofloxacin** (5%) and **tobramycin** (3%) over the last five years.

Blood, urine, wound/pus and respiratory: S. aureus

- In *S. aureus* from hospital patients, resistance was high for **clindamycin** (including inducible resistance, 18%). This indicates that culture and susceptibility testing are advised before starting treatment with this drug. We also analyzed **clindamycin** resistance without considering inducible resistance (3%). According to EUCAST, clindamycin may still be used for short-term therapy in less severe skin and soft tissue infections if tested susceptible, even in the presence of an inducible resistance mechanism.
- Resistance to **levofloxacin**, **doxycycline**, and **co-trimoxazole** was less than 4%.
- **MRSA** was found in 3% of *S. aureus* isolates of hospital patients which remained stable over the previous 5 years.

Blood, urine, blood, wound/pus, and respiratory: β-haemolytic Streptococcus spp. groups A, B and C/G

- Resistance to **clindamycin** (including inducible resistance) and **erythromycin** in β-haemolytic *Streptococcus* spp. group A remained stable at 6% for **clindamycin** (including inducible resistance) and 9% for **erythromycin**. Resistance to **doxycycline/tetracycline** was 25%.
- In β-haemolytic *Streptococcus* spp. groups B, resistance levels for **clindamycin** (including inducible resistance) increased significantly to 19%. Resistance to **doxycycline/tetracycline** remained high at 75%.
- In β-haemolytic *Streptococcus* spp. groups C and G, resistance levels for **clindamycin** (including inducible resistance) remained stable at 14% and for **erythromycin** stable at 17%.
- In *S. mitis* group species the resistance level for **amoxicillin/ampicillin** significantly increased to 4%.

Blood, urine, blood, wound/pus, and respiratory: Anaerobes

- The European Committee on Antimicrobial Susceptibility Testing (EUCAST) publishes clinical breakpoints for anaerobes. Prior to 2022, these breakpoints were not species-specific. However, when all test values over the last five years, including those from previous years, were reinterpreted according to EUCAST breakpoints version 14.1, resistance in *B. fragilis* and *C. perfringens* remained stable and low, except for 68% resistance to **clindamycin** in *C. perfringens* and 16% in *B. fragilis* complex.
- Resistance to **metronidazole** in both *B. fragilis* and *C. perfringens* remained low at 1% and were not influenced by the new species-specific breakpoints.

5.1.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood, cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to intensive care units in 2024 is presented in table 5.1.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2024 are presented in tables 5.1.3.3.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex and *P. aeruginosa*), 5.1.3.3.3 (*E. faecalis* and *E. faecium*), 5.1.3.3.4 (*S. aureus*) and 5.1.3.3.5 (β-haemolytic *Streptococcus* spp. group A and B, and *S. anginosus* group). Five-year trends in resistance are shown in figures 5.1.3.3.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex and *P. aeruginosa*), 5.1.3.3.2 (*E. faecalis* and *E. faecium)*, 5.1.3.3.3 (*S. aureus*) and 5.1.3.3.4 (β-haemolytic *Streptococcus* spp. group A and B, and *S. anginosus* group). For β-haemolytic *Streptococcus* spp. groups C and G and*, S. mitis* group, *B. fragilis* complex, and *C. perfringens*, resistance levels and trends were not calculated because data were available for less than 100 isolates each year.

In intensive care units in the Netherlands, diagnostic (infection related) samples are taken from almost all patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Bias due to selective sampling of patients is therefore expected to be very low.

Table 5.1.3.3.1 *Distribution of isolated pathogens in diagnostic samples from patients admitted to intensive care units, ISIS-AR 2024*

	Blood	Cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	228 (7)	2 (2)	347 (8)	518 (38)	407 (13)
K. pneumoniae	41 (1)	0 (0)	165 (4)	106 (8)	94 (3)
P. mirabilis	8 (0)	0 (0)	51 (1)	69 (5)	51 (2)
E. cloacae complex	34 (1)	4 (3)	225 (5)	34 (2)	124 (4)
Other Enterobacterales[1]	109 (3)	3 (2)	770 (18)	150 (11)	312 (10)
P. aeruginosa	48 (1)	2 (2)	301 (7)	67 (5)	199 (6)
Other non-fermenters[2]	28 (1)	5 (4)	446 (11)	13 (1)	72 (2)
Other Gram-negatives[3]	31 (1)	6 (5)	410 (10)	2 (0)	67 (2)
E. faecalis	106 (3)	3 (2)	19 (0)	183 (13)	280 (9)
E. faecium	311 (9)	2 (2)	23 (1)	108 (8)	358 (11)
S. aureus	283 (8)	11 (9)	1,002 (24)	31 (2)	397 (13)
ß-haemolytic *Streptococcus* spp. group A	26 (1)	0 (0)	70 (2)	0 (0)	81 (3)
ß-haemolytic *Streptococcus* spp. group B	12 (0)	2 (2)	42 (1)	18 (1)	23 (1)
S. anginosus group	21 (1)	3 (2)	31 (1)	3 (0)	162 (5)
Other Gram-positives[4]	2,142 (62)	83 (66)	280 (7)	72 (5)	537 (17)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Serratia* spp., *Citrobacter* spp., *Morganella* spp., *Proteus* spp. (non-*mirabilis*), *Raoultella* spp., *Hafnia* spp., *Pantoea* spp., *Providencia* spp., *Enterobacter* spp. (non-*cloacae* complex), *Salmonella* spp., *Escherichia* spp. (non-*coli*), *Mixta* spp., *Yersinia* spp.
[2] In order of frequency: *S. maltophilia*, *M. catarrhalis*, *Acinetobacter* spp., *Pseudomonas* spp. (non-*aeruginosa*), *B. cepacia*.
[3] In order of frequency: *H. parainfluenzae*, *H. influenzae*, *B. fragilis complex*, *B. fragilis*, *N. meningitidis*, *C. coli*, *C. jejuni*.
[4] In order of frequency: *Staphylococcus* spp. (non-*aureus*), *S. mitis*, *S. dysgalactiae* n.n.g., *S. peroris*, *ß-haemolytische Streptokokken groep G n.n.g.*, *S. gordonii*, *S. infantis*, *S. canis*, *S. oralis*, *S. dysgalactiae* subsp. *equisimilis*, *S. pneumoniae*, *streptococcus dysgalactiae subsp. dysgalactiae*, *ß-haemolytische Streptokokken groep C n.n.g.*, *Streptococcus sanguinis groep n.n.g.*, *Streptococcus mitis groep n.n.g.*, *Streptococcus mitis/oralis n.n.g.*, *Enterococcus* spp. (non-*faecalis*, non-*faecium*), *A. urinae*, *C. perfringens*, *L. monocytogenes*.

Table 5.1.3.3.2 *Resistance levels (%) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex and P. aeruginosa from patients admitted to intensive care units, ISIS-AR 2024*

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	43	-	16 ↓	-	-
co-amoxiclav[a]	31 ↓	19 ↓	7	-	-
piperacillin-tazobactam	6	15 ↓	0.5	-	14
cefuroxime	17	21	3 ↑	-	-
cefotaxime/ceftriaxone[b]	11	17	3	-	-
ceftazidime	7	14	0.6	-	9
meropenem/imipenem[b]	0.1	2 ↑	-	0.2	-
meropenem[b]	-	-	0.0	-	4
imipenem	-	-	-	-	9 ↑
ciprofloxacin[b]	12	14	13	3 ↓	11
gentamicin	7	8	3	4 ↓	-
tobramycin	7	10	4	4 ↓	3
co-trimoxazole	21	15	22	4	-
Empiric therapy combinations					
cefuroxime + gentamicin	3	8	2	-	-
cefuroxime + ciprofloxacin[b]	8	12	1.7	-	-
cefotaxime/ceftriaxone + gentamicin[b]	3	7	3	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	7	11	2	-	-
ceftazidime + tobramycin	-	-	-	-	1.9
Multidrug resistance					
MDOT[1]	5	8	4	-	-

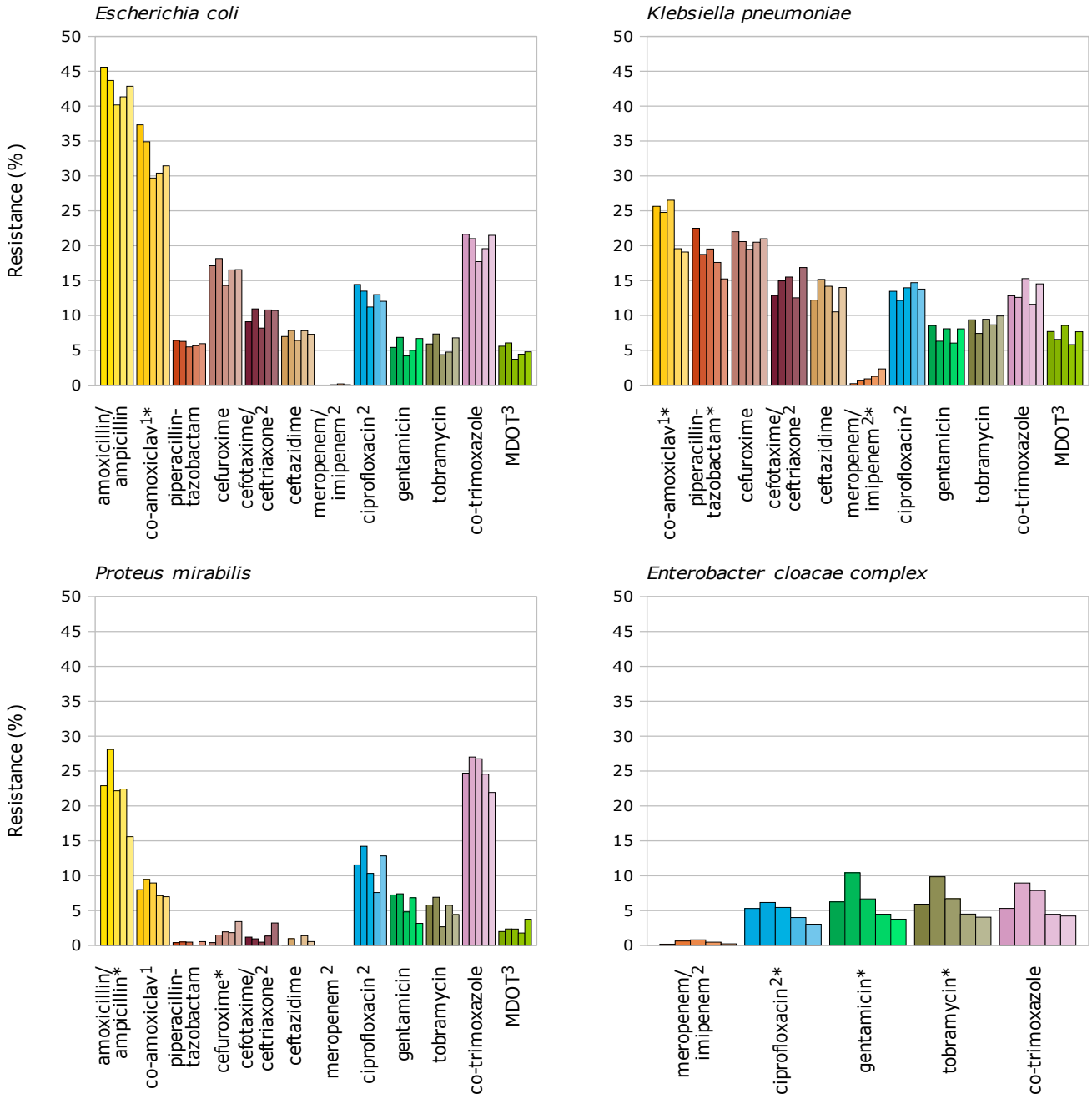
10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.

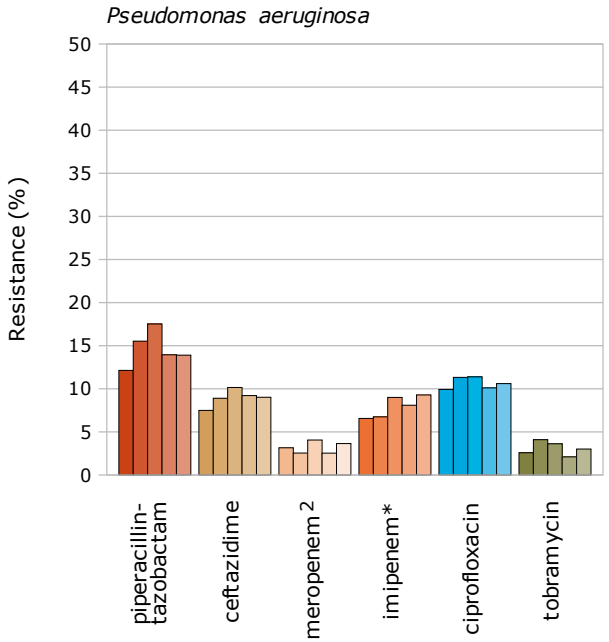
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae complex*). For more details see section 5.1.1.1.

Figure 5.1.3.3.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex and P. aeruginosa from patients admitted to intensive care units in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Figure 5.1.3.3.1 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex and P. aeruginosa from patients admitted to intensive care units in ISIS-AR*



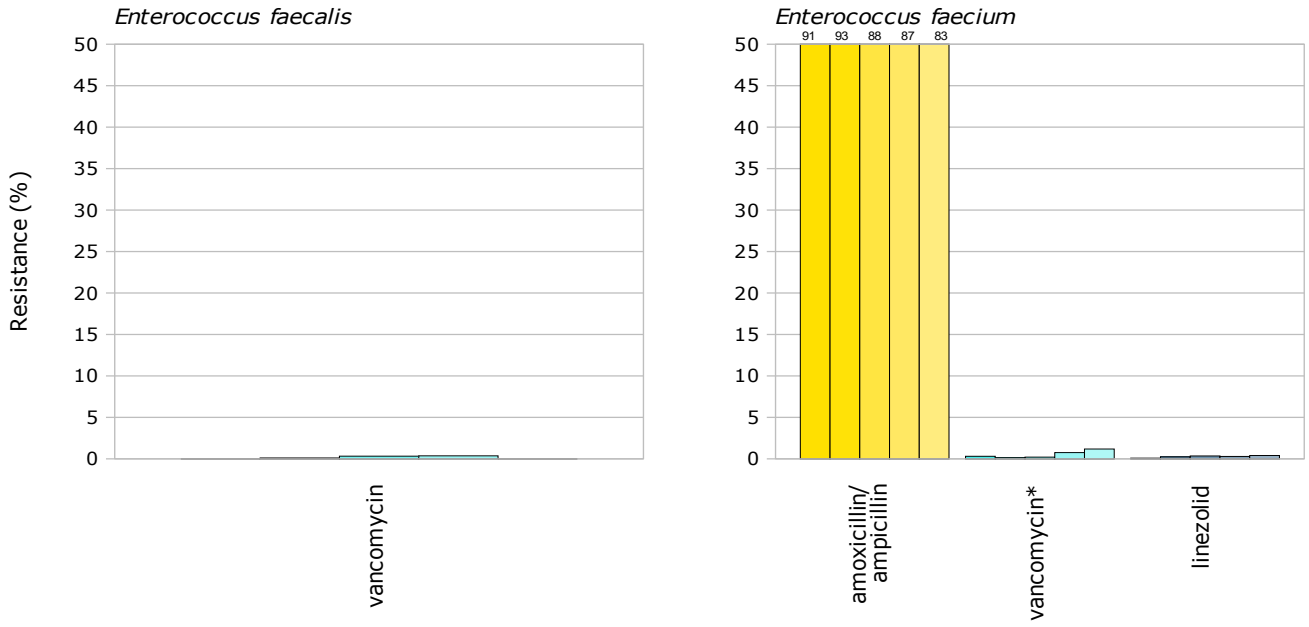
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Table 5.1.3.3.3 *Resistance levels (%) among diagnostic isolates of E. faecalis and E. faecium from patients admitted to intensive care units, ISIS-AR 2024*

	E. faecalis	***E. faecium***
Antibiotic		
amoxicillin/ampicillin	-	83
vancomycin	0.0	1.2 ↑
linezolid	-	0.4

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.3.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of E. faecalis and E. faecium from patients admitted to intensive care units in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

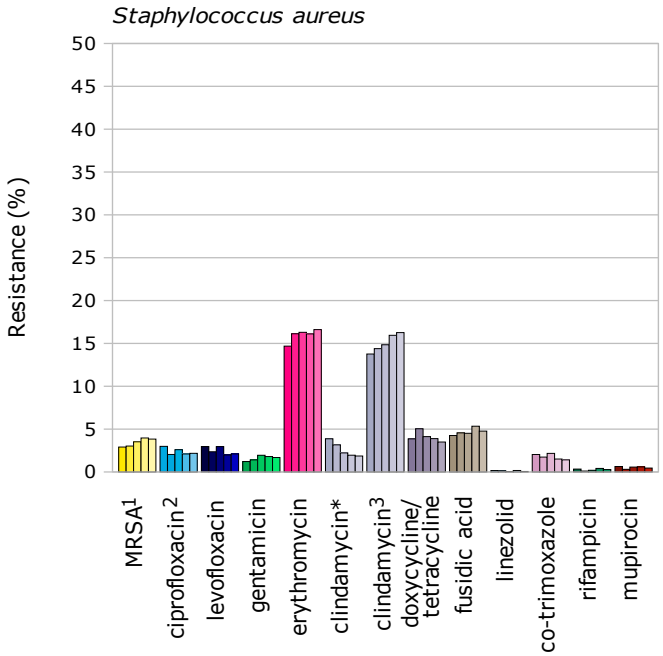
Table 5.1.3.3.4 *Resistance levels (%) among diagnostic isolates of S. aureus from patients admitted to intensive care units, ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	4
ciprofloxacin[2]	2
levofloxacin	2
gentamicin	1.7
erythromycin	17
clindamycin	1.9 ↓
clindamycin (including inducible resistance)[3]	16
doxycycline/tetracycline	3
fusidic acid	5
linezolid	0.1
co-trimoxazole	1.4
rifampicin	0.3
mupirocine	0.5

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 0 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.3.3 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of S. aureus from patients admitted to intensive care units in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.

[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.

[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Table 5.1.3.3.5 *Resistance levels (%) among diagnostic isolates of β-haemolytic Streptococcus spp. group A and B, and S. anginosus group from patients admitted to intensive care units, ISIS-AR 2024*

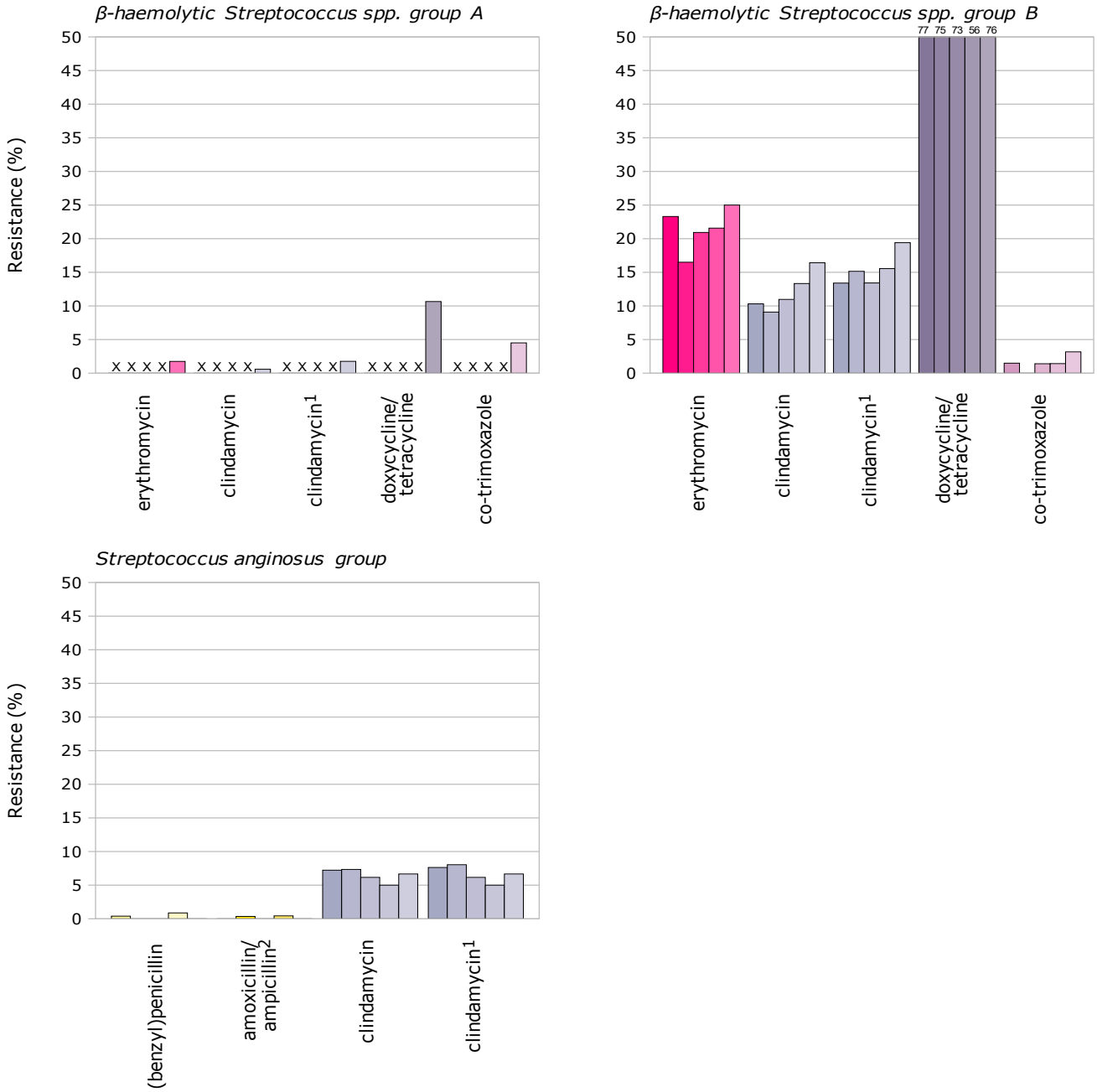
	ß-haemolytic *Streptococcus* spp. group A	**ß-haemolytic *Streptococcus* spp. group B**	***S. anginosus* group**
Antibiotic			
(benzyl)penicillin	-	-	0.0
amoxicillin/ampicillin[1]	-	-	0.0
erythromycin	1.8°	25	-
clindamycin	0.6°	16	7
clindamycin (including inducible resistance)[2]	1.8°	19	7
doxycycline/tetracycline	11°	76	-
co-trimoxazole	5°	3	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

[2] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.3.4 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of β-haemolytic Streptococcus spp. group A and B, and S. anginosus group from patients admitted to intensive care units in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
[2] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

Key results

Intensive Care Units

Blood, urine, blood, wound/pus, and respiratory: Enterobacterales and P. aeruginosa

- For *Enterobacterales* and *P. aeruginosa*, resistance levels for **beta-lactam antibiotics** were higher in ICU patients than in isolates from non-ICU patients.
- In *K. pneumoniae*, resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 21% and 17%, respectively, and increased slightly over the last five years. In *E. coli,* resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 17% and 11%, respectively. This means that ICU patients with infections due to *K. pneumoniae* and *E. coli* had considerable risk of non-adequate empiric treatment with a **second** or a **third generation cephalosporin**. In case of severe infection, in *E. coli*, **empiric combination therapy with an aminoglycoside**, reducing likelihood of resistance to 3%, which might be a suitable strategy. For *K. pneumonia*, **empiric combination therapy with an aminoglycoside** may result in 8% risk of treatment failure, which represents a substantial risk that warrants close monitoring over the coming years.
- A worrisome increase in resistance to **meropenem/imipenem** in *K. pneumoniae* was found. Resistance increased from 0.3% to 2% over the last five years.
- In *P. aeruginosa* isolates from ICU patients, resistance to **piperacillin-tazobactam** and **ceftazidime**, the two first choice agents for the treatment of severe *P. aeruginosa* infections, was 14% for **piperacillin-tazobactam** and 9% for **ceftazidime**, which is much higher than in isolates from other hospital departments. This might complicate empirical treatment of severe infections due to *P. aeruginosa* in the ICU.

Blood, urine, blood, wound/pus, and respiratory: S. aureus and E. faecium

- **MRSA** percentage in clinical *S. aureus* isolates of ICU patients increased to 4% over the last five years, but not significantly.
- The percentage of **vancomycin** resistance in clinical *E. faecium* isolates of ICU patients increased significantly to 1.2% over the last five years.

5.1.3.4 Blood isolates from inpatient departments (incl. intensive care units)

The distribution of pathogens isolated from blood of patients admitted to non-intensive care inpatient departments (non-ICU) and intensive care units (ICU) in 2024 is presented in table 5.1.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2024 are presented in tables 5.1.3.4.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 5.1.3.4.3 (*E. faecalis* and *E. faecium*), 5.1.3.4.4 (*S. aureus*), 5.1.3.4.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus* group, and *S. mitis* group), and 5.1.3.4.6 (*B. fragilis* complex and *C. perfringens*). Five-year trends in resistance are presented in figures 5.1.3.4.1 (*E. coli, K. pneumoniae, P. mirabilis, E. cloacae* complex, and *P. aeruginosa*), 5.1.3.4.2 (*E. faecalis* and *E. faecium*), 5.1.3.4.3 (*S. aureus*), 5.1.3.4.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus* group, and *S. mitis* group), and 5.1.3.4.5 (*B. fragilis* complex and *C. perfringens*).

In most hospitals, blood samples are taken from all patients suspected of having sepsis and susceptibility testing is performed as part of routine diagnostics. Bias due to selective sampling of patients is therefore unlikely.

Table 5.1.3.4.1 *Distribution of pathogens in diagnostic blood samples from patients admitted to non-intensive care inpatient departments (non-ICU) and intensive care units (ICU), ISIS-AR 2024*

	Non-ICU	**ICU**
Pathogen	**N (%)**	**N (%)**
E. coli	7,653 (23)	219 (6)
K. pneumoniae	1,517 (5)	44 (1)
P. mirabilis	466 (1)	9 (0)
E. cloacae complex	522 (2)	43 (1)
Other Enterobacterales[1]	2,040 (6)	150 (4)
P. aeruginosa	736 (2)	59 (2)
Other non-fermenters[2]	367 (1)	28 (1)
B. fragilis complex	358 (1)	19 (1)
Other Gram-negatives[3]	401 (1)	13 (0)
E. faecalis	1,041 (3)	104 (3)
E. faecium	633 (2)	331 (10)
S. aureus	3,496 (11)	241 (7)
ß-haemolytic *Streptococcus* spp. group A	573 (2)	26 (1)
ß-haemolytic *Streptococcus* spp. group B	443 (1)	13 (0)
ß-haemolytic *Streptococcus* spp. groups C and G	355 (1)	2 (0)
S. anginosus group	604 (2)	16 (0)
S. mitis group	805 (2)	38 (1)
C. perfringens	123 (0)	4 (0)
Other Gram-positives[4]	11,094 (33)	2,088 (61)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Citrobacter* spp., *Serratia* spp., *Salmonella* spp., *Morganella* spp., *Pantoea* spp., *Raoultella* spp., *Proteus* spp. (non-*mirabilis*), *Providencia* spp., *Hafnia* spp., *Escherichia* spp. (non-*coli*), *Enterobacter* spp. (non-*cloacae* complex), *Yersinia* spp., *Mixta* spp., *Shigella* spp., *Cronobacter* spp.
[2] In order of frequency: *Acinetobacter* spp., *Pseudomonas* spp. (non-*aeruginosa*), *S. maltophilia*, *M. catarrhalis*, *B. cepacia*.
[3] In order of frequency: *H. parainfluenzae*, *H. influenzae*, *N. meningitidis*, *C. lari*, *C. coli*, *C. jejuni*.
[4] In order of frequency: *Staphylococcus* spp. (non-*aureus*), *S. dysgalactiae* subsp. *equisimilis*, *S. pneumoniae*, *S. dysgalactiae* n.n.g., *A. urinae*, *Enterococcus* spp. (non-*faecalis*, non-*faecium*), *L. monocytogenes*.

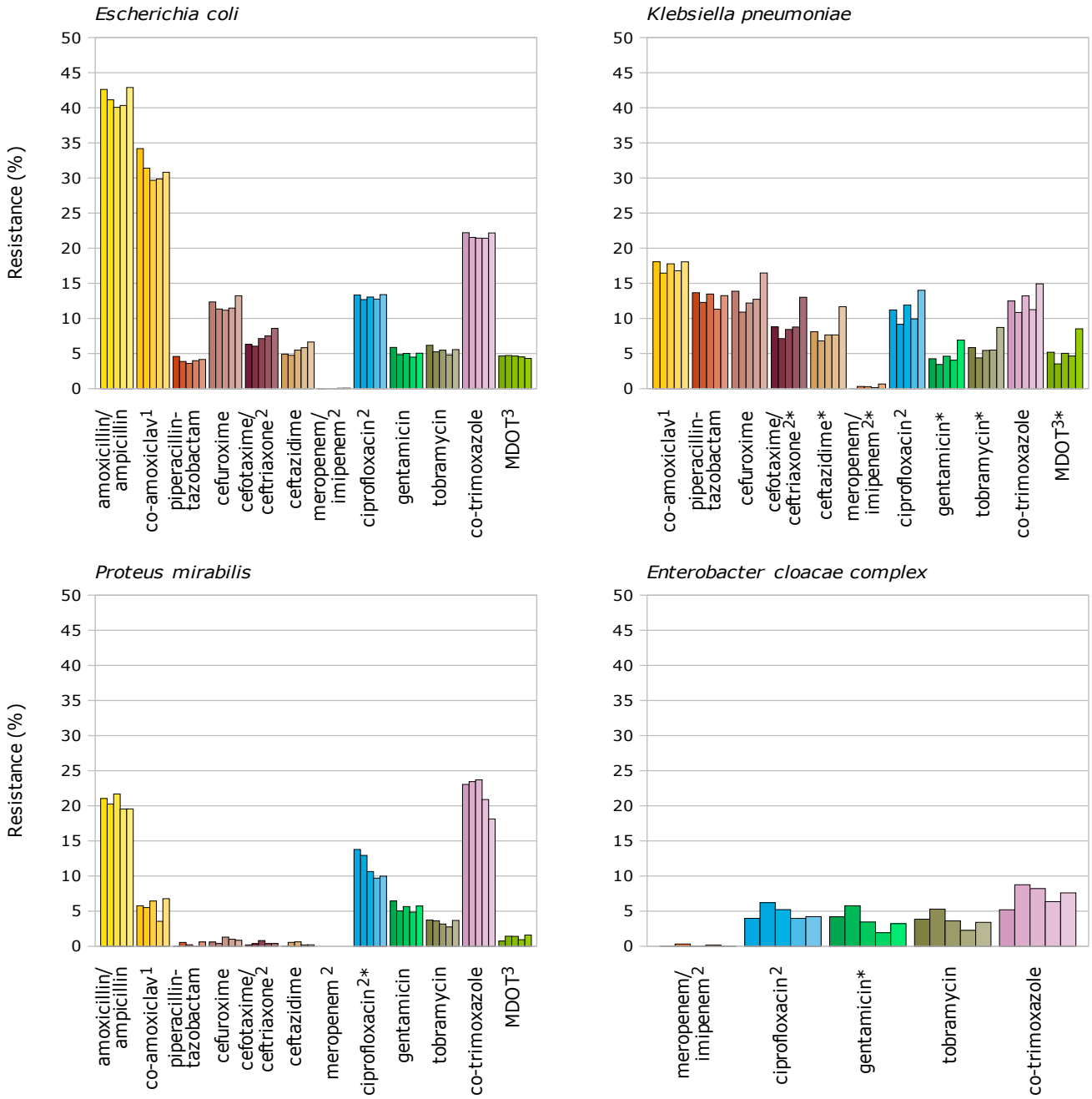
Table 5.1.3.4.2 *Resistance levels (%) among diagnostic blood isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, and P. aeruginosa from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2024*

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	43	-	20	-	-
co-amoxiclav[a]	31	18	7	-	-
piperacillin-tazobactam	4	13	0.6	-	7
cefuroxime	13	16	0.8	-	-
cefotaxime/ceftriaxone[b]	9	13 ↑	0.4	-	-
ceftazidime	7	12 ↑	0.2	-	4
meropenem/imipenem[b]	0.1	0.7 ↑	-	0.0	-
meropenem[b]	-	-	0.0	-	1.0
imipenem	-	-	-	-	5
ciprofloxacin[b]	13	14	10 ↓	4	7
gentamicin	5	7 ↑	6	3 ↓	-
tobramycin	6	9 ↑	4	3	1.1
co-trimoxazole	22	15	18	8	-
Empiric therapy combinations					
cefuroxime + gentamicin	2	6 ↑	0.5	-	-
cefuroxime + ciprofloxacin[b]	7	11 ↑	0.6	-	-
cefotaxime/ceftriaxone + gentamicin[b]	1.9	6 ↑	0.2	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	6	10 ↑	0.4	-	-
ceftazidime + tobramycin	-	-	-	-	0.5
Multidrug resistance					
MDOT[1]	4	9 ↑	1.6	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

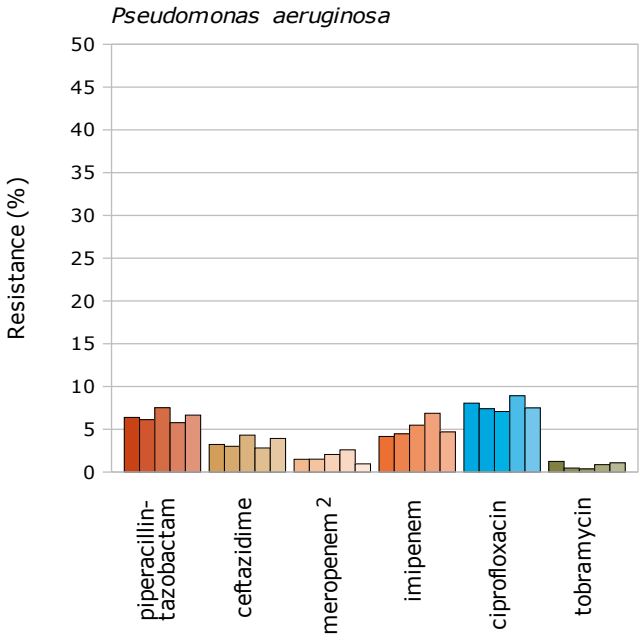
[1] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae complex*). For more details see section 5.1.1.1.

Figure 5.1.3.4.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, and P. aeruginosa from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Figure 5.1.3.4.1 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of E. coli, K. pneumoniae, P. mirabilis, E. cloacae complex, and P. aeruginosa from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



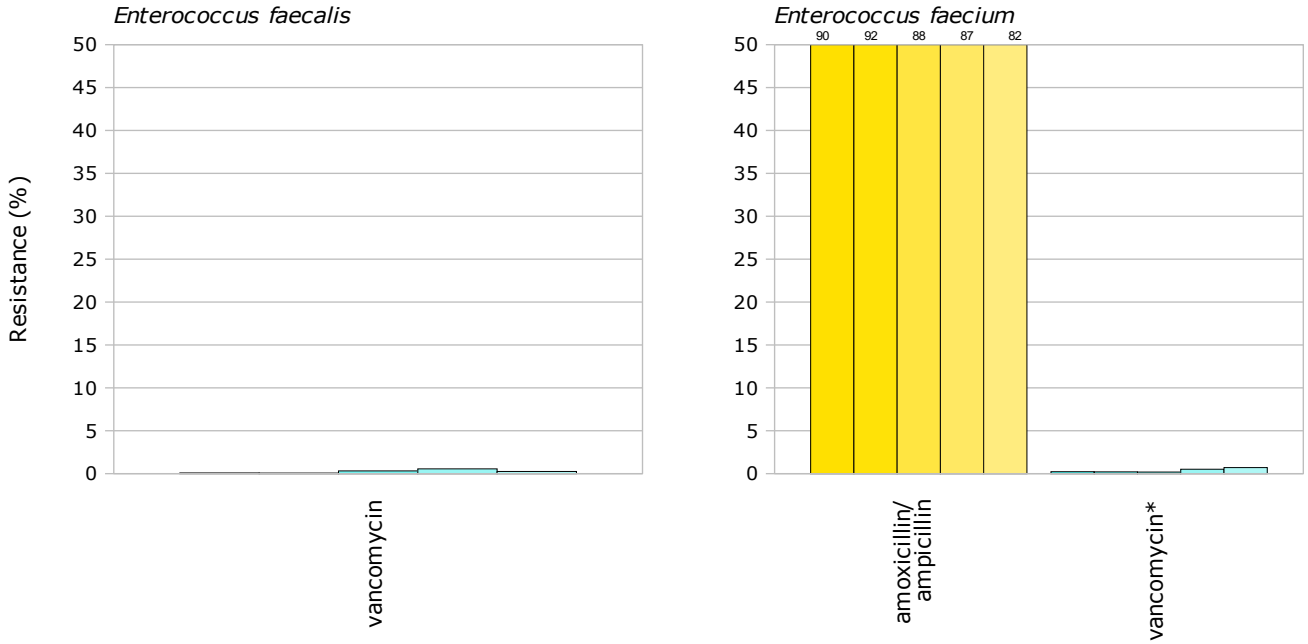
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Table 5.1.3.4.3 *Resistance levels (%) among diagnostic blood isolates of E. faecalis and E. faecium from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2024*

	E. faecalis	***E. faecium***
Antibiotic		
amoxicillin/ampicillin	-	82
vancomycin	0.3	0.7 ↑

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.4.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of E. faecalis and E. faecium from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

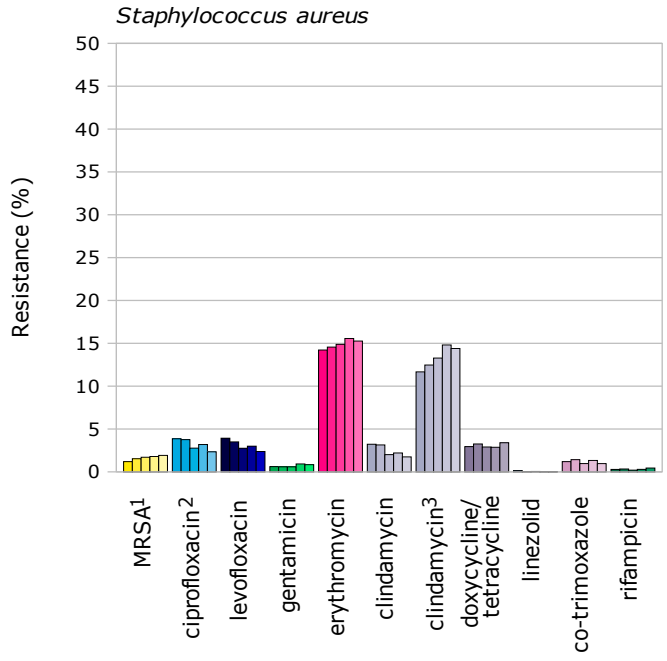
Table 5.1.3.4.4 *Resistance levels (%) among diagnostic blood isolates of S. aureus from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	1.9
ciprofloxacin[2]	2
levofloxacin	2
gentamicin	0.8
erythromycin	15
clindamycin	1.7
clindamycin (including inducible resistance)[3]	14
doxycycline/tetracycline	3
linezolid	0.0
co-trimoxazole	1.0
rifampicin	0.4

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 1 out of 7 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.4.3 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of S. aureus from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.

[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.

[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

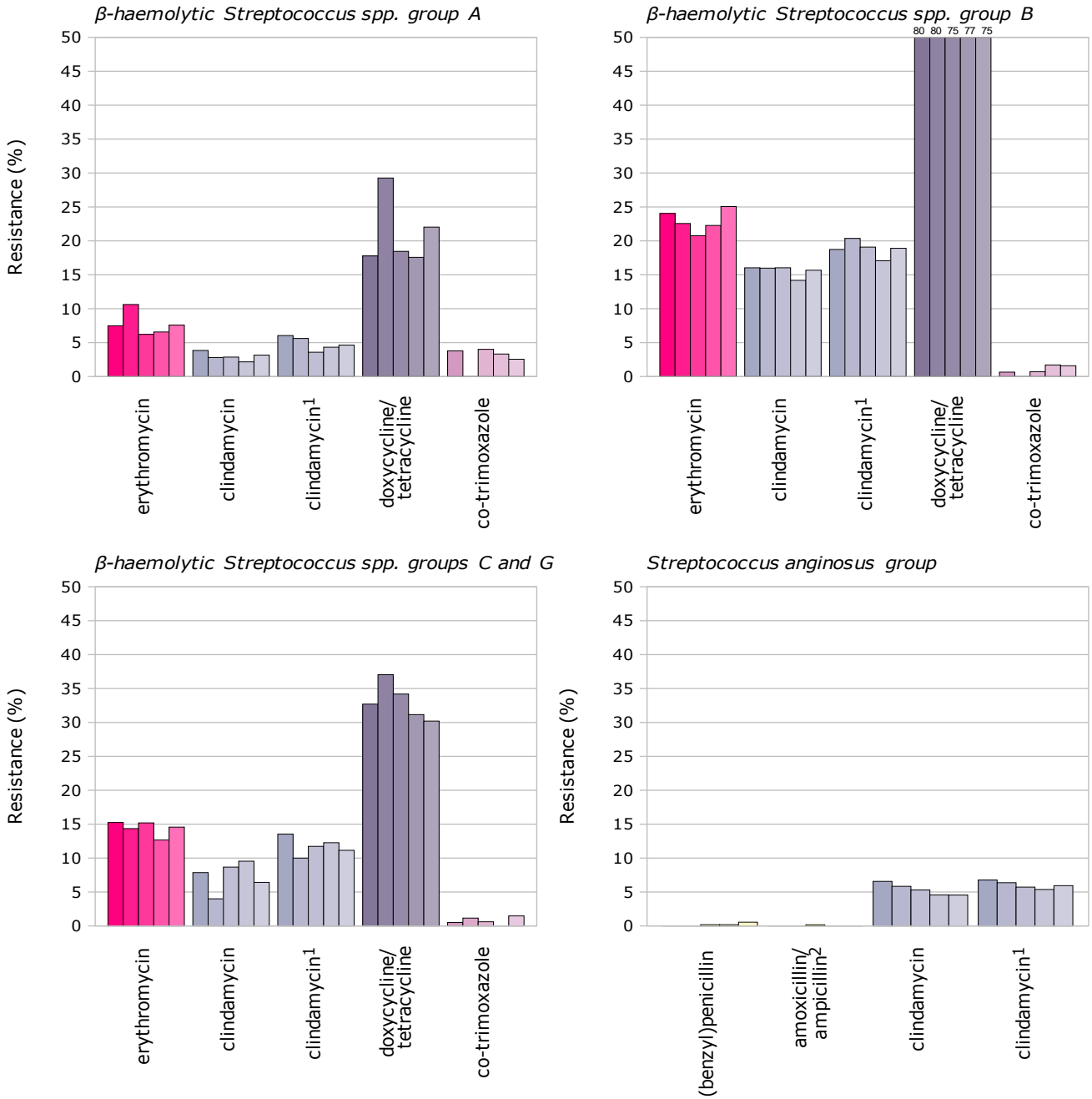
Table 5.1.3.4.5 *Resistance levels (%) among diagnostic blood isolates of β-haemolytic Streptococcus spp. groups A, B, C and G, S. anginosus group, and S. mitis group from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2024*

	ß-haemolytic *Streptococcus* spp. group A	**ß-haemolytic *Streptococcus* spp. group B**	**ß-haemolytic *Streptococcus* spp. groups C and G**	***S. anginosus* group**	***S. mitis* group**
Antibiotic					
(benzyl)penicillin	-	-	-	0.6	4
amoxicillin/ampicillin[1]	-	-	-	0.0	4 ↑
erythromycin	8	25	15	-	-
clindamycin	3	16	6	5	5
clindamycin (including inducible resistance)[2]	5	19	11	6	5
doxycycline/tetracycline	22	75	30	-	-
co-trimoxazole	3	1.6	1.5	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

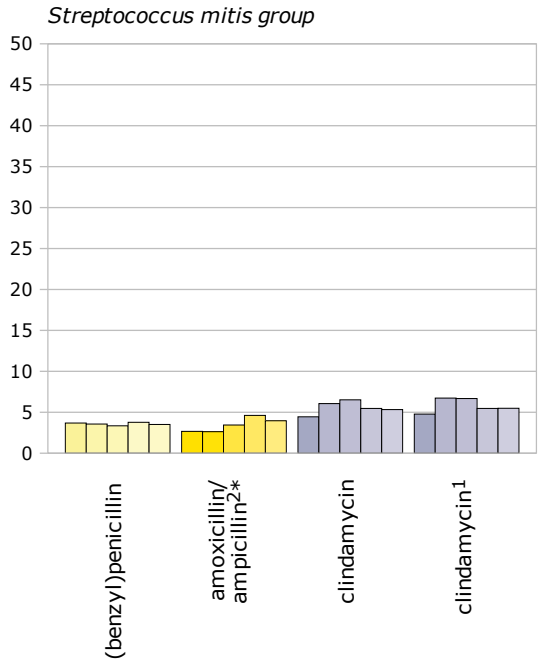
[1] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.
[2] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.3.4.4 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of β-haemolytic Streptococcus spp. groups A, B, C and G, S. anginosus group, and S. mitis group from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
[2] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

Figure 5.1.3.4.4 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of β-haemolytic Streptococcus spp. groups A, B, C and G, S. anginosus group, and S. mitis group from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



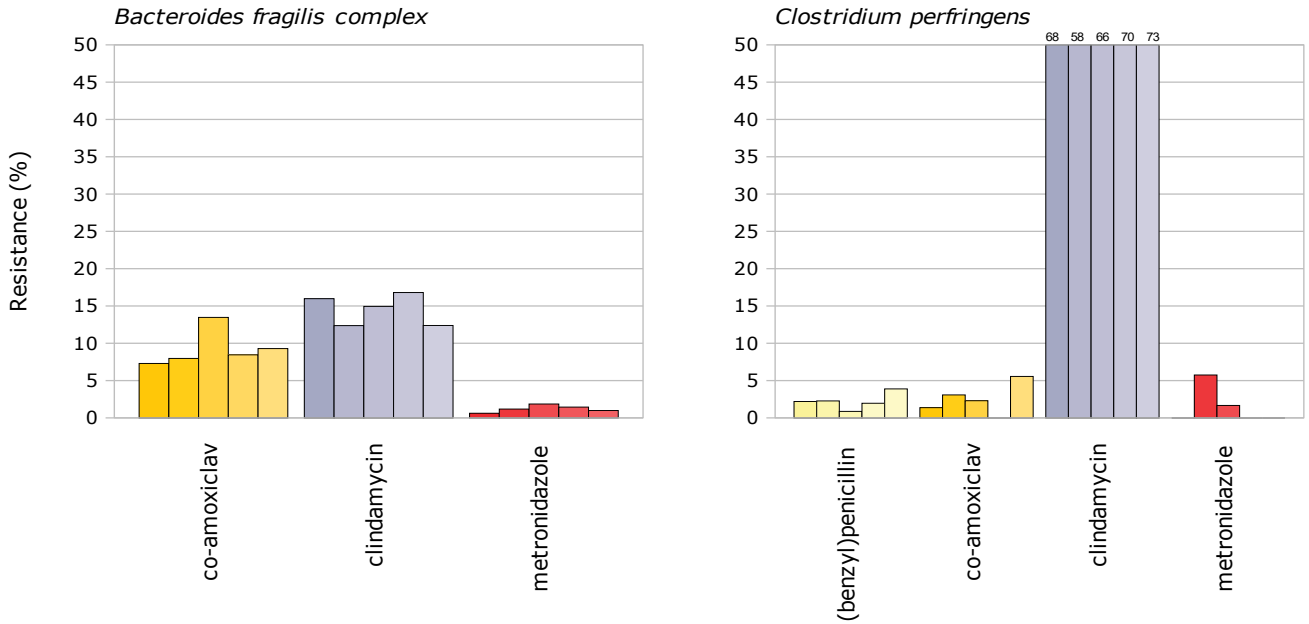
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
[2] Resistance to amoxicillin/ampicillin in *S. anginosus* group and *S. mitis* group was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

Table 5.1.3.4.6 *Resistance levels (%) among diagnostic blood isolates of B. fragilis complex and C. perfringens from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2024*

	***B. fragilis* complex**	***C. perfringens***
Antibiotic		
(benzyl)penicillin	-	4
co-amoxiclav	9	6
clindamycin	12	73
metronidazole	1.0	0.0

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.4.5 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic blood isolates of B. fragilis complex and C. perfringens from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

Key results

Blood isolates from inpatient departments (incl. intensive care units)

Enterobacterales and P. aeruginosa in blood cultures

- Resistance levels to **second** and **third generation cephalosporins** and **aminoglycosides** remained stable in *E. coli* blood culture isolates over the last five years.
- However, in *K. pneumoniae* resistance to **third generation cephalosporins** and **aminoglycosides** increased significantly over the last five years.
- In *K. pneumoniae*, resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 16% and 13%, and in *E. coli,* resistance to **cefuroxime** and **cefotaxime/ceftriaxone** was 13% and 9%. Patients with a bloodstream infection with *K. pneumoniae* or *E. coli* thus have a considerable risk of non-adequate empiric treatment with a **second** or (to a lesser extent) **third generation cephalosporin**. In case of severe infection, **empiric combination therapy with an aminoglycoside**, reducing likelihood of resistance to 2% in *E. coli*. which might be a suitable option. However, for *K. pneumonia*, **empiric combination therapy with an aminoglycoside** may result in 6% risk of treatment failure, a significant increase compared to last year (3%), which represents a substantial risk that warrants close monitoring over the coming years.
- Empirical combination therapy with **cephalosporins** and **ciprofloxacin** appears to provide little additional benefit over treatment with a **second**- or **third-generation cephalosporin** alone.
- *K. pneumoniae* from blood cultures showed a significant increase in resistance to **meropenem/imipenem** of 0.7%.
- After initial iv treatment, in patients with *E. coli* bacteremia, a switch to either **ciprofloxacin**, **co-trimoxazole**, or **co-amoxiclav** was most often possible given the relatively low (≤4%) combined resistance rates for these oral agents. However, in patients with *K. pneumoniae* bacteremia, there was a significant increase in **multidrug resistance** of these oral agents to 9% over the last five years.
- Compared to *P. aeruginosa* isolates from ICU patients, resistance to **ceftazidime**, **piperacillin-tazobactam** and **ciprofloxacin** in *P. aeruginosa* isolates from blood cultures was much lower (≤7%).

S. aureus and E. faecium in blood cultures

- **MRSA** was found in 2% of *S. aureus* isolates in blood cultures which remained stable over the previous 5 years.
- **Vancomycin** resistance was found in 0.7% of *Enterococcus faecium* isolates in blood cultures which increased significantly over the previous 5 years.

5.1.3.5 Urology services

The distribution of pathogens in urine samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in 2024 is presented in table 5.1.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2024 are presented by type of department in tables 5.1.3.5.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 5.1.3.5.3 (*E. faecalis* and *E. faecium*). Five-year trends in resistance are shown in figure 5.1.3.5.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 5.1.3.5.2 (*E. faecalis* and *E. faecium*).

In urology departments of Dutch hospitals, a urine sample is routinely taken from patients presenting with complicated urinary tract infections and susceptibility testing is performed as part of routine diagnostics. However, guidelines do not indicate sampling in case of uncomplicated urinary tract infections. As a result, for those infections often only a sample is taken after therapy failure, and the presented resistance levels are likely to be higher than those for all patients with urinary tract infections at urology departments.

Table 5.1.3.5.1 *Distribution of isolated pathogens in diagnostic urine samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2024*

	OPD	IPD
Pathogen	**N (%)**	**N (%)**
E. coli	14,295 (37)	999 (27)
K. pneumoniae	3,739 (10)	295 (8)
P. mirabilis	1,742 (4)	155 (4)
Other Enterobacterales[1]	6,856 (18)	717 (20)
P. aeruginosa	1,536 (4)	243 (7)
Other non-fermenters[2]	672 (2)	83 (2)
Other Gram-negatives[3]	23 (0)	4 (0)
E. faecalis	3,935 (10)	505 (14)
E. faecium	278 (1)	139 (4)
Other Gram-positives[4]	5,976 (15)	504 (14)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., *Morganella* spp., *Providencia* spp., *Proteus* spp. (non-*mirabilis*), *Raoultella* spp., *Pantoea* spp., *Escherichia* spp. (non-*coli*), *Hafnia* spp., *Salmonella* spp., *Mixta* spp.

[2] In order of frequency: *Acinetobacter* spp., *S. maltophilia*, *Pseudomonas* spp. (non-*aeruginosa*).

[3] In order of frequency: *H. parainfluenzae*, *H. influenzae*, *B. fragilis*, *N. meningitidis*.

[4] In order of frequency: *Staphylococcus* spp., *A. urinae*, *ß-haemolytische Streptokokken groep G n.n.g.*, *S. constellatus*, *S. gordonii*, *Streptococcus mitis/oralis n.n.g.*, *S. anginosus*, *ß-haemolytische Streptokokken groep A n.n.g.*, *S. dysgalactiae* subsp. *equisimilis*, *S. equi*, *Streptococcus anginosus groep n.n.g.*, *S. intermedius*, *S. pneumoniae*, *S. agalactiae*, *S. pyogenes*, *S. dysgalactiae* n.n.g., *streptococcus dysgalactiae subsp. dysgalactiae*, *S. canis*, *ß-haemolytische Streptokokken groep C n.n.g.*, *S. oralis*, *Streptococcus mitis groep n.n.g.*, *S. mitis*, *Enterococcus* spp. (non-*faecalis*, non-*faecium*), *C. perfringens*.

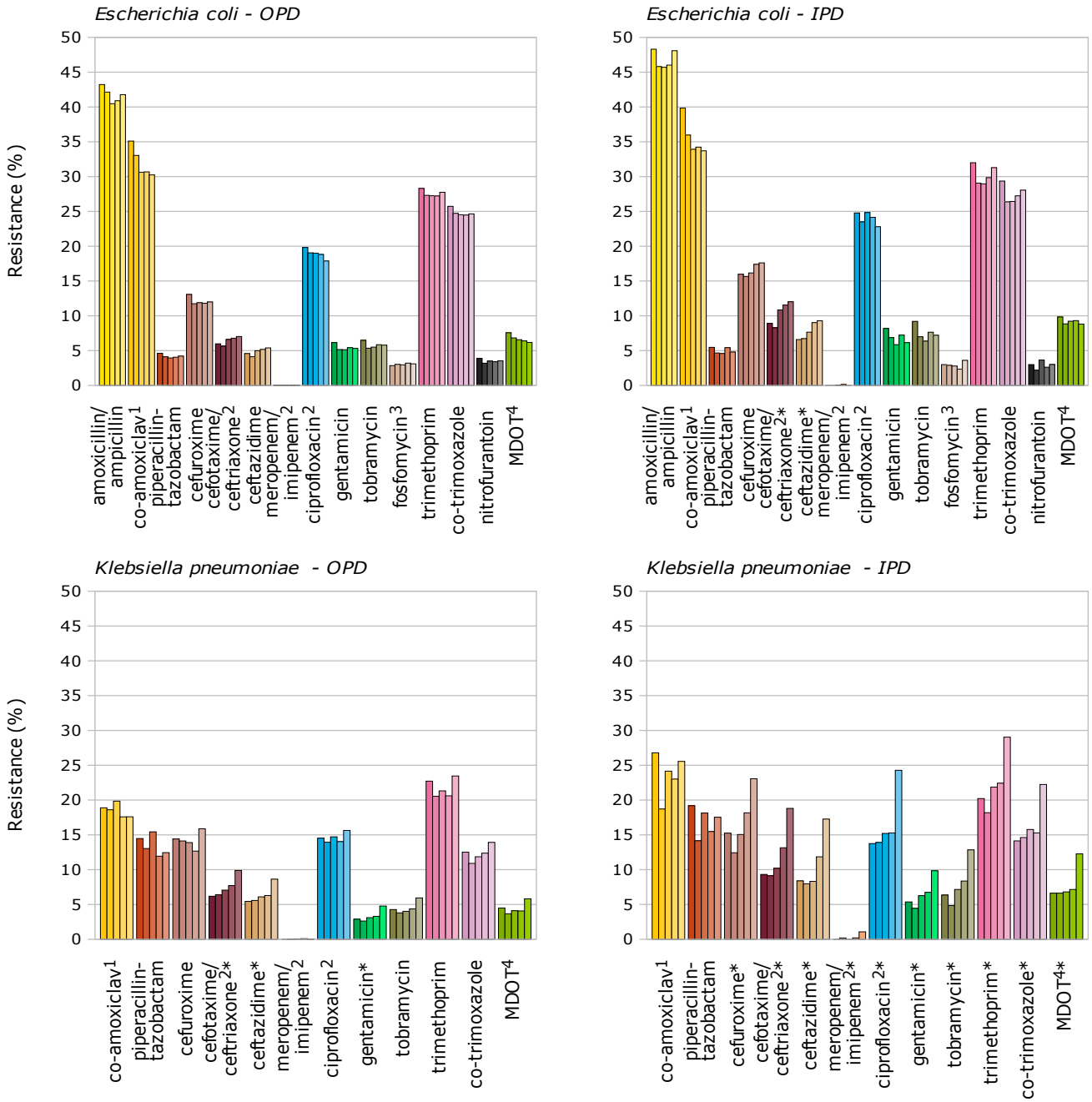
Table 5.1.3.5.2 *Resistance levels (%) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2024*

	E. coli		***K. pneumoniae***		***P. mirabilis***		***P. aeruginosa***	
	OPD	**IPD**	**OPD**	**IPD**	**OPD**	**IPD**	**OPD**	**IPD**
Antibiotic								
amoxicillin/ampicillin	42	48	-	-	20	21	-	-
co-amoxiclav[a]	30	34	18	26	7	9	-	-
piperacillin-tazobactam	4	5	12	18	0.4	1.0	5	5
cefuroxime	12	18	16	23 ↑	1.8	3	-	-
cefotaxime/ceftriaxone[b]	7	12 ↑	10 ↑	19 ↑	1.3	2	-	-
ceftazidime	5	9 ↑	9 ↑	17 ↑	0.2	0.6	1.6	3
meropenem/imipenem[b]	0.0	0.0	0.0	1.1 ↑	-	-	-	-
meropenem[b]	-	-	-	-	0.0	0.0	1.2	0.2
imipenem	-	-	-	-	-	-	6	6
ciprofloxacin[b]	18	23	16	24 ↑	14	17	13	13
gentamicin	5	6	5 ↑	10 ↑	7	9	-	-
tobramycin	6	7	6	13 ↑	5	7	0.8	0.0
fosfomycin[1]	3	4	-	-	-	-	-	-
trimethoprim	28	31	23	29 ↑	32	30	-	-
co-trimoxazole	25	28	14	22 ↑	25	23	-	-
nitrofurantoin	4	3	-	-	-	-	-	-
Empiric therapy combinations								
cefuroxime + gentamicin	1.9	3	4 ↑	9 ↑	0.7	1.5	-	-
cefuroxime + ciprofloxacin[b]	7	12	10	17 ↑	0.8	2	-	-
cefotaxime/ceftriaxone + gentamicin[b]	1.6	3	4 ↑	9 ↑	0.6	1.4	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	5	9 ↑	7 ↑	15 ↑	0.7	2	-	-
ceftazidime + tobramycin	-	-	-	-	-	-	0.3	0.0
Multidrug resistance								
MDOT[2]	6	9	6	12 ↑	2	5 ↑	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[2] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, and *P. mirabilis*). For more details see section 5.1.1.1.

Figure 5.1.3.5.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

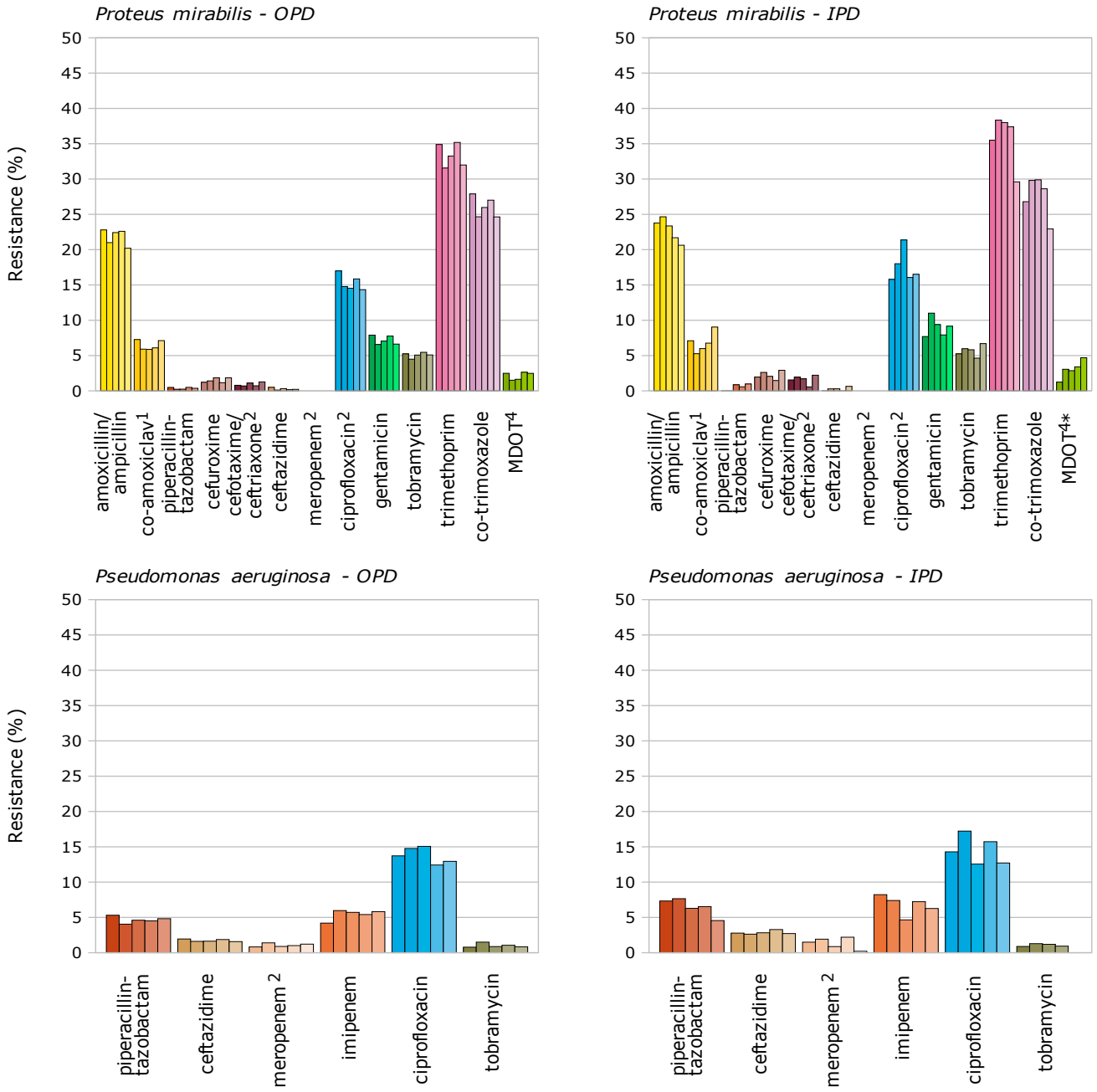
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.

[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.

[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.

[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Figure 5.1.3.5.1 (continued) *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR*



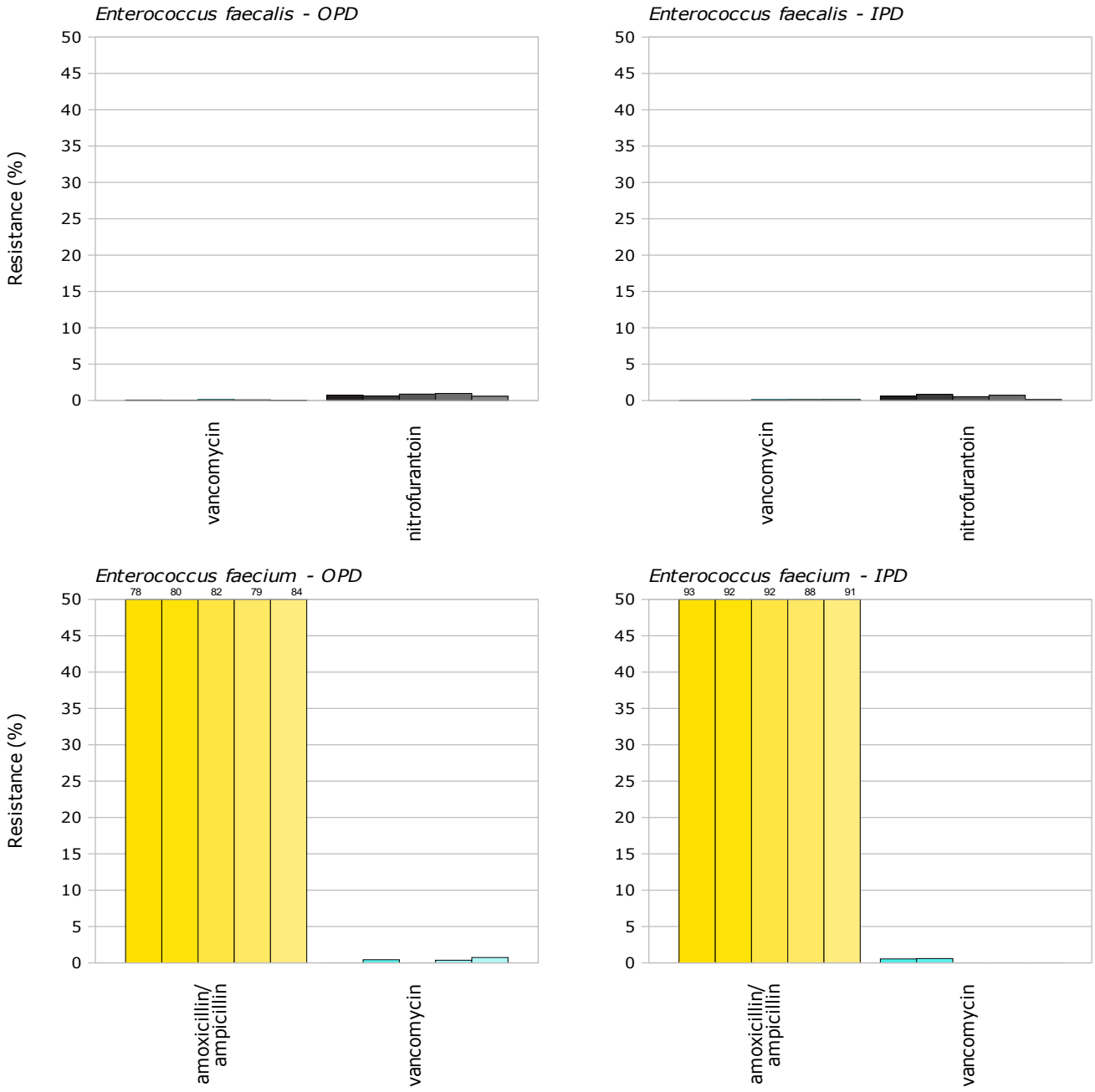
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

Table 5.1.3.5.3 *Resistance levels (%) among diagnostic urine isolates of E. faecalis and E. faecium from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2024*

	E. faecalis		***E. faecium***	
	OPD	**IPD**	**OPD**	**IPD**
Antibiotic				
amoxicillin/ampicillin	-	-	84	91
vancomycin	0.0	0.1	0.7	0.0
nitrofurantoin	0.6	0.1	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.1.3.5.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. faecalis and E. faecium from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

Key results
Urology services

Urine: Enterobacterales and P. aeruginosa

- Resistance levels in Enterobacterales and *P. aeruginosa* from patients in urology services traditionally have been higher than in non-urology patients.
- Within urology services, resistance levels were higher in isolates from patients that were admitted compared to patients seen in OPD.
- Resistance to **ciprofloxacin** (23%) and **co-trimoxazole** (28%) in *E. coli* from admitted patients remains a problem.
- A worrisome increase in resistance to **almost all antibiotics** was seen for *K. pneumoniae* from admitted patients over the last five years: resistance to **ciprofloxacin** and **co-trimoxazole** increased to 24% and 22%; resistance to **cefuroxime** increased to 23% and **cefotaxime/ceftriaxone** to 19%; resistance to **meropenem/imipenem** increased to 1.1% and **gentamicin** to 10%.
- Resistance to **ciprofloxacin** in *P. aeruginosa* in admitted patients has increased 13% over the last five years. This is a problem as **ciprofloxacin** is the only available oral agent to treat *P. aeruginosa* infections.

5.1.4 Long-term care facilities

The distribution of pathogens in diagnostic urine and wound or pus samples from residents of long-term care facilities (LTCF) in 2024 is presented in table 5.1.4.1. The resistance levels in 2024 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 5.1.4.2 and for *S. aureus* isolates from wound or pus samples in table 5.1.4.3. Five-year trends in resistance are shown in figures 5.1.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), and 5.1.4.2 (*S. aureus*).

In 2018 a new sampling guideline for urinary tract infections was implemented in LTCFs, in which urinary culture and susceptibility testing is advised always when a urine dipstick test is positive. Although the indications to take a urine dipstick test were narrowed down, this may have resulted in lower resistance levels than before implementation of the guideline, when urine culture was only advised in case of treatment failure. Because it is not clear whether all LTCFs have adopted the new guideline fully and at the same time, resistance percentages may still be somewhat higher than those for all residents with urinary tract infections caused by Enterobacterales or *P. aeruginosa*, and falsely decreasing time trends may be found. Therefore, the trends in resistance for these infections should be interpreted with caution.

LTCFs usually send wound or pus samples for culture and susceptibility testing in case of antimicrobial therapy failure. As a result, the presented resistance levels are likely to be higher than those for all residents with wound infections or pus caused by *S. aureus* presenting in LTCFs.

Because the urinary, wound, or pus infections that were cultured may be a selection of all infections in LTCF residents, residents from whom samples were taken are hereafter referred to as ‘selected residents of long-term care facilities’.

Table 5.1.4.1 *Distribution of isolated pathogens in diagnostic urine and wound or pus samples from selected residents of long-term care facilities, ISIS-AR 2024*

	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**
E. coli	11,013 (42)	154 (6)
K. pneumoniae	2,682 (10)	53 (2)
P. mirabilis	2,797 (11)	213 (9)
Other Enterobacterales[1]	3,041 (12)	224 (9)
P. aeruginosa	1,413 (5)	301 (12)
Other non-fermenters[2]	185 (1)	43 (2)
Other Gram-negatives[3]	0 (0)	22 (1)
S. aureus	885 (3)	1,113 (45)
Other Gram-positives[4]	4,186 (16)	358 (14)

[1] In order of frequency: *Klebsiella* spp. (non-*pneumoniae*), *Citrobacter* spp., *Enterobacter* spp., *Morganella* spp., *Proteus* spp. (non-*mirabilis*), *Serratia* spp., *Providencia* spp., *Raoultella* spp., *Pantoea* spp., *Escherichia* spp. (non-*coli*), *Hafnia* spp., *Salmonella* spp., *Cronobacter* spp.
[2] In order of frequency: *Acinetobacter* spp., *Pseudomonas* spp. (non-*aeruginosa*), *S. maltophilia*, *M. catarrhalis*.
[3] In order of frequency: *B. fragilis*, *H. influenzae*.
[4] In order of frequency: *Enterococcus* spp., *A. urinae*, *S. constellatus*, *S. dysgalactiae* subsp. *equisimilis*, *S. intermedius*, *ß-haemolytische Streptokokken groep A n.n.g.*, *S. anginosus*, *ß-haemolytische Streptokokken groep G n.n.g.*, *S. equi*, *Streptococcus anginosus groep n.n.g.*, *S. oralis*, *Streptococcus mitis/oralis n.n.g.*, *S. agalactiae*, *streptococcus dysgalactiae subsp. dysgalactiae*, *S. dysgalactiae* n.n.g., *ß-haemolytische Streptokokken groep C n.n.g.*, *S. canis*, *S. pyogenes*, *Streptococcus mitis groep n.n.g.*, *S. pneumoniae*, *Staphylococcus* spp. (non-*aureus*), *C. perfringens*.

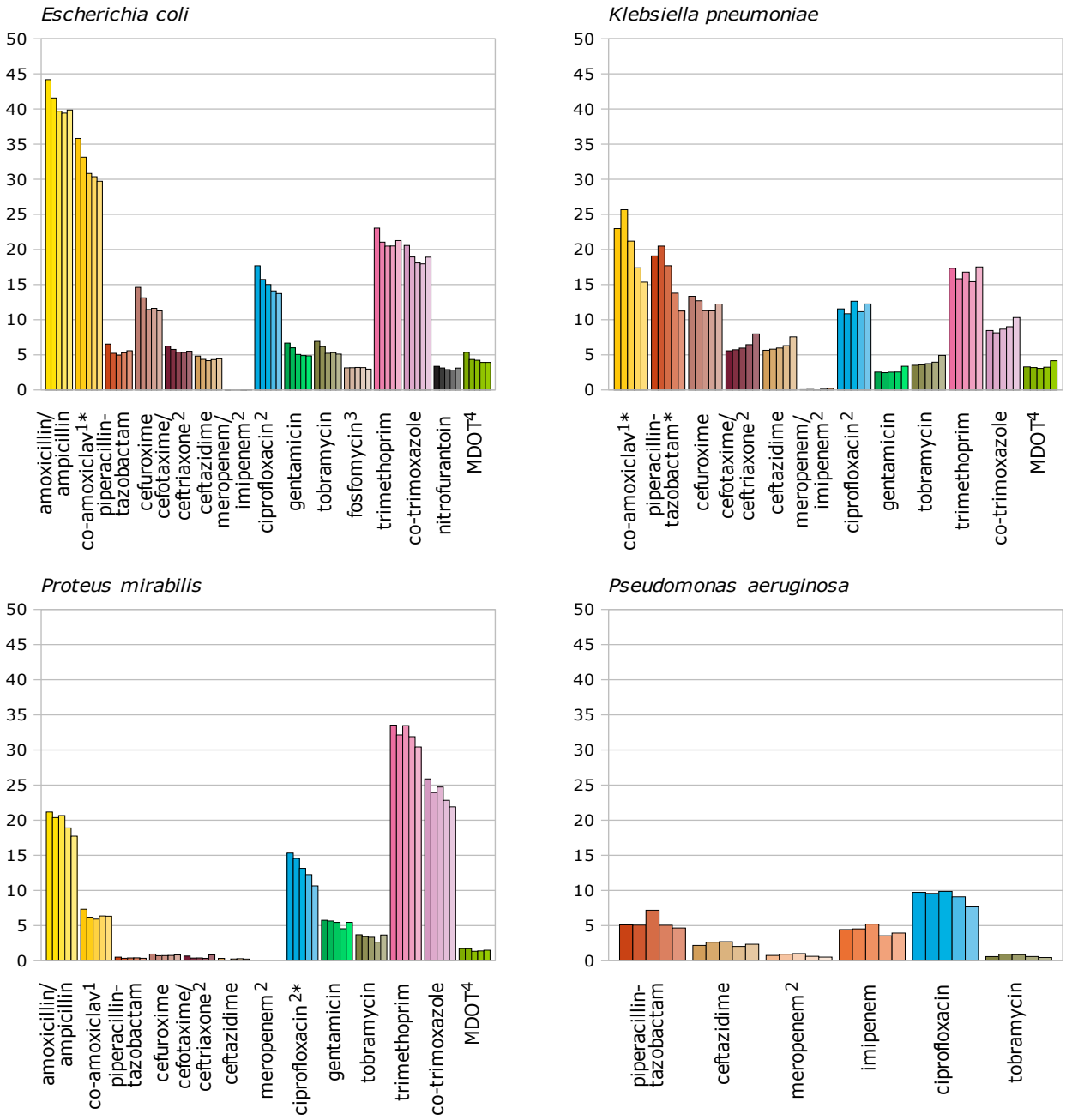
Table 5.1.4.2 *Resistance levels (%) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected residents of long-term care facilities, ISIS-AR 2024*

	E. coli	***K. pneumoniae***	***P. mirabilis***	***P. aeruginosa***
Antibiotic				
amoxicillin/ampicillin	40	-	18	-
co-amoxiclav[a]	30 ↓	15 ↓	6	-
piperacillin-tazobactam	6	11 ↓	0.4	5
cefuroxime	11	12	0.8	-
cefotaxime/ceftriaxone[b]	6	8	0.8	-
ceftazidime	4	8	0.2	2
meropenem/imipenem[b]	0.0	0.2	-	-
meropenem[b]	-	-	0.0	0.5
imipenem	-	-	-	4
ciprofloxacin[b]	14	12	11 ↓	8
gentamicin	5	3	5	-
tobramycin	5	5	4	0.5
fosfomycin[1]	3	-	-	-
trimethoprim	21	18	30	-
co-trimoxazole	19	10	22	-
nitrofurantoin	3	-	-	-
Multidrug resistance				
MDOT[2]	4	4	1.5	-

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[2] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.
[a] According to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration. For more details see section 5.1.1.1.
[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *E. coli*, *K. pneumoniae*, and *P. mirabilis*). For more details see section 5.1.1.1.

Figure 5.1.4.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic urine isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected residents of long-term care facilities in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] According to breakpoint for oral treatment of infections originating from the urinary tract. For more details see section 5.1.1.1.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance percentage calculated using an MIC cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 5.1.1.1.
[4] MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract or for intravenous administration), ciprofloxacin (according to breakpoint for indications other than meningitis), and co-trimoxazole.

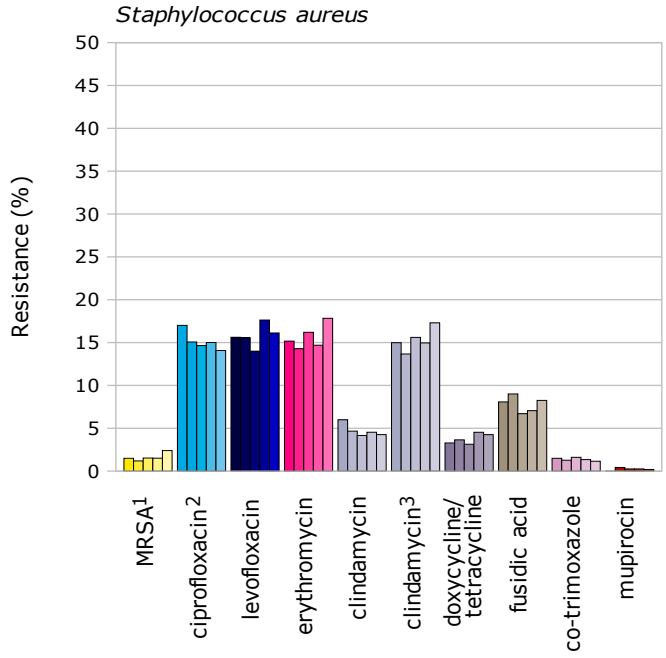
Table 5.1.4.3 *Resistance levels (%) among diagnostic wound or pus isolates of S. aureus from selected residents of long-term care facilities, ISIS-AR 2024*

	S. aureus
Antibiotic	
MRSA[1]	2
ciprofloxacin[2]	14
levofloxacin	16
erythromycin	18
clindamycin	4
clindamycin (including inducible resistance)[3]	17
doxycycline/tetracycline	4
fusidic acid	8
co-trimoxazole	1.2
mupirocine	0.2

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.
[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[3] For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Figure 5.1.4.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic wound or pus isolates of S. aureus from selected residents of long-term care facilities in ISIS-AR*



Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

[1] MRSA = Methicillin resistant *S. aureus*. For the estimation method of MRSA see section 5.1.1.1.; Within the *S. aureus* complex 0 out of 1 *S. argenteus* and 0 out of 0 *S. schweitzeri* were methicillin resistant.

[2] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.

[3] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.

Key results
Long-term care facilities

Urine: Enterobacterales and P. aeruginosa

- Resistance levels in *E. coli*, *K. pneumoniae* and *P. aeruginosa* urine isolates from selected LTCF residents were higher than resistance levels in selected GP patients and comparable to resistance levels in OPD and hospital patients.
- Resistance levels for **nitrofurantoin** and **fosfomycin** in *E. coli*, first and second choice antibiotics for the treatment of uncomplicated UTI in adults, were low (both 3%).
- Resistance levels for **ciprofloxacin**, first choice antibiotic for the empiric oral treatment of complicated UTI in adults, was 14% in *E. coli*, 12% in *K. pneumoniae* and 8% in *P. aeruginosa*. Resistance levels for **co-amoxiclav**, second choice antibiotic for the treatment of complicated UTI significantly decreased to 30% in *E. coli* and to 15% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third choice antibiotic for this indication, was 19% in *E. coli* and 10% in *K. pneumoniae*. **Combined resistance** for co-amoxiclav, ciprofloxacin, and co-trimoxazole in all Enterobacterales was low (≤4%).

Wound/pus: S. aureus

- Resistance levels in *S. aureus* isolates from selected LTCF residents were higher than resistance levels in selected GP patients and comparable to resistance levels in OPD and hospital patients, with the exception of resistance to **levofloxacin** (16%), which was much higher in *S. aureus* from selected LTCF residents than in *S. aureus* from OPD (3%), hospital (3%) and ICU patients (2%), which might be the result of either selective sampling or intensive use of fluoroquinolones in this setting.

5.1.5 Respiratory pathogens

The distribution of pathogens isolated from diagnostic lower and upper respiratory tract samples from general practitioners' (GP) patients and from diagnostic blood or cerebrospinal fluid, and lower and upper respiratory tract samples from hospital patients (outpatients and inpatients, including intensive care patients) in 2024 is presented in table 5.1.5.1. Resistance levels for respiratory pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) in 2024 are presented by patient group in table 5.1.5.2. Five-year trends in resistance are shown in figure 5.1.5.1.

Although patients from general practitioners are assumed to be representative of the community with respect to resistance levels of pathogens, in accordance with the NHG guidelines, general practitioners do not routinely take a sample when respiratory tract infection is suspected. Therefore, the results may be biased towards higher resistance levels due to overrepresentation of more severe or recurrent cases of respiratory tract infections.

In hospitals in the Netherlands, according to the guidelines a sample should be taken for routine diagnostic purposes when lower respiratory tract infection is suspected. Although often it is not possible to take a sample because a patient does not produce sputum, it is not expected that this is correlated to resistance, and selective sampling bias is expected to be small. Nevertheless, resistance levels in hospital patients may be higher than in the community, as hospital patients are likely to be more severely ill and patients with previous treatment failure, chronic obstructive pulmonary diseases (COPD), and cystic fibrosis (CF) may be overrepresented.

Table 5.1.5.1 *Distribution of isolated pathogens in diagnostic respiratory samples from general practitioners' patients (GP) and in diagnostic blood or cerebrospinal fluid and respiratory samples from hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2024*

	GP		Hospital departments		
	Lower respiratory tract	**Upper respiratory tract**	**Blood or cerebrospinal fluid**	**Lower respiratory tract**	**Upper respiratory tract**
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
S. pneumoniae	209 (8)	12 (0)	1,714 (4)	2,552 (8)	177 (2)
Other Gram-positives[1]	306 (11)	2,246 (86)	21,304 (55)	5,155 (16)	4,767 (62)
H. influenzae	1,076 (40)	73 (3)	259 (1)	9,815 (30)	689 (9)
M. catarrhalis	291 (11)	39 (1)	32 (0)	2,636 (8)	214 (3)
Other non-fermenters[2]	357 (13)	31 (1)	1,207 (3)	4,884 (15)	481 (6)
Enterobacterales[3]	396 (15)	204 (8)	13,358 (35)	6,796 (21)	1,298 (17)
Other Gram-negatives[4]	47 (2)	9 (0)	585 (2)	697 (2)	82 (1)

[1] In order of frequency: *Staphylococcus* spp., *Streptococcus mitis/oralis n.n.g.*, *S. australis*, *ß-haemolytische Streptokokken groep C n.n.g.*, *S. mitis*, *S. agalactiae*, *S. peroris*, *S. dysgalactiae* n.n.g., *streptococcus dysgalactiae subsp. dysgalactiae*, *S. constellatus*, *S. cristatus*, *S. infantis*, *Streptococcus mitis groep n.n.g.*, *S. intermedius*, *S. gordonii*, *S. oralis*, *S. anginosus*, *S. pyogenes*, *ß-haemolytische Streptokokken groep A n.n.g.*, *Streptococcus sanguinis groep n.n.g.*, *S. canis*, *S. dysgalactiae* subsp. *equisimilis*, *ß-haemolytische Streptokokken groep G n.n.g.*, *Streptococcus anginosus groep n.n.g.*, *S. equi*, *Enterococcus* spp., *A. urinae*, *C. perfringens*, *L. monocytogenes*.
[2] In order of frequency: *Pseudomonas* spp., *S. maltophilia*, *Acinetobacter* spp., *B. cepacia*.
[3] In order of frequency: *Escherichia* spp., *Klebsiella* spp., *Serratia* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Morganella* spp., *Raoultella* spp., *Salmonella* spp., *Pantoea* spp., *Hafnia* spp., *Providencia* spp., *Yersinia* spp., *Mixta* spp., *Shigella* spp., *Cronobacter* spp.
[4] In order of frequency: *H. parainfluenzae*, *B. fragilis complex*, *B. fragilis*, *N. meningitidis*, *C. coli*, *C. lari*, *C. jejuni*.

Table 5.1.5.2 *Resistance levels (%) among diagnostic isolates of S. pneumoniae, H. influenzae, and M. catarrhalis from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2024*

	S. pneumoniae		***H. influenzae***		***M. catarrhalis***	
	GP	**Hospital**	**GP**	**Hospital**	**GP**	**Hospital**
Antibiotic						
(benzyl)penicillin (R)[1]	0.0	0.3	-	-	-	-
(benzyl)penicillin (I)[1]	9	9 ↑	-	-	-	-
amoxicillin/ampicillin[1,a]	-	-	29	28	-	-
co-amoxiclav[1,a]	-	-	11	12	0.7	1
cefuroxime[1,a]	-	-	7	14 ↑	-	-
cefotaxime/ceftriaxone[1,b]	0.0	0.0	-	-	-	-
ciprofloxacin[b]	-	-	1.6 ↓	3	4	7°
levofloxacin/moxifloxacin[2]	0.0	0.5	-	-	-	-
erythromycin	17	9	-	-	9	8
doxycycline/tetracycline	13	10	1.7	2	0.4	1,6
co-trimoxazole	10	10	21	21	5	8 ↑

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

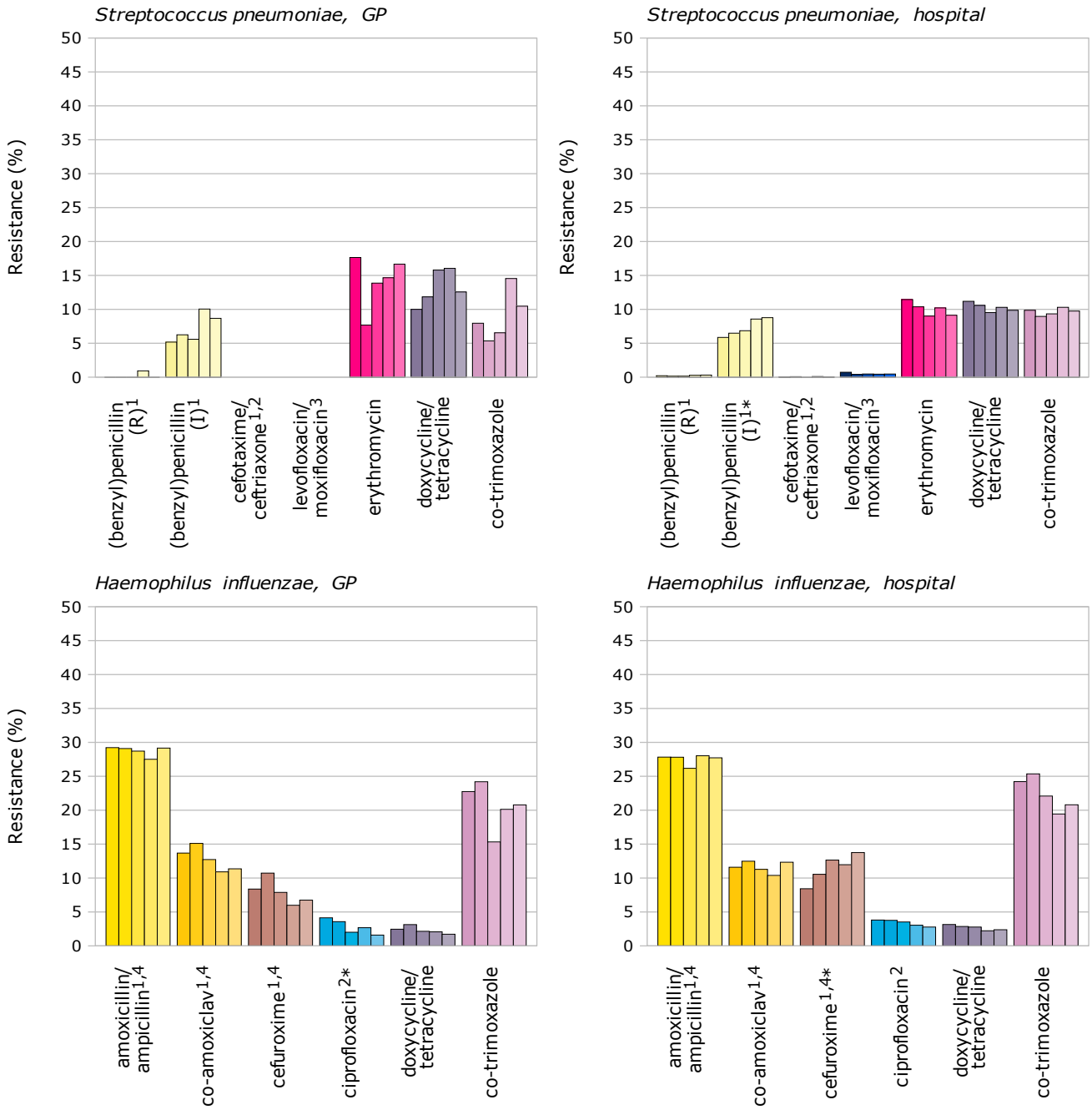
[1] Resistance to beta-lactam antibiotics in *S. pneumoniae* and *H. influenzae* was calculated based on a flow chart according to directions in the EUCAST guidelines for indications other than meningitis (for details see section 5.1.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in *S. pneumoniae* (for details see section 5.1.1.1). Resistance percentages may therefore be biased toward a lower level.

[2] Resistance to levofloxacin/moxifloxacin in *S. pneumoniae* was calculated based on norfloxacin and levofloxacin/moxifloxacin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

[a] According to breakpoint for intravenous administrations (for co-amoxiclav this only applies to *H. influenzae*). For more details see section 5.1.1.1.

[b] According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to *H. influenzae*). For more details see section 5.1.1.1.

Figure 5.1.5.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of S. pneumoniae, H. influenzae, and M. catarrhalis from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).

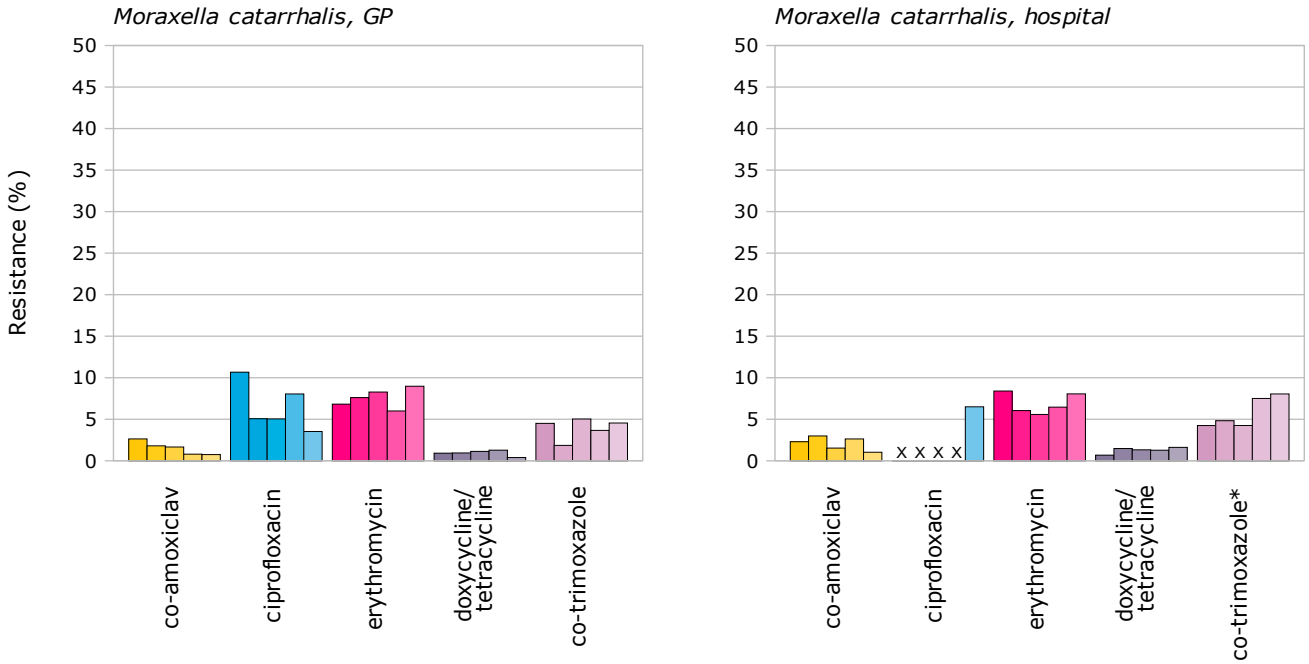
[1] Resistance to beta-lactam antibiotics in *S. pneumoniae* and *H. influenzae* was calculated based on a flow chart according to directions in the EUCAST guidelines for indications other than meningitis (for details see section 5.1.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in *S. pneumoniae* (for details see section 5.1.1.1). Resistance percentages may therefore be biased toward a lower level.

[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.

[3] Resistance to levofloxacin/moxifloxacin in *S. pneumoniae* was calculated based on norfloxacin and levofloxacin/moxifloxacin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.

[4] According to breakpoint for intravenous administration. For more details see section 5.1.1.1.

Figure 5.1.5.1 (continued)*Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic isolates of S. pneumoniae, H. influenzae, and M. catarrhalis from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units) in ISIS-AR*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Resistance to beta-lactam antibiotics in *S. pneumoniae* and *H. influenzae* was calculated based on a flow chart according to directions in the EUCAST guidelines for indications other than meningitis (for details see section 5.1.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in *S. pneumoniae* (for details see section 5.1.1.1). Resistance percentages may therefore be biased toward a lower level.
[2] According to breakpoint for indications other than meningitis. For more details see section 5.1.1.1.
[3] Resistance to levofloxacin/moxifloxacin in *S. pneumoniae* was calculated based on norfloxacin and levofloxacin/moxifloxacin according to directions in the EUCAST guidelines. For details see section 5.1.1.1.
[4] According to breakpoint for intravenous administration. For more details see section 5.1.1.1.

Key results
Respiratory pathogens

S. pneumoniae

- The proportion of *S. pneumoniae* isolates in the I-category for **(benzyl)penicillin** appears to be increasing and significantly in hospital patients to 9%.
- Resistance to **doxycycline/tetracycline** in *S. pneumoniae* was 13% for GP and 10% for hospital isolates.
- Resistance to **erythromycin** in *S. pneumoniae* was 17% for GP and and 9% for hospital isolates.

H. influenzae

- resistance to **co-amoxiclav** in H. influenzae isolates from hospital patients remained stable at 12%. However, resistance to **cefuroxime** increased significantly over the last five years to 14%

5.2 Specific AMR surveillance

5.2.1 Extended spectrum β-lactamases

Introduction

Extended spectrum β-lactamase producing Enterobacterales (ESBL-E) have become a major concern worldwide. The prevalence of ESBL-E carriage has become quite widespread, also in the WHO European Region.[1] The percentage of ESBLs among clinical isolates of Enterobacterales in the Netherlands was estimated for the period 2020-2024 using the ISIS-AR database. Here we present data from ISIS-AR for *Escherichia coli* and *Klebsiella pneumoniae*.

Methods

Data were extracted from the ISIS-AR database. The percentages of ESBL-producing *E. coli* and *K. pneumoniae* were estimated based on positivity of confirmation tests (available >99% of the ESBL positive isolates), or, if data from these tests were lacking, inferred from the MICs for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime) based on the EUCAST 2024 clinical resistance breakpoints.

Results

In table 5.2.1.1 and 5.2.1.2, the estimated percentages of ESBL-carrying *E. coli* and *K. pneumoniae* are shown by healthcare setting or department, i.e. general practice (GP), outpatient departments, inpatient departments, and intensive care units (ICUs), in 2024. In figure 5.2.1.1, trends in ESBL percentages (from left to right 2020 to 2024) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown.

Overall, the percentages of ESBL for *E. coli* and *K. pneumoniae* seem to increase during the previous five years, with a microbiologically relevant increasing trend in ESBL *K. pneumoniae* in patients cultured at general practitioners and outpatient departments in hospitals. The ESBL percentages in both *E. coli* and *K. pneumoniae* from diagnostic ICU isolates remain high in the previous five years, up to 10% in *E. coli* and 15% in *K. pneumoniae* in 2024.

Table 5.2.1.1 *Extended spectrum β-lactamase (ESBL) producing E. coli in the Netherlands in 2024 in diagnostic isolates, based on ISIS-AR data*

Type of department	Tested isolates, N	ESBL, number (%)[1]
GP	147,220	6,322 (4)
Outpatient departments	27,519	1,751 (6)
Inpatient departments excl. intensive care units	36,357	2,562 (7)
Intensive care units	1,435	143 (10)
Total	**212,531**	**10,778 (5)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

[1] The percentage of ESBL producing *E. coli* was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 2 mg/L for both cefotaxime and ceftriaxone or 17 mm for cefotaxime and 22 mm for ceftriaxone) and/or ceftazidime (according to a cut-off of 4 mg/L or 19 mm), based on re-interpretation of testvalues according to EUCAST 2024

Table 5.2.1.2 *Extended spectrum β-lactamase (ESBL) producing K. pneumoniae in the Netherlands in 2024 in diagnostic isolates, based on ISIS-AR data*

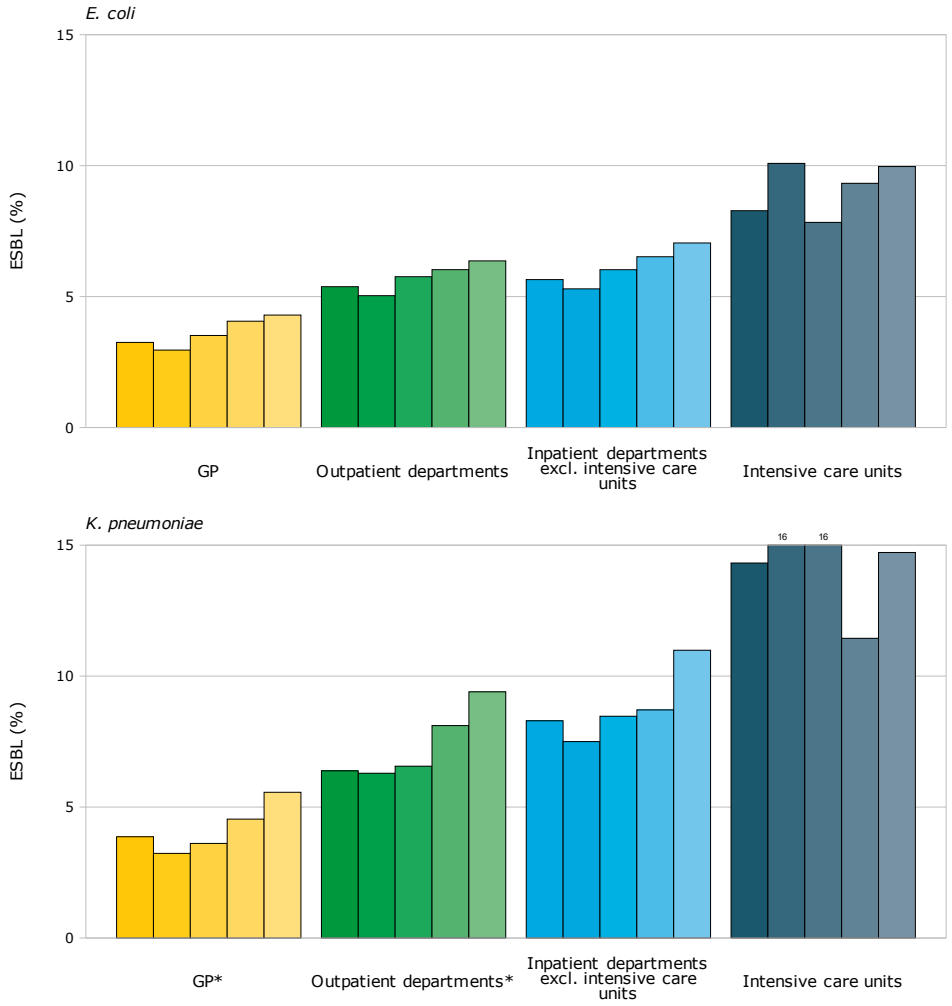
Type of department	Tested isolates, N	ESBL, number (%)[1]
GP	21,583	1,201 (6) ↑
Outpatient departments	6,426	604 (9) ↑
Inpatient departments excl. intensive care units	7,645	840 (11)
Intensive care units	394	58 (15)
Total	**36,048**	**2,703 (7)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *K. pneumoniae* isolate per patient was selected.

[1] The percentage of ESBL producing *K. pneumoniae* was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 2 mg/L for both cefotaxime and ceftriaxone or 17 mm for cefotaxime and 22 mm for ceftriaxone) and/or ceftazidime (according to a cut-off of 4 mg/L or 19 mm), based on re-interpretation of testvalues according to EUCAST 2024

Figure 5.2.1.1 *Trends in extended spectrum β-lactamase (ESBL) producing E. coli and K. pneumoniae in the Netherlands (from left to right 2020 to 2024), based on ISIS-AR data*



Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *E. coli* and *K. pneumoniae* isolate per patient per year was selected.
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] The percentage of ESBL producing *E. coli* and *K. pneumoniae* was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 2 mg/L for both cefotaxime and ceftriaxone or 17 mm for cefotaxime and 22 mm for ceftriaxone) and/or ceftazidime (according to a cut-off of 4 mg/L or 19 mm), based on re-interpretation of testvalues according to EUCAST 2024

Discussion

Over the past 5 years, ESBL-producing *K. pneumoniae* showed a significantly increasing trend in GP and outpatient department populations. During the COVID-years (2020 and 2021), the percentages of both ESBL-producing *K. pneumoniae* and *E. coli* had been lower, consequently resulting in a rise in resistance after the COVID-period in all healthcare departments. However, looking at the 10-year-trends, the percentages of ESBL-positive *E. coli* and *K. pneumoniae* in 2024 are up or above the percentages in the pre-COVID-years in all types of healthcare departments. The percentage ESBL-producing *K. pneumoniae* in the inpatients department population shows a peak in 2024, which is for the first time above 10%. This increase can partly be explained by the increased detection of a single MLST sequence type (ST). Several

hospitals have reported the emergence of an ESBL-producing *K. pneumoniae* ST15, that is also resistant to aminoglycosides, which jeopardizes empirical antibiotic policy in sepsis patients. The pathways of expansion of this type are not fully clear. Molecular surveillance on ESBL-producing *K. pneumoniae* is being performed by several institutions based on local or regional initiatives. However, there is no structural national surveillance covering both molecular and patient related data to monitor high-risk lineages, identify transmission pathways, and set out infection control interventions to stop the transmission.
In 2024, the "Bijzonder resistente micro-organismen (BRMO) | SRI-richtlijnen" have been published. These guidelines state that, given the limited treatment options, the nosocomial spread of ESBL-producing *K. pneumoniae* is undesirable. In the event of an unexpected finding, considering source and contact investigation is recommended. The increasing trend in 2024 shows the urgency for infection control interventions to preserve the effectiveness of third generation cephalosporins as empiric treatment of sepsis by *K. pneumoniae*.

Conclusions

- From 2020-2024, there has been a significant increase in ESBL-producing *K. pneumoniae* in GP and outpatient department populations.
- ESBL-producing *K. pneumoniae* percentages showed for the first time a peak above 10% in the inpatient department population.
- In the 10-year-trends, the percentages of ESBL-producing *K. pneumoniae* in all healthcare departments are up or above the levels in the pre-COVID-years.
- ESBL-producing *E. coli* percentages are slightly increasing from 2020-2024 and percentages in 2024 are also higher compared to the levels in the pre-COVID-years (data not shown).

References

1. Surveillance of antimicrobial resistance in Europe, https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2023-data-executive-summary.

5.2.2 *Carbapenem-resistant and carbapenemase-producing Enterobacterales*

Introduction

Carbapenem-resistant Enterobacterales (CRE) and carbapenemase-producing Enterobacterales (CPE) have been reported all over the world. Because carbapenems represent a group of antibiotics of last resort for treatment of many bacterial infections, resistance poses a significant challenge to clinicians and negatively impacts patient care.[1] In Europe, CRE were first described in the early 2000s and their prevalence has increased since then.[2] The current epidemiology in Europe varies from sporadic imported cases, to sporadic hospital outbreaks, to (inter-) regional spread between hospitals, to CRE being endemic in healthcare settings.[3] In the Netherlands, CRE are mainly found in hospitalised patients so far, but community-spread has been described. CRE are therefore considered a growing public health threat.[4]
Measured prevalence of CRE is influenced by test procedures and methods. Up to 2021, the Dutch national guideline suggested a gradient strip test as the first step in further investigation of isolates with an elevated MIC based on automated tests.[5] However, the guideline has been adapted in 2021 and now suggests to directly perform tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic) when further investigation is necessary.[6]
This chapter describes the prevalence of CRE/CPE, (molecular) epidemiology and outbreaks of CPE in the Netherlands.

Methods

Data on CRE/CPE were obtained from the ISIS-AR and the Type-Ned databases, mandatory notifications in OSIRIS, and outbreaks reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR).[7,8]

Prevalence of carbapenem-resistant and carbapenemase-producing Enterobacterales based on ISIS-AR

These analyses focus on all Enterobacterales, divided into 4 categories: *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter cloacae* complex and all other Enterobacterales species. The category *E. cloacae* complex in the ISIS-AR database contains the following species: *E. cloacae*, *E. hormaechei*, *E. asburiae*, *E. cancerogenus*, *E. kobei*, *E. roggenkampii*, and *E. cloacae* complex not further specified. We searched the ISIS-AR database (years 2020-2024) for diagnostic (infection-related) isolates that were tested for meropenem and/or imipenem by an automated system and/or gradient strip test. For *Proteus* spp., *Providencia* spp., *Serratia* spp. and *Morganella morganii*, only meropenem test results were included and analysed because of intrinsic low activity of imipenem against these species.[6] If results from an automated system and a gradient strip test were available then the result of the gradient strip was used.

Several breakpoints are used in this chapter: i) the screening breakpoint as defined by the Dutch national guideline6 (which is >0.25 mg/L for meropenem and >1 mg/L for imipenem), and ii) the clinical breakpoints according to EUCAST, namely the clinical susceptible (S, which is ≤2 mg/L for both imipenem and meropenem) and the clinical resistant (R) breakpoint, which is >8 mg/L for meropenem and >4 mg/L for

imipenem. Based on the crude automated and gradient strip test values, we categorized them as having either an:

i) MIC ≤ the screening breakpoint,
ii) MIC > the screening breakpoint and ≤ the EUCAST clinical S breakpoint,
iii) MIC > the clinical S breakpoint and ≤ the clinical R breakpoint (the clinical I category, i.e., susceptible with Increased dosing or exposure), or
iv) MIC > the clinical R breakpoint (also referred to as CRE).

Categories ii, iii and iv are together referred to as elevated MIC. If meropenem and imipenem were tested then the antibiotic with the highest category was used.

Subsequently, for all isolates, we searched the ISIS-AR and Type-Ned database for data on confirmatory tests (i.e., tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic)). We included only one isolate per patient per bacterial species or complex per year. An isolate with data on confirmatory tests (further referred to as CPE determined by positive phenotypic and/or genotypic testing confirmed) was prioritized over an isolate with only an automated test and/or gradient strip test result. If, subsequently, multiple isolates were eligible for inclusion, we prioritized the most resistant isolate. We calculated numbers of isolates with automated and/or gradient strip MIC in the respective categories in 2024, the number of isolates with available data on confirmatory tests and the number of isolates that were CPE confirmed per micro-organism species or complex. Based on data from 36 laboratories that continuously submitted data to ISIS-AR from 2020 to 2024, we assessed the annual percentage of isolates i) with an elevated MIC based on automated testing and/or gradient strip testing, and ii) categorized as CRE, and describe trends over time.

Molecular characteristics of carbapenemase-producing Enterobacterales and patient related characteristics based on Type-Ned
For the enhanced surveillance of CPE via Type-Ned, all -except one- Dutch medical microbiology laboratories participate (n=46). Dutch laboratories are requested to submit screening or diagnostic Enterobacterales isolates to the RIVM with a positive confirmatory test for carbapenemase production and/or a detected carbapenemase-encoding gene. A restriction is that the laboratory can only send the first species/carbapenemase gene combination per person per year. The RIVM allows consecutive isolates from the same person if these are Enterobacterales species with other carbapenemase-encoding gene combinations when compared to the first isolate. The RIVM confirms the species by MALDI-ToF, determines the MIC for meropenem by Etest, and tests for carbapenemase production by the carbapenem inactivation method (CIM).[9] The presence of carbapenemase-encoding genes (carba-PCR on *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, bla$_{VIM}$, and *bla*$_{OXA-48}$-like) and mobile colistin resistance gene (*mcr*-1) are assessed by PCR. Next-generation sequencing (NGS) and Nanopore long-read sequencing is performed for all isolates that are CIM positive to describe trends in molecular epidemiology and to identify genetic clusters.[10] The data described in this chapter are based on the first unique CIM-positive Enterobacterales

species/carbapenemase-encoding allele combination per person for the period 2020-2024.

Since 2022, due to the Russia-Ukraine war, a substantial number of CPE isolates were cultured from patients originating from Ukraine, who had migrated or been evacuated to the Netherlands. Normally, these isolates would be excluded from further analysis because of the lack of a person ID (encrypted BSN). However, in the current analysis CPE from persons without a person ID were included for NGS and further analysis if it represented a unique person, based on gender, age and postal code and when country of origin was Ukraine and/or if Ukrainian origin was confirmed by the MML.

Whole-genome multi-locus sequence typing (wgMLST) was used to detect genetic clusters consisting of genetically highly related *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *Citrobacter freundii* complex isolates and clusters are systematically assigned consecutive cluster numbers. A genetic cluster is defined per bacterial species and includes ≥2 highly related isolates of one species that differ typically ≤20 wgMLST alleles (25 for *E. coli*).

Genetic clusters based on Type-Ned
Assigning genetic clusters started in 2018 and all sequenced isolates available from the national surveillance since 2014 were included in the wgMLST cluster analysis. Clusters solely consisting of multiple isolates from the same patient, included over different years and/or submitted by different laboratories, were not counted. Since the end of 2019, genetic clusters for CPE are reported to the submitting laboratory in Type-Ned in order control further spread.

Clinical/epidemiological characteristics of persons with carbapenemase-producing Enterobacterales based on mandatory notifications in OSIRIS
From 1 July 2019 onwards, CPE, either phenotypically or genotypically confirmed, is mandatorily notifiable on person level (not on isolate level). Since then, epidemiological patient data are reported by Municipal Health Services (MHS) via the national web-based system for notifiable diseases (OSIRIS). Notifications with a sampling date between 1 January and 31 December 2024 that are approved by the RIVM are included in this chapter, while for trends approved notifications with a sampling date between 1 January 2020 and 31 December 2024 are included. Notifications are stratified into persons with diagnostic and screening isolates, as reported by de MHS.

Outbreaks based on SO-ZI/AMR
The SO-ZI/AMR database (see chapter 5.2.7 for more details) was interrogated for CPE outbreaks that were reported in 2024.[8]

Results

Prevalence of carbapenem-resistant and carbapenemase-producing Enterobacterales based on ISIS-AR

Absolute numbers of isolates and categorization according to automated and gradient strip MICs in 2024 are presented in Table 5.2.2.1. Of a total number of 271,618 isolates with an automated test or gradient strip test value for meropenem or imipenem (175,458 *E. coli,* 29,048 *K. pneumoniae,* 9,907 *E. cloacae* complex*,* and 57,205 other Enterobacterales species), an elevated MIC was found in 2.0% of isolates (5,544). CPE confirmed isolates with an elevated MIC were mostly found in *K. pneumoniae* (81/340, 23.8% of isolates with an elevated MIC), followed by *E. coli* (72/512, 14.1%), *E. cloacae* complex (10/305, 3.3%) and other Enterobacterales (30/4,387, 0.7%). Most CPE confirmation tests were performed for species in the category *E. cloacae* complex (1.4% of all isolates in the category), followed by other Enterobacterales (0.9%), *K. pneumoniae* (0.5%), and *E. coli* (0.1%).

Figure 5.2.2.1 shows the percentage of isolates with an elevated MIC and the percentage of isolates defined as CRE of the past 5 years. The overall prevalence of carbapenem resistant *E. coli* strains slightly increased between 2020 and 2024 from 0.00% to 0.01% (Figure 5.2.2.1a). For *K. pneumoniae*, the prevalence of carbapenem resistant strains increased between 2020 and 2024 from 0.04% to 0.21% (Figure 5.2.2.1a).

As for *E. cloacae* complex, the overall prevalence of carbapenem resistant strains has fluctuated between 2020 and 2024 around an average of 0.09% (Figure 5.2.2.1b). For the other Enterobacterales species, the prevalence of carbapenem resistant strains increased between 2020 and 2024 from 0.01% to 0.04% (Figure 5.2.2.1b).

Molecular characteristics of carbapenemase-producing Enterobacterales and patient related characteristics based on Type-Ned

Carbapenemase-production was confirmed in 696 Enterobacterales isolates (unique species/carbapenemase allele combinations per person) obtained in 2024 from 573 patients with and 123 without an encrypted person ID. Forty-seven (38%, 47/123) unique patients without a person ID were from Ukraine, and the remainder patients without person ID had unknown or variable countries of origin at time of submission. The screening and diagnostic isolates were submitted to the RIVM by 47 Dutch medical microbiology laboratories. The number of unique CPE isolates submitted to the RIVM were 225 and 245 in the COVID-19 years 2020 and 2021, respectively, and increased with 44.3% from 482 in 2022 and 581 in 2023, to 696 in 2024 (Figure 5.2.2.2a). Of the 696 CPE isolates in 2024, 321 (46%) were *E. coli*, 246 (35%) *K. pneumoniae*, 53 (8%) *E. cloacae* complex, and 23 (3%) *C. freundii* complex and the remaining 53 (8%) isolates belonged to other species (Figure 5.2.2.2a). Of the CPE analysed in 2024, 24% (165/696) carried a $bla_{\text{OXA-48}}$-like gene ($bla_{\text{OXA-162}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-232}}$, $bla_{\text{OXA-244}}$, $bla_{\text{OXA-245}}$, $bla_{\text{OXA-484}}$, $bla_{\text{OXA-1205}}$ and $bla_{\text{OXA-1207}}$). The $bla_{\text{NDM-5}}$ gene ranked the second (18%, 123/696) carbapenemase-encoding gene in CPE isolates from 2024,

Table 5.2.2.1 *Results of MIC and confirmatory carbapenem susceptibility testing among diagnostic (infection-related) Enterobacterales isolates in 2024, in 36 laboratories participating in ISIS-AR*

	MIC*				
	MIC <= screening breakpoint **	**MIC > screening** and <= clinical S breakpoint *****	**MIC > clinical S and <= clinical R breakpoint *****	**MIC > clinical R breakpoint *****	**Total**
E. coli					
Total (N)	174.946	437	31	44	175.458
CPE confirmatory test performed (N (% of total))	71(0)	53(12.1)	22(71)	37(84.1)	183(0.1)
CPE confirmed (N(% of total))	17(0)	23(5.3)	16(51.6)	33(75)	89(0.1)
K. pneumoniae					
Total (N)	28.708	236	24	80	29.048
CPE confirmatory test performed (N (% of total))	8(0)	41(17.4)	21(87.5)	67(83.8)	137(0.5)
CPE confirmed (N(% of total))	0(0)	8(3.4)	7(29.2)	66(82.5)	81(0.3)
***E. cloacae* complex**					
Total (N)	9.602	253	37	15	9.907
CPE confirmatory test performed (N (% of total))	41(0.4)	64(25.3)	26(70.3)	10(66.7)	141(1.4)
CPE confirmed (N(% of total))	2(0)	2(0.8)	1(2.7)	7(46.7)	12(0.1)
Other Enterobacterales****					
Total (N)	52.818	4.305	48	34	57.205
CPE confirmatory test performed (N (% of total))	88(0.2)	356(8.3)	31(64.6)	27(79.4)	502(0.9)
CPE confirmed (N(% of total))	0(0)	6(0.1)	8(16.7)	16(47.1)	30(0.1)
All isolates					
Total (N)	266.074	5.231	140	173	271.618

CPE confirmed = confirmation through phenotypical carbapenemase production test and/or genotypical carbapenemase gene test.
* Both automated and gradient strip MIC were used to define the 4 categories. If MICs of both test were available then the gradient strip MIC was used, even if the gradient strip MIC was lower than the automated MIC.
** Screening breakpoint according to NVMM guideline Laboratory detection of highly resistant microorganisms (MIC meropenem > 0.25 mg/L and/or MIC imipenem > 1 mg/L) (published November 2021).
*** Clinical breakpoints according to EUCAST guideline v.14.0.
**** All other Enterobacterales species present in the ISIS-AR database. For species within the *Proteus* spp., *Serratia* spp., *Providencia* spp. and *Morganella morganii* results of imipenem were excluded. Top 5 species are: *Proteus mirabilis*, *Klebsiella oxytoca*, *Citrobacter koseri*, *Serratia marcescens*, *Morganella morganii*.

Figure 5.2.2.1a *Proportion of isolates with elevated MIC and proportion of CRE isolates (%) in E. coli and K. pneumoniae by year, in 36 laboratories, ISIS-AR 2020-2024*

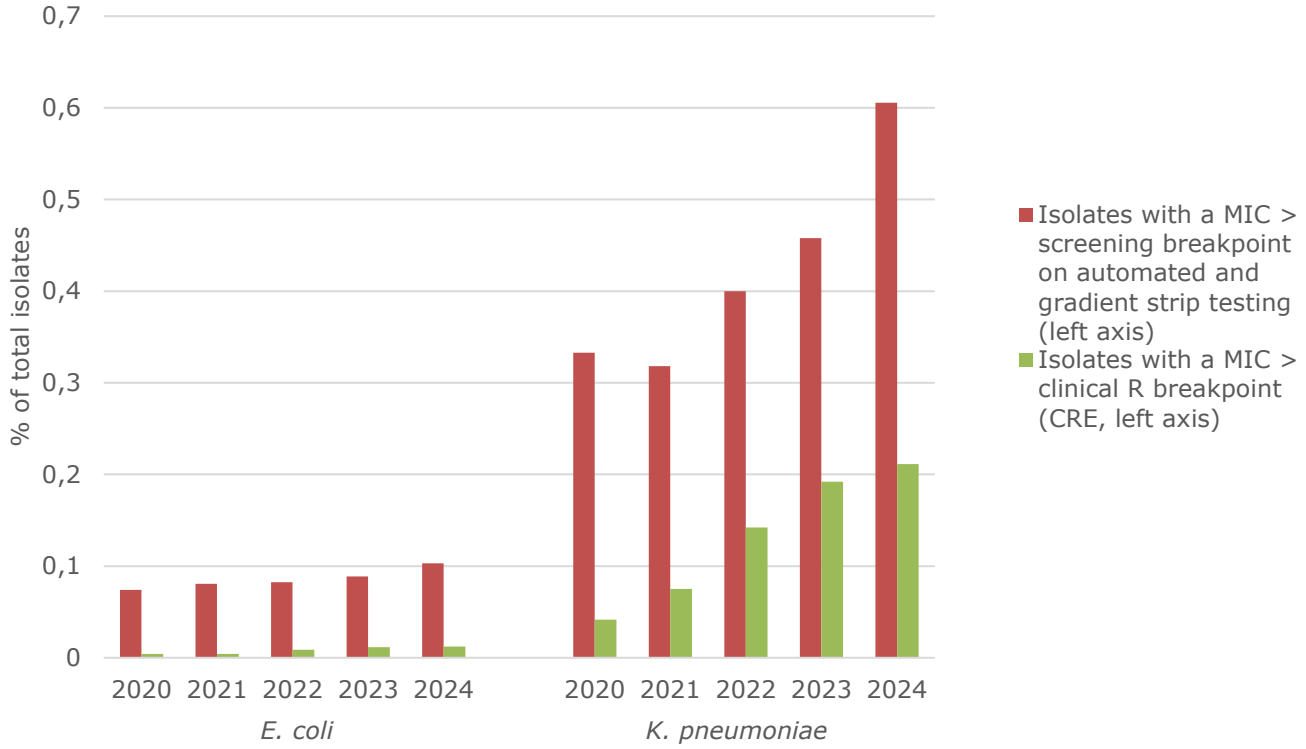
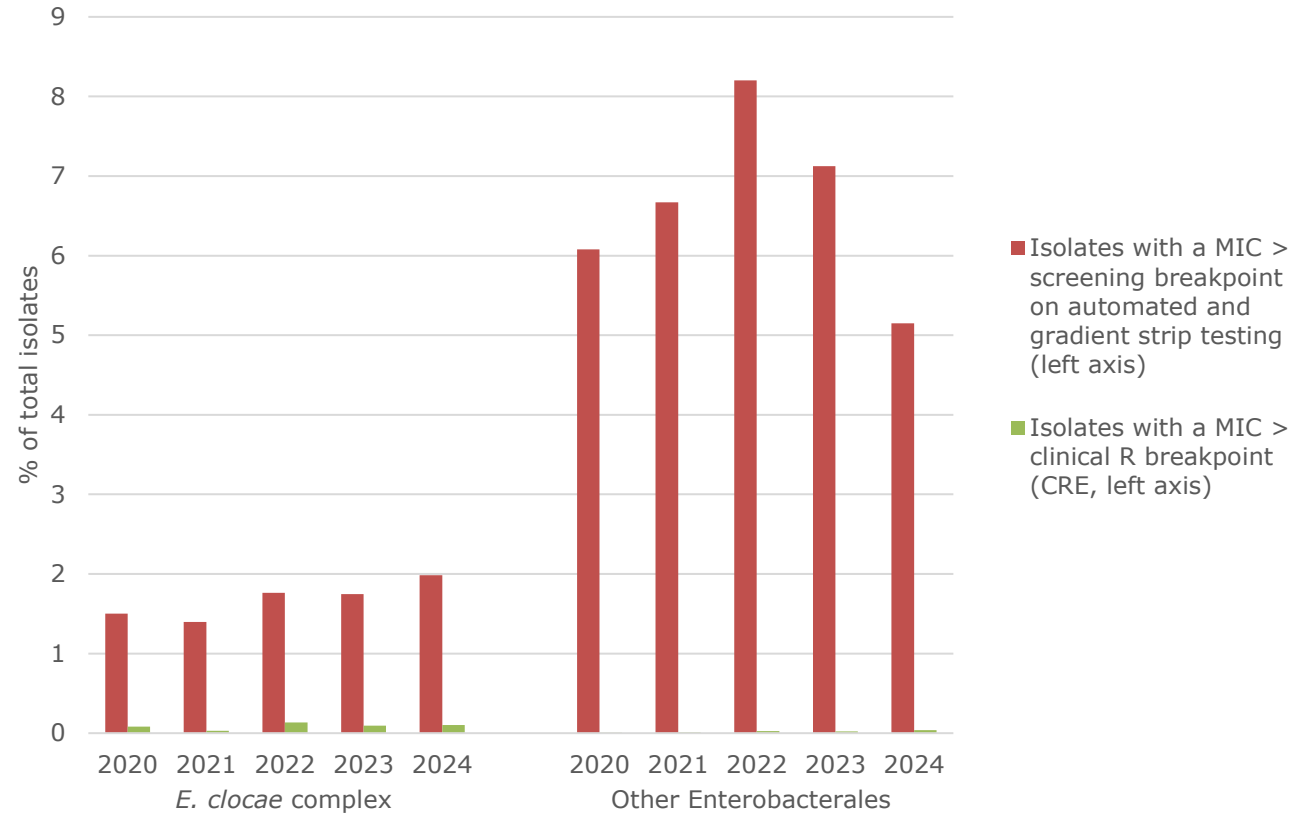


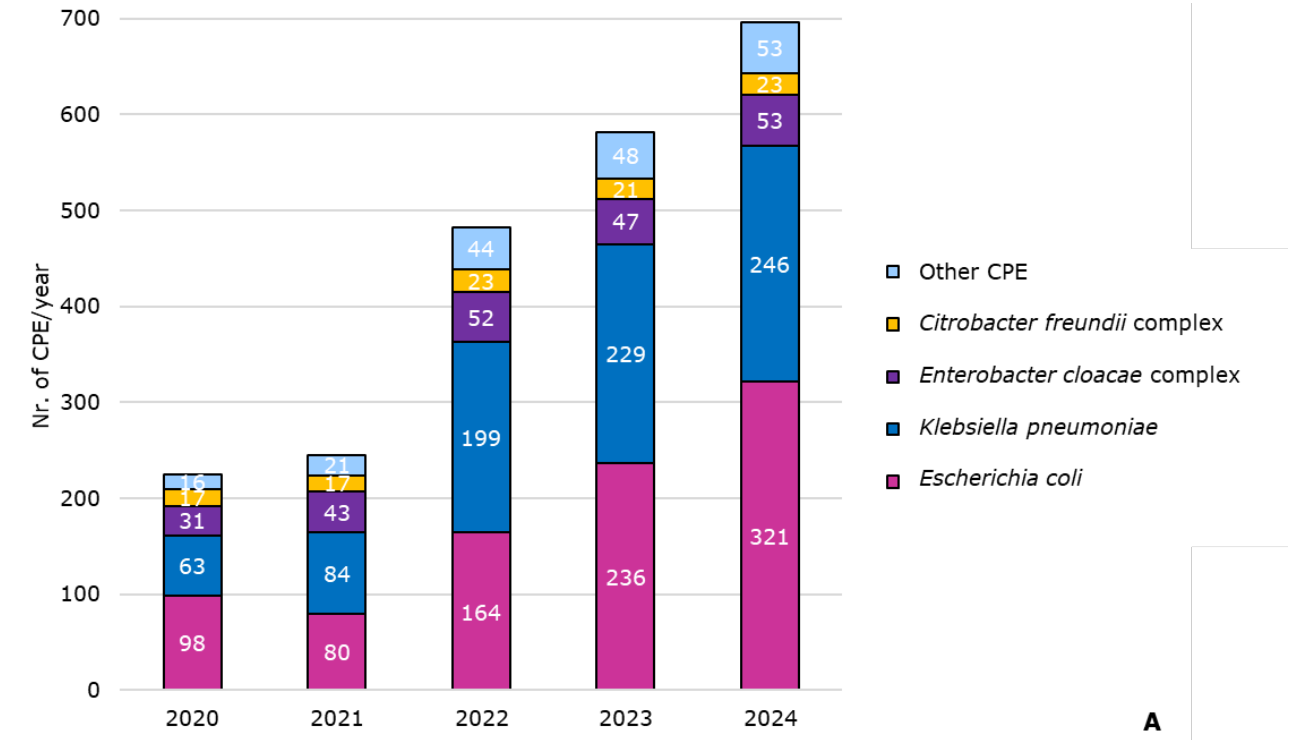
Figure 5.2.2.1b *Proportion of isolates with elevated MIC and proportion of CRE isolates (%) in E. cloacae complex and other Enterobacterales by year, in 36 laboratories, ISIS-AR 2020-2024*



while bla$_{OXA-48}$ ranked third with 15% (101/696) (Figure 5.2.2.2b). The bla$_{OXA-48}$-like alleles were found in 39% and 11% of the *E. coli* and *K. pneumoniae* isolates, respectively. The bla$_{NDM-5}$ gene was found in 30% (95/321) and 7% (18/246) of the *E. coli* and *K. pneumoniae* isolates, respectively. Conversely, bla$_{NDM-1}$ was found predominantly in *K. pneumoniae* isolates (15%, 37/246) and only in 4% (12/321) of the *E. coli* isolates. While the absolute number of OXA-48-producing CPE isolates remained stable the past 5 years (Figure 5.2.2.2b), the overall trend is a proportional decline of the presence of bla$_{OXA-48}$ among CPE from 31% in 2020 to 15% in 2024 (Figure 5.2.2.2c). In contrast, there was a proportional increase from 10% in 2021 to 24% in 2024 of OXA-48-like-carrying CPE, thereby surpassing bla$_{OXA-48}$ carrying CPE (Figure 5.2.2.2b).

When the EUCAST clinical breakpoints were applied, 261/696 (38%) of the CPE isolates were resistant (MIC >8 mg/L) to meropenem and 344/696 (49%) were susceptible for meropenem (MIC ≤2 mg/L). Thirteen percent (91/696) of the CPE were susceptible with increased exposure (MIC >2 and ≤8 mg/L). Notably, a large fraction of 209/696 (30%) of the CPE had a MIC for meropenem of <0.5 mg/L, as determined by E-test, questioning the lower limit of the MIC range for meropenem of semi-automated systems like Vitek (Biomerieux) and Phoenix (BD) of ≤0.25 mg/L for careful detection of all possible CPE's. In general, a larger proportion of the *K. pneumoniae* isolates (63%, 156/246) was meropenem resistant compared to the *E. coli* isolates (31%, 66/216), irrespective of the carbapenemase-encoding genes present. Ninety of the 91 (98%) *bla*$_{OXA-244}$ *E. coli* isolates submitted in 2024 and 24/25 (96%) of the *bla*$_{OXA-181}$ carrying *E. coli* had MICs for meropenem ≤2 mg/L. Only 21/101 (21%) of the CPE carrying *bla*$_{OXA-48}$ had a MIC above the clinical breakpoint for meropenem resistance (MIC >8 mg/L), while this was 71/123 (58%) for CPE carrying *bla*$_{NDM-5}$. Ten percent (71/696) of the CPE carried two carbapenemase-encoding genes, and in these cases 87% (62/71) of the isolates were resistant for meropenem. In 22/696 (3%) of the isolates no carbapenemase-encoding gene was detected. Of these isolates, 16 (73%) were Enterobacter spp. and 6 (38%) from other species. The nature of the apparent carbapenemase production in Enterobacter spp. is weak carbapenemase activity of Ambler class C-type (AmpC) of enzymes such as *bla*$_{MIR}$ and *bla*$_{ACT}$ combined with the absence of porins (van Gorp et al., 2025 in revision).

Figure 5.2.2.2 *Numbers of carbapenemase-producing Enterobacterales (CPE), per species (a), per carbapenemase allele (b), and relative contribution of detected carbapenemase genes (c), based on Type-Ned, 2020-2024*



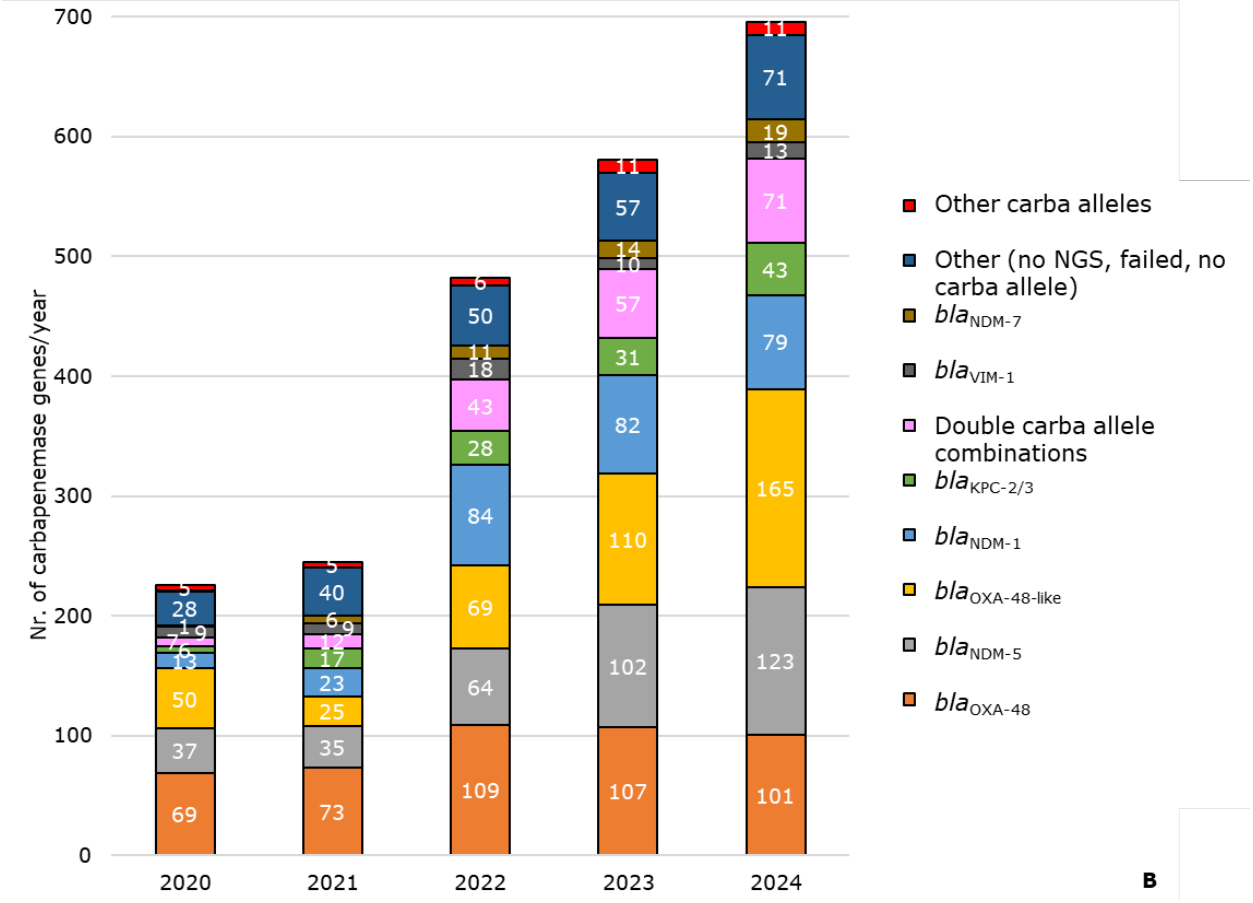
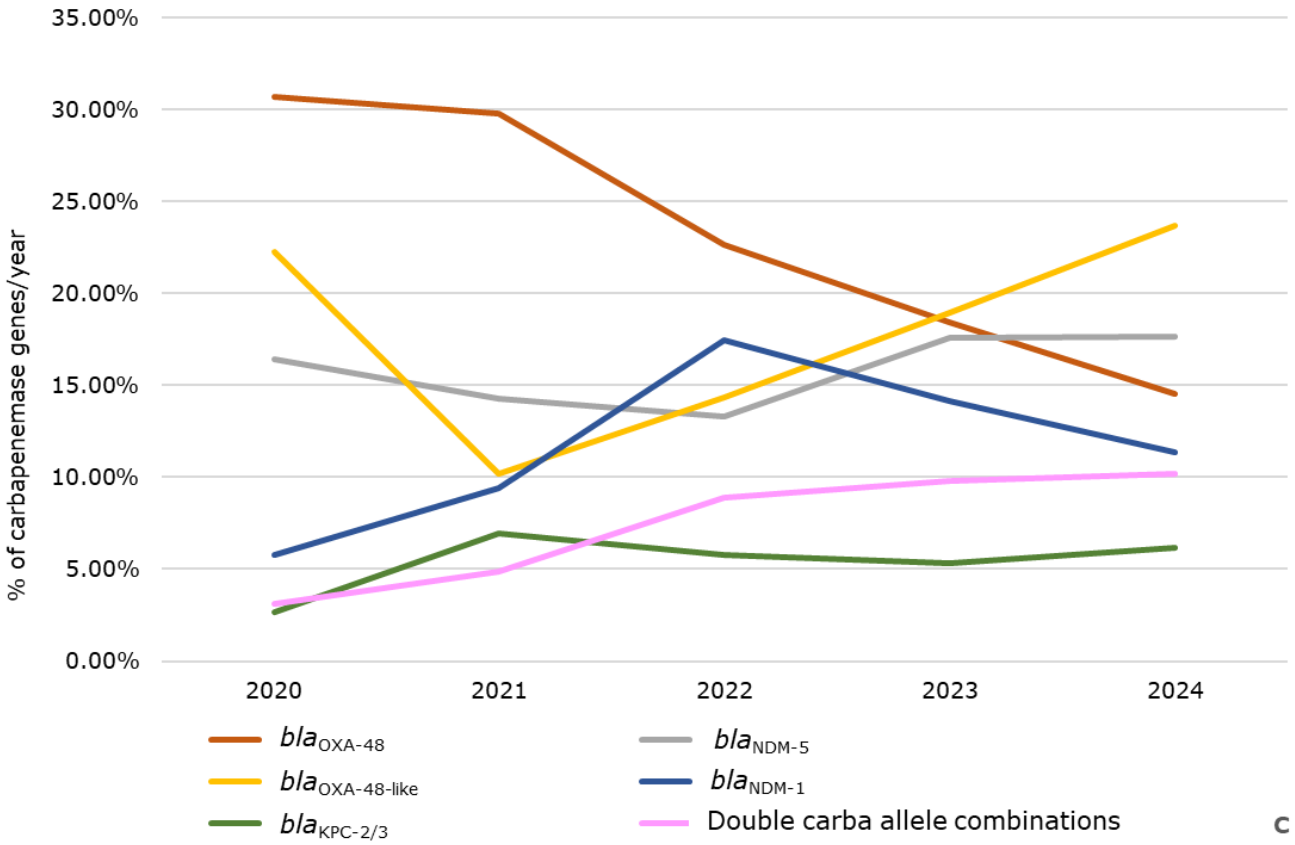


Figure 5.2.2.2 (continued) *Numbers of carbapenemase-producing Enterobacterales (CPE), per species (a), per carbapenemase allele (b), and relative contribution of detected carbapenemase genes (c), based on Type-Ned, 2020-2024*



Relatedness and genetic clusters among carbapenemase-producing Enterobacterales based on Type-Ned

Between 2020 and 2024, the CPE that could be typed by multilocus sequence typing (MLST) revealed a waxing and waning of the vast majority of distinct MLST sequence types through the years (not shown). However, there is persistence of globally epidemic and multidrug-resistant lineages in the top 15 of most commonly detected MLST sequence types, such as *K. pneumoniae* ST147, ST307, ST395 and *E. coli* ST131, ST167, ST361, ST405 and ST410 in the Netherlands (Table 5.2.2.2).

Based on species-specific whole-genome multilocus sequence typing (wgMLST), 960 of the 2229 (43%) isolates from 2020-2024 fell in one of the detected 297 genetic clusters, including clusters partially containing older isolates (<2020). The species most involved were: *E. coli* (126 clusters), *K. pneumoniae* (125 clusters), *E. cloacae* complex (19 clusters), *C. freundii* complex (9 clusters) and *Proteus mirabilis* (6 clusters). The ten largest clusters are shown in Table 5.2.2.3.

Table 5.2.2.2 *Results of multilocus sequence typing (MLST) of 2229 CPE isolates from 2020-2024**

Species	MLST sequence type	2020	2021	2022	2023	2024	Total
K. pneumoniae	147	12	6	35	60	56	169
K. pneumoniae	307	5	10	32	26	28	101
E. coli	38	19	8	13	18	21	79
E. coli	167	4	5	12	22	34	77
K. pneumoniae	395	2	3	18	21	19	63
E. coli	410	6	2	14	15	20	57
E. coli	10	5	3	9	14	17	48
E. coli	131	6	2	7	11	19	45
E. clocacae complex	121	8	10	13	6	5	42
K. pneumoniae	11	2	7	8	10	14	41
E. coli	405	5	2	5	15	11	38
E. coli	69	6	2	5	6	18	37
E. coli	361		1	8	14	13	36
E. coli	648	3	6	5	8	10	32
K. pneumoniae	16	2	4	8	9	9	32
Not shown	Various	140	174	290	326	402	1332
Total		225	245	482	581	696	2229

* Red indicates high number of isolates with specific MLST sequence type, while blue indicates low number of isolates with MLST sequence type.

Table 5.2.2.3 *The ten largest genetic clusters based on the number of patients between 2020 and 2024*

Species	MLST	Carba-alleles	Year start	n (patients)	n 2020-2024	Remarks
K. pneumoniae	ST147	*bla*$_{NDM-1}$, *bla*$_{OXA-48}$	2022	41	41	multi-institutional Ukraine
E. cloacae complex	ST121	*bla*$_{OXA-48}$	2016	48	38	>80% 1 institute
E. coli	ST38	*bla*$_{OXA-244}$	2016	28	25	multi-institutional 14 Türkiye/ Morocco/ Syria
C. freundii complex	ST22	*bla*$_{NDM-5}$	2018	57	22	>80% 1 institute
E. coli	ST127	*bla*$_{OXA-48}$	2015	31	22	multi-institutional
E. coli	ST38	*bla*$_{OXA-48}$	2015	21	15	multi-institutional 6 Türkiye/ Iran
E. coli	ST13730	*bla*$_{OXA-244}$	2022	14	14	multi-institutional 9 Türkiye/ Iraq/ Syria
K. pneumoniae	ST307	*bla*$_{NDM-7}$	2017	14	14	multi-institutional Morocco
K. pneumoniae	ST39	*bla*$_{KPC-2}$	2022	13	14	multi-institutional Ukraine
P. stuartii	N.A.	*bla*$_{NDM-1}$	2022	12	12	multi-institutional Ukraine

Multi-institutional: at least 5 different submitting laboratories, no lab/hospital > 50%.
Countries mentioned: mix of recent hospitalisation (< 2 months ago), visit or country of birth.
N.A.: not assigned

Clinical/epidemiological characteristics of persons with carbapenemase-producing Enterobacterales based on mandatory notifications in OSIRIS
Additional epidemiological questionnaire data was available in OSIRIS for 578 CPE positive persons with a sampling date between 1 January 2024 and 31 December 2024 (Table 5.2.2.4). This is much higher than the previous years (n=170 in 2020, n=201 in 2021, n=377 in 2022, and n=486 in 2023). The median age of the 578 persons was 63 (range 0 - 94) years and 340 (59%) were male.
In 24% (138/578) of the notified persons in 2024 a sample was taken for diagnostic purposes (Table 5.2.2.4), compared to 28% in 2020 (48/170), 25% (50/201) in 2021, 23% (86/377) in 2022, and 24% (118/486) in 2023. The most common reported infection among patients with a diagnostic isolate was urinary tract infection (51%, 70/138), followed by wound infection (10%, 14/138). Most patients with a diagnostic isolate had no known risk factor or it was unknown whether the person had a risk factor (65%) (Figure 5.2.2.3). Hospitalization abroad for at least 24 hours during two months prior to sampling occurred in 8% of patients with a diagnostic isolate, which was comparable to 2023 (10%), 2021 (8%), and 2020 (10%), but lower than in 2022 (21%) (Table 5.2.2.4 and Figure 5.2.2.3). Ukraine (n=3) and Greece (n=2) were most often reported as countries of hospitalization (Table 5.2.2.5). Persons with a diagnostic isolate without a known risk factor or it was unknown whether the person had a risk factor were younger than persons with a risk factor (median age 71 versus 54 years, respectively), but no other differences were observed.
Screening as part of routine screening (e.g., on admission, because of prolonged hospital stay or as part of selective decontamination regimens) or targeted screening because of suspected CPE carriage was the reason for sampling in 74% (427/578) of the persons in 2024 and this percentage was stable over time. Hospitalization abroad for at least 24 hours within two months before sampling was the most common reported risk factor for CPE among persons with a screening isolate in 2024 (49%) which was lower than 2022 (62%) and 2023 (61%), comparable to 2021 (49%), and higher than 2020 (42%) (Table 5.2.2.4 and Figure 5.2.2.3). Ukraine (n=37), Türkiye (n=32), Egypt (n=28) and Morocco (n=19) were most often reported as countries where hospitalization had occurred (Table 5.2.2.5). When combining the category ‘recent hospitalization abroad’ with ‘contact with a hospital abroad during the last year before sampling’, equal percentages as in previous years were found: 60% in 2024 compared to 68-70% in 2022-2023. No risk factor (and thus detected by routine screening and not by targeted screening), or it was unknown whether the person had a risk factor, was reported in 26%, which was comparable to 2021 (26%), but higher than in 2022 (18%) and 2023 (19%).

Table 5.2.2.4 *Epidemiological data of notifications of persons carrying CPE, stratified by reason for sampling, OSIRIS, 2024*

Characteristic	Total[a] N (%)	Diagnostic N (%)	Screening N (%)
N	578	138	427
Location of sampling			
Outpatient/emergency departments or by a general practitioner	215 (37.2)	88 (63.8)	124 (29.0)
Inpatient departments (excl. Intensive care units)	290 (50.2)	42 (30.4)	243 (56.9)
Intensive care units	47 (8.1)	6 (4.3)	40 (9.4)
Other/unknown	26 (4.5)	2 (1.4)	20 (4.7)
Residence			
Living independently	404 (69.9)	113 (81.9)	288 (67.4)
Rehabilitation centre	15 (2.6)	4 (2.9)	11 (2.6)
Nursing or elderly home/facilities for small-scale housing for elderly	35 (6.1)	10 (7.2)	22 (5.2)
Asylum seekers centre	35 (6.1)	2 (1.4)	33 (7.7)
Other/unknown	89 (15.4)	9 (6.5)	73 (17.1)
Invasive medical procedure/device within twelve months prior to sampling[b]			
No	163 (28.2)	50 (36.2)	110 (25.8)
Surgery	157 (27.2)	30 (21.7)	125 (29.3)
Other (incl. endoscopy, cystoscopy, urinary catheter, renal dialysis)	95 (16.4)	28 (20.3)	67 (15.7)
Yes but unknown which invasive procedure(s)	12 (2.1)	1 (0.7)	11 (2.6)
Unknown	151 (26.1)	29 (21.0)	114 (26.7)
Identified risk factors[b]			
Hospitalization abroad for >24 hours during two months prior to sampling	220 (38.1)	11 (8.0)	208 (48.7)
Already known carrier of CPE	19 (3.3)	4 (2.9)	15 (3.5)
Contact with a hospital abroad twelve months prior to sampling in a different way than >24 hours within two months prior to sampling	63 (10.9)	14 (10.1)	48 (11.2)
Travelling abroad twelve months prior to sampling without hospitalization or visiting a hospital	59 (10.2)	19 (13.8)	40 (9.4)
(Prior) stay in a healthcare facility where a (possible) CPE outbreak is/was occurring	5 (0.9)	0 (0.0)	5 (1.2)
No risk factor known/unknown	212 (36.7)	90 (65.2)	111 (26.0)

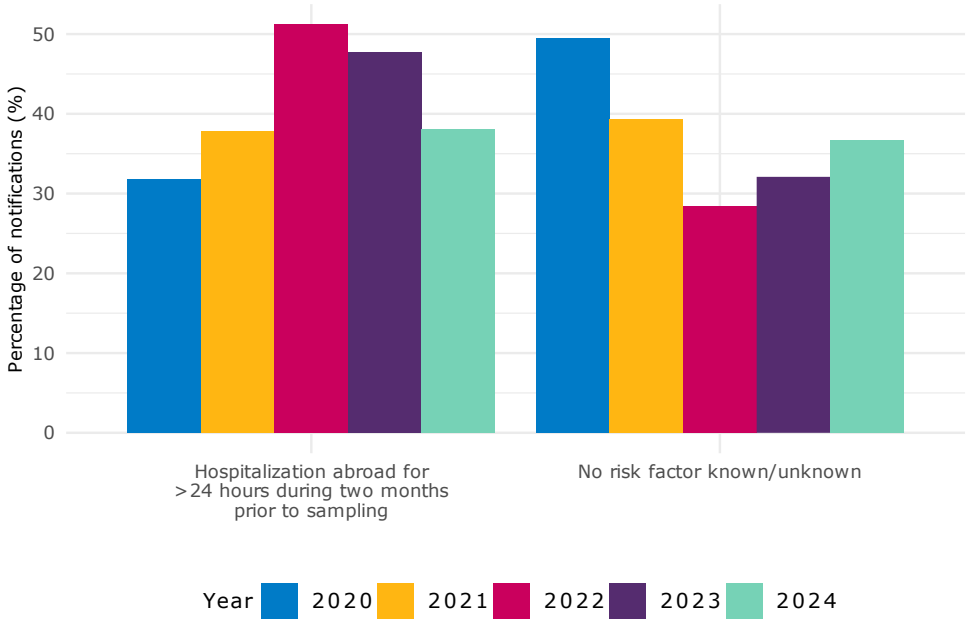
[a] Including 13 persons for whom the reason for sampling was unknown
[b] Listed in order of considered importance: in case of multiple answers the person is included in the highest listed answer

Table 5.2.2.5 *Top 5 parts of the world where persons with hospitalization abroad for at least 24 hours during two months prior to sampling were admitted, OSIRIS, 2024*

Characteristic	Total[a] N (%)	Diagnostic N (%)	Screening N (%)
N	220	11	208
Hospitalized in a country in			
Northern Africa	48 (21.8)	1 (9.1)	47 (22.6)
Eastern Europe	43 (19.5)	3 (27.3)	40 (19.2)
Western Asia	42 (19.1)	1 (9.1)	40 (19.2)
Southern Europe	29 (13.2)	3 (27.3)	26 (12.5)
Southern Asia	13 (5.9)	0 (0.0)	13 (6.2)
Other region of the world/>1 region/unknown	45 (20.5)	3 (27.3)	42 (20.2)

[a] Including persons for whom the reason for sampling was unknown

Figure 5.2.2.3 *Hospitalization abroad for at least 24 hours during two months prior to sampling and no risk factor known/unknown among CPE positive persons (diagnostic and screening combined), OSIRIS, 2020-2024*



Outbreaks based on SO-ZI/AMR

In 2024, two new outbreaks with CPE in hospitals were reported to SO-ZI/AMR (Table 5.2.2.6). See chapter 5.2.7 for more details about SO-ZI/AMR.

Table 5.2.2.6 *Outbreaks of carbapenemase-producing Enterobacterales reported in 2024 to the Early warning and response meeting for Healthcare-associated Infections and Antimicrobial Resistance (SO-ZI/AMR)*

Region	Facility	Micro-organism	Gene(s)	No. of patients
West	Hospital	*K. pneumoniae*	$bla_{\text{OXA-48+NDM-5}}$	3
West	Hospital	*E. coli*	$bla_{\text{NDM-5}}$	8

Discussion

In ISIS-AR, the prevalence of carbapenem resistance of *E. coli* was 0.01% in 2024 which was comparable to the previous years. Carbapenem resistance in *K. pneumoniae* is significantly higher compared to *E. coli*, and has increased from 0.04% in 2020 to 0.2% in 2024. *E. cloacae* complex species and other Enterobacterales show similar patterns as *E. coli*. In 2021, the NVMM national guideline, was revised and suggests to perform tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic) of isolates with an elevated MIC (>0.25 mg/L for meropenem and/or >1.0 mg/L for imipenem) based on semi-automated tests.[6] The percentage of CPE confirmatory tests performed differed between the various species categories within Enterobacterales. Even among the isolates with MIC > the clinical R breakpoint, the percentage of CPE confirmatory tests performed differed between the species, but overall stayed between 65-85%. This was partly a result of lab-specific algorithms for additional testing after automated antimicrobial susceptibility testing, which was not always in accordance with the national guideline.

In 2024, the number of carbapenemase-producing Enterobacterales isolates that was submitted to the RIVM was considerably higher than in previous years, as were the numbers of notifications of persons with CPE, and was succeeding pre-COVID years. In 2020-2021 the decrease of the number of CPE was presumably the result of the COVID-19 pandemic associated measures, such as travel restrictions, social isolation, and a reduction in regular healthcare, but present numbers have exceeded the pre-COVID levels of 2019. The increase in 2022-2024 is partially attributable to the transfer of Ukrainian patients to the Netherlands due to the Ukraine/ Russia war. We indeed noted an increase in CPE isolates originating from patients from Ukraine since 2022,[11,12] shown by Ukraine being the first country of all reported countries where recent hospitalization abroad had occurred.

A considerable part (43%) of the isolates from 2020-2024 fell in one of the detected 297 genetic clusters, including clusters partially containing older isolates (<2020). The ten largest clusters had a diversity in species, carbapenem alleles and starting year. Some clusters were a single institute cluster, suggesting nosocomial spread, some had a link to foreign hospitals and some showed a mix.

The introduction of next-generation sequencing and Nanopore long-read-generation sequencing on all carbapenemase-producing isolates allows the identification of genetic clusters that may indicate transmission within and between healthcare centres. Genetic clustering does not prove direct transmission or an outbreak. Isolates that cluster together based on wgMLST may still be different in plasmid content and/or resistome and may lack an epidemiological link in time and place. For some genetic clusters, sampling dates are several years apart. To identify transmission, information on epidemiological links would be needed. The absolute number of persons with a sample taken for diagnostic purposes as well as for routine or targeted screening increased in 2024 compared to previous years. However, the relative distribution of diagnostic vs screening isolates is stable over the last three years. The increase of absolute numbers of targeted and routine screening samples in the last three years is potentially related to the increased travel after releasing the COVID-19 measures and to the transfer of patients from Ukraine.

Conclusions

- The overall percentage of Enterobacterales isolates with elevated MIC value for meropenem or imipenem (i.e., > the screening breakpoint) was 2.0% in 2024. The prevalence of CRE/CPE confirmed isolates among *E. coli* was 0.1%, among *K. pneumoniae* 0.3%, *E. cloacae* complex 0.1% and other Enterobacterales 0.1%.
- In 2024, the number of CPE isolates submitted to the RIVM was considerably higher than in previous years and was succeeding pre- and post-COVID totals. The increase is partially attributable to the transfer of Ukrainian patients to the Netherlands.
- The predominant CPE species in 2024 were *E. coli*, *K. pneumoniae*, *E. cloacae* complex, and *C. freundii* complex, respectively.
- The most frequently identified carbapenemase encoding genes in Enterobacterales were *bla*$_{\text{OXA-48}}$-like (24%), *bla*$_{\text{NDM-5}}$ (18%), *bla*$_{\text{OXA-48}}$ (15%), and *bla*$_{\text{NDM-1}}$ (11%), respectively.
- There is an increase of *bla*$_{\text{OXA-48}}$-like (i.e., *bla*$_{\text{OXA-162}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{OXA-232}}$, *bla*$_{\text{OXA-244}}$, *bla*$_{\text{OXA-245}}$, *bla*$_{\text{OXA-484}}$, *bla*$_{\text{OXA-1205}}$ and *bla*$_{\text{OXA-1207}}$) CPE in the Netherlands.
- Notably, a large fraction (30%) of the CPE had a MIC for meropenem of <0.5 mg/L, as determined by E-test, questioning the lower limit of the MIC range for meropenem in semi-automated systems like Vitek and Phoenix of ≤0.25 mg/L for careful detection of alle possible CPE's.
- The MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates.
- There is persistence of globally epidemic multidrug-resistant *E. coli* and *K. pneumoniae* lineages in the top 15 most MLST-typed CPE.
- Twenty-four percent of CPE cases were identified in diagnostic - mainly urine - samples, 74% were identified upon routine screening or targeted screening because of suspected CPE carriage, and for 2% a different/unknown reason was reported.
- In 38% of patients, there is a relation with hospitalization abroad for more than 24 hours during the two months prior to sampling (8% and 49% among persons with a diagnostic and screening isolate, respectively), which was lower than in 2022 and 2023 (48-51%), but equal to 2021. Türkiye, Ukraine, Egypt, and Morocco are the countries that are most often reported. However, when combining the category 'recent hospitalization abroad' with 'contact with a hospital abroad during the last year before sampling', equal percentages as in previous years were found.
- In 37% of the CPE positive persons no known CPE risk factor was identified or it was unknown whether the person had a risk factor (65% and 26% among persons with a diagnostic and screening isolate, respectively), which is higher than in 2022 and 2023 (28-32%), but comparable to 2022 (39%). Persons with a diagnostic isolate without a known risk factor or it was unknown whether the person had a risk factor were younger than those with a risk factor (median age 71 versus 54 years, respectively), but no other differences were observed.

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5.2.3 *Carbapenem-resistant and carbapenemase-producing Pseudomonas aeruginosa*

Introduction
Pseudomonas aeruginosa is a common nosocomial pathogen, intrinsically resistant to various broad-spectrum antibiotics. The emergence of multidrug-resistant (MDR) *P. aeruginosa* by acquired resistance mechanisms is a problem of global concern, and in 2024 the World Health Organization classified carbapenem-resistant *P. aeruginosa* as 'high-priority' pathogen.[1]

Methods
Data on carbapenem-resistant and carbapenemase-producing *P. aeruginosa* (CPPA) were obtained from ISIS-AR and the national enhanced CPPA surveillance via Type-Ned.

Prevalence of carbapenem-resistant and MDR P. aeruginosa based on ISIS-AR
From the ISIS-AR database, the first *P. aeruginosa* isolate per patient per year was extracted for the period 2020-2024. To avoid overestimation of the percentage carbapenem-resistant *P. aeruginosa* caused by active screening for highly resistant isolates, only data on diagnostic cultures (as categorized by the reporting laboratory) from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus were included in the analysis. Carbapenem resistance was defined as (1) a positive test for carbapenemase gene detection and/or carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (applying the cut-off of >8 mg/L or <14 mm) and/or imipenem (cut-off >4 mg/L or <20 mm). In addition, the percentage of *P. aeruginosa* that was MDR was calculated. Multidrug resistance was defined as resistance to ≥3 antimicrobials or antimicrobial groups among fluoroquinolones (resistance to ciprofloxacin and/or levofloxacin), aminoglycosides (resistance to tobramycine), carbapenems (resistance to meropenem and/or imipenem), ceftazidime, and piperacillin-tazobactam, based on re-interpretation of test-values according to the EUCAST 2024 breakpoints. Only isolates which were tested for all five groups of antimicrobials were included in the latter analysis. The numbers were based on a selection of 39 laboratories (out of a total of 47 laboratories in the Netherlands), which provided complete data on the last five years (2020 to 2024).

Molecular characteristics of carbapenemase-producing P. aeruginosa and patient related characteristics based on Type-Ned
In 2020 the enhanced CPPA surveillance via Type-Ned was implemented. All but one Dutch medical microbiology laboratories (MMLs) participate (n=46), however, not all laboratories have submitted eligible isolates. MMLs are requested to submit *P. aeruginosa* isolates to the RIVM with an MIC for meropenem of >2 mg/L and/or an MIC for imipenem >4 mg/L and/or carbapenemase production and/or a detected carbapenemase-encoding gene. A restriction is that the laboratory can only send the first isolate per person per year. The RIVM allows consecutive isolates from the same person if these are *P. aeruginosa*

isolates with different carbapenemase-encoding gene combinations when compared to the first isolate. The RIVM confirms the species by MALDI-ToF, determines the MIC for meropenem by Etest, and detects carbapenemase production by the carbapenem inactivation method (CIM).[2] The presence of carbapenemase-encoding genes is assessed by PCR (carba-PCR on *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{OXA}$), next-generation sequencing (NGS) and Nanopore sequencing for all isolates that are CIM positive. The data described in this chapter are based on the first unique CIM-positive *P. aeruginosa* isolate/carbapenemase-encoding allele combination per person with a person ID for the period 2020-2024, and all of these isolates collected in 2024 were included. As in 2022-2023, also samples without a person ID from persons who had country of origin Ukraine were included for further analysis if it was confirmed that these represented a unique person, based on sex, age and postal code. Isolates without a person ID were excluded. Based on the results of whole-genome multi-locus sequence typing (wgMLST), closely related CPPA isolates were grouped in genetic clusters and assigned consecutive cluster numbers. A genetic cluster was defined as ≥2 CPPA isolates that differ ≤15 wgMLST alleles.[3] Assigning genetic cluster numbers in the surveillance started in 2020, but the genetic cluster numbers in the results of this report include all sequenced *P. aeruginosa* isolates available from (pilot) surveillance studies in the RIVM. When multiple isolates within the same cluster were submitted for the same patient, only the first isolate was included in the analysis, also if the different isolates were submitted in different years and/or by different laboratories. In addition to submitting an isolate, Dutch laboratories are also requested to fill in a clinical/epidemiological questionnaire on characteristics of the patient from whom the CPPA isolate was obtained.

Results

Prevalence of carbapenem-resistant and MDR P. aeruginosa based on ISIS-AR

In the ISIS-AR database, 5% (953/20,011) of the diagnostic *P. aeruginosa* isolates were carbapenem-resistant in 2024 (Table 5.2.3.1). The percentage of carbapenem-resistant *P. aeruginosa* isolates was higher among *P. aeruginosa* isolates from patients in intensive care units (ICUs) compared to other departments. The observed proportion of resistance appears to be relatively stable over the 2020-2024 time period, except for an increase in the proportion of carbapenem resistance in *P. aeruginosa* isolates from ICUs, especially after the COVID-years 2020 and 2021, when a decrease was observed compared to the pre-COVID-years (Figure 5.2.3.1). Of the total number of 953 carbapenem-resistant *P. aeruginosa* isolates, for 144 (15%) isolates, data on tests for carbapenemase production was available, of which 25 (17%) showed a positive result. According to the Dutch HRMO guideline[4], a test for carbapenemase production is only required if phenotypic resistance to carbapenems is present in combination with resistance to tobramycin. Of the 809 carbapenem-resistant *P. aeruginosa* isolates without test results on carbapenemase production, 90% (734/809) were sensitive for tobramycin.

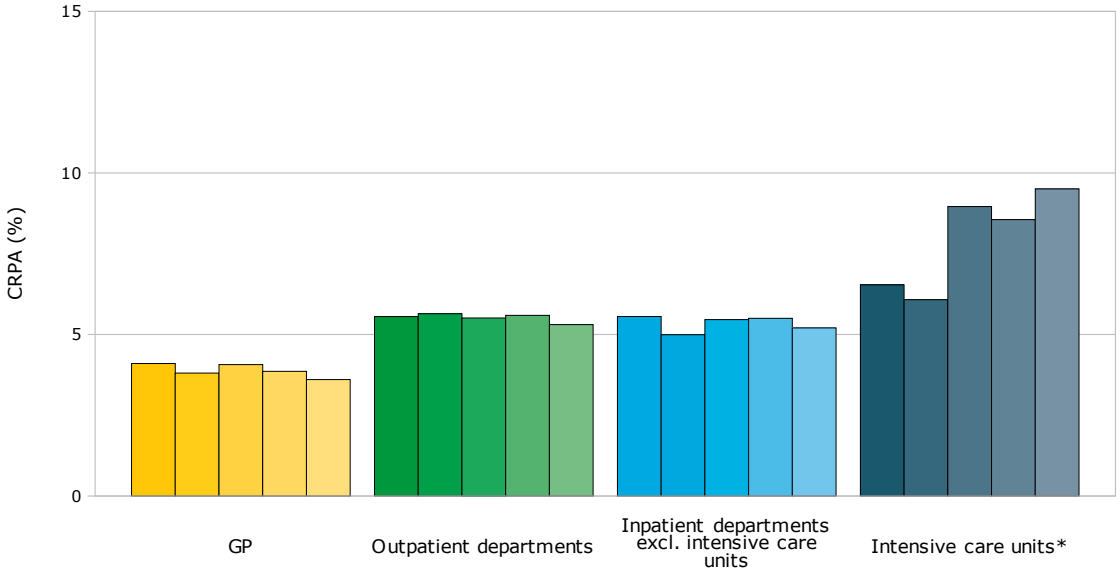
Table 5.2.3.1 *Carbapenem-resistant P. aeruginosa among diagnostic P. aeruginosa isolates in the Netherlands in 2024, based on ISIS-AR data*

Type of department	Tested isolates, N	Carbapenem-resistant *P. aeruginosa*, N (%)
General practitioners	7,296	263 (4)
Outpatient departments	5,597	297 (5)
Inpatient departments excl. intensive care units	6,592	343 (5)
Intensive care units	526	50 (10) ↑
Total	**20,011**	**953 (5)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *P. aeruginosa* isolate per patient was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase (production) and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (applying the cut-off of >8 mg/L or <14 mm) and/or imipenem (cut-off >4 mg/L or <20 mm).

Figure 5.2.3.1 *Percentages of carbapenem-resistant P. aeruginosa among diagnostic P. aeruginosa isolates in the Netherlands (from left to right 2020 to 2024), based on ISIS-AR data*



Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *P. aeruginosa* isolate per patient per year was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase (production) and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (applying the cut-off of >8 mg/L or <14 mm) and/or imipenem (cut-off >4 mg/L or <20 mm).
GP: General practitioners

Additional analyses in the 2024 ISIS-AR database showed that 1.6% (n=320) of 19,586 diagnostic *P. aeruginosa* isolates tested for all five (groups of) antimicrobials included in the MDR definition, were MDR (Table 5.2.3.2) and 68% (219/320) of the MDR isolates were carbapenem-resistant.

Table 5.2.3.2 *Multidrug-resistant (MDR) P. aeruginosa among diagnostic P. aeruginosa isolates in the Netherlands in 2024, based on ISIS-AR data*

Type of department	Tested isolates, N	MDR *P. aeruginosa*, N (%)	Carbapenem-resistant MDR *P. aeruginosa*, N (%)
General practitioners	6,927	43 (0.6)	28 (65)
Outpatient departments	5,573	123 (2)	81 (66)
Inpatient departments excl. intensive care units	6,547	123 (1.9)	85 (69)
Intensive care units	539	31 (6)	25 (81)
Total	**19,586**	**320 (1.6)**	**219 (68)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

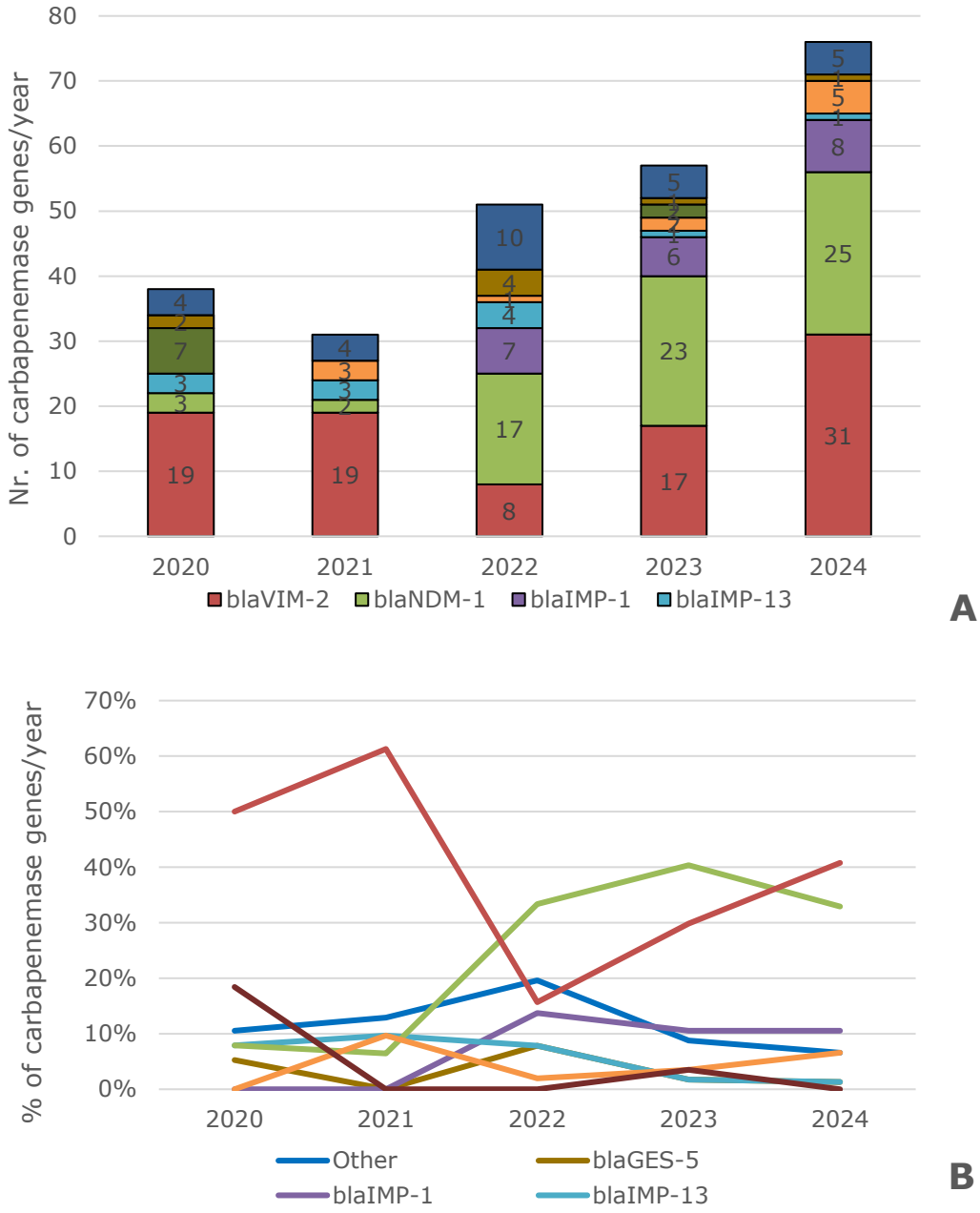
Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *P. aeruginosa* isolate per patient was selected.
Multidrug resistance was defined as resistant to ≥3 antimicrobials or antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam, based on re-interpretation of test-values according to EUCAST 2024; Carbapenem resistance was defined as (1) a positive test for carbapenemase (production) and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (applying the cut-off of >8 mg/L or <14 mm) and/or imipenem (cut-off >4 mg/L or <20 mm).

Molecular characteristics of carbapenemase-producing P. aeruginosa and patient related characteristics based on Type-Ned

In the enhanced surveillance, the RIVM received 76 *P. aeruginosa* isolates from samples collected in 2024 through Type-Ned, all of which produced carbapenemase according to the CIM. The CPPA were from 74 patients and these isolates were submitted by 29 MMLs. One patient carried two unrelated CPPA strains harbouring either *bla*$_{\text{NDM-1}}$ or *bla*$_{\text{VIM-2}}$, and one Ukrainian patient also carried two unrelated CPPA strains harbouring either *bla*$_{\text{NDM-1}}$ or *bla*$_{\text{IMP-1}}$. Seventeen of the 76 CPPA isolates (22%) were obtained from sixteen Ukrainian patients. From all of the 76 isolates whole-genome sequencing data was available upon analysis and revealed that 31 of the 76 (41%) CPPA isolates carried a *bla*$_{\text{VIM-2}}$ allele, 25 harboured *bla*$_{\text{NDM-1}}$ (33%) and eight *bla*$_{\text{IMP-1}}$ (11%)(Figure 5.2.3.2). Five isolates carried *bla*$_{\text{VIM-11}}$ (7%), and *bla*$_{\text{IMP-13}}$, *bla*$_{\text{IMP-26}}$, *bla*$_{\text{VIM-28}}$ or *bla*$_{\text{GES-5}}$ were detected in one single isolate each (5%). Of the seventeen CPPA isolates from Ukrainian patients, nine harboured a *bla*$_{\text{NDM-1}}$ allele, six *bla*$_{\text{IMP-1}}$, one *bla*$_{\text{VIM-2}}$ and one was unknown. The overall trend in the past 5 years is an increase of the presence of *bla*$_{\text{NDM-1}}$ among CPPA from 8% in 2020 to 33% in 2024 (Figure 5.2.3.2). This increase could partly be attributed to 37% (26/70) of NDM-1-producing CPPA associated with Ukrainian patients, but there was also an increase among non-Ukrainian

patients. Nineteen of the 21 CPPA with *bla*$_{IMP-1}$ were found in Ukrainian patients between 2022 and 2024. In 2021 CPPA carrying *bla*$_{VIM-2}$ decreased from 61% to 16% in 2022, followed by annual increase to 41% in 2024.
The genetic relations of the 76 sequenced CPPA were assessed by performing MLST and wgMLST (Figure 5.2.3.3). This revealed that most

Figure 5.2.3.2 *Numbers of carbapenemase-producing Pseudomonas aeruginosa (CPPA) per carbapenemase allele (A), and trend in detected carbapenemase genes (B) based on Type-Ned, 2020-2024*



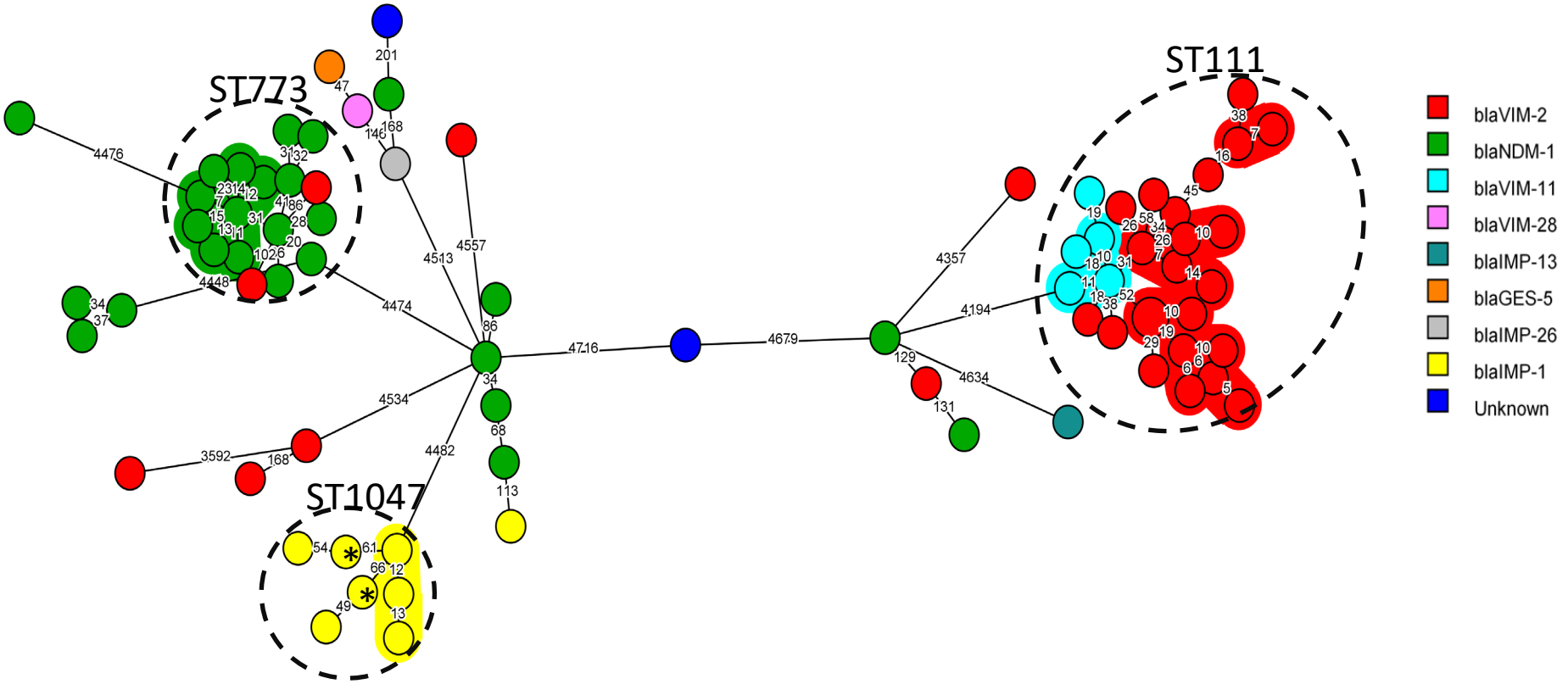
of the isolates belonged to three distinct groups, generally according to the carbapenemase allele they harbored and MLST sequence type ST111, ST773 and ST1047. In 2024, one group of eight CPPA ST773 isolates, two groups of two isolates (ST111), four groups of three isolates (ST111 and ST1047) and one group of five CPPA ST111 isolates differed in ≤15 wgMLST alleles and could therefore be regarded as genetic clusters.[3] The isolates of the large CPPA ST773 cluster and one ST1047 cluster were found among Ukrainian (n=9) and Dutch (n=1) patients in ten different hospitals, while for the ST111 clusters, isolates were found in patients in the same or two hospitals. Between 2020 and 2024, the CPPA that could be typed by MLST revealed a waxing and waning of distinct MLST sequence types through the years (Table 5.2.3.3). However, since the start of the war in Ukraine in 2022, there is persistence of globally epidemic ST773 and ST1047 lineages in the top 3 of most commonly detected MLST sequence types. MLST ST111 remains the most commonly found CPPA sequence type in the Netherlands. Of the CPPA isolates, 83% (63/76) had MICs for meropenem above the clinical EUCAST breakpoint of 8 mg/L, whereas thirteen CPPA isolates with a *bla*$_{VIM-2}$ allele (19%) had an MIC between 1 and 8 mg/L. The following sample materials were reported: twenty CPPA were from screening swabs, ten from sputum, eight were from wound samples, seventeen from urine samples, seven from urine catheter, and the remainder from other isolation sites. The majority (70/76) of the CPPA were obtained from materials sampled in hospitals.

Table 5.2.3.3 *Results of multilocus sequence typing (MLST) of 253 CPPA isolates from 2020-2024**

Species	MLST sequence type	2020	2021	2022	2023	2024	Total
P. aeruginosa	111	14	19	5	17	28	83
	773	2	2	14	18	17	53
	1047	1		7	4	5	17
	235	3		5	1	5	14
	621	3	3	4	2	1	13
	654	2		4	4	3	13
	357			3	1	3	7
	233	1	2	1			4
	308				1	3	4
	2592			1	1	2	4
	2844	3			1		4
	253	1	1	1			3
	446			1	1	1	3
	395	1	1			1	3
	5211				2	1	3
	1006	3					3
	Various	4	3	5	4	6	22
	Total	**38**	**31**	**51**	**57**	**76**	**253**

* Red indicates high number of isolates with specific MLST sequence type, while blue indicates low number of isolates with MLST sequence type.

Figure 5.2.3.3 *wgMLST-based minimum spanning tree of 76 CPPA isolates from patients sampled in 2024, based on enhanced CPPA surveillance data*



Each node represents an isolate, the numbers on the connecting lines indicate allelic distances between isolates. A colored halo indicates ≥2 isolates differing ≤15 wgMLST alleles.

Clinical/epidemiological characteristics of patients with carbapenemase-producing P. aeruginosa
Clinical/epidemiological questionnaire data in Type-Ned were available for 47 of the 74 CPPA-carrying persons, of whom 42 were in-hospital patients. Thirty-two patients (68%) were male and the median age was 65 years (range 21-89 years). Seventeen patients (17/47, 36%) were admitted to the ICU at the moment of sampling, 25 (53%) were admitted to a non-ICU hospital department, and five (11%) samples were taken at the outpatient department or in a non-hospital setting. For eight of the fifteen Ukrainian patients, complete questionnaires were available. For 2/8 (25%) of those Ukrainian patients, clinical signs of an infection was mentioned as the reason for taking the sample. Seven of these patients (7/8, 88%) had been admitted >24 hours in a hospital abroad in the previous two months (six in Ukraine and one in Türkiye). Thirty-nine questionnaires concerned non-Ukrainian patients. In these patients, 9/39 samples (23%) were taken for diagnostic purposes, while for the other thirty (77%), the reason for sampling was screening. For two patients though, for whom screening was mentioned as the sampling reason, the isolates were cultured in blood samples, suggesting a diagnostic sampling reason rather than screening. Twelve patients (12/39, 31%) had been admitted >24 hours in a hospital abroad in the previous two months, namely four in Southern Europe, two in South America, two in Eastern Africa, two in Western Asia, one in Northern Africa, and one in Southeast Asia. Sixteen of the non-Ukrainian patients were admitted to the ICU at the moment of sampling, and 21 patients (including seven of the ICU patients) had (severe) comorbidities.

Discussion

In 2024, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 15% of these isolates, data on carbapenemase tests (phenotypically or genotypically) performed by the participating MMLs, were available in the ISIS-AR database. The majority of the isolates without carbapenemase test results were sensitive for tobramycin, which might explain the absence of these data, since these isolates are not tested for carbapenemase in accordance with the Dutch national guideline for detection of highly resistant microorganisms[4]. Of the 144 carbapenem-resistant isolates with carbapenemase test results, 25 were positive for carbapenemase production. Not all phenotypically carbapenem-resistant *P. aeruginosa* isolates are routinely tested on carbapenemase production or carbapenemase genes in the MMLs, and such results are also not always routinely included in the data submitted to the surveillance system.
The proportion of carbapenem-resistant *P. aeruginosa* in ICUs returned to pre-COVID-19 levels in 2022 and remained more or less stable in 2023 and 2024, after two years in which this proportion was remarkably lower. An important source for acquisition of carbapenemase-carrying *P. aeruginosa* in ICU patients, is contaminated environmental sources or acquisition originating from patient-to-patient transfer.[5] Possibly, intensified hygienic measures in ICUs during the COVID-19-pandemic have decreased the transmission from environment to patients or between patients.
Due to the Ukraine/Russia war, Ukrainian patients migrated or were transferred to multiple European countries, sometimes carrying highly

resistant microorganisms.[6] Consequently, the 2024 results of the enhanced CPPA surveillance submitted via Type-Ned were very different to those of 2020 and 2021. Twenty-two percent of the patients with CPPA isolates in the enhanced surveillance in 2024 were Ukrainian patients, thereby introducing *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{NDM-1}}$ carrying CPPA in the Netherlands, and thus leading to a changed molecular epidemiology. Up until 2021 the most predominant (61%) carbapenemase-encoding allele in CPPA was *bla*$_{\text{VIM-2}}$, in 2022 this was reduced to 16% and dominated the *bla*$_{\text{NDM-1}}$ allele in 33% of the isolates until 2024. CPPA with *bla*$_{\text{IMP-1}}$ were only found in Ukrainian patients.
For more than one-third of the CPPA-positive persons in Type-Ned CPE/CPPA no additional epidemiological data were available. However, based on the available information we can conclude that more than 40% of the patients had been hospitalized abroad and more than half had comorbidities reported.
Unfortunately, it is not yet possible to get a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because not all laboratories routinely perform tests for carbapenemase production (which is in accordance with the Dutch national guideline), and because only a selection of the relevant isolates and data were submitted to one or both of the surveillance systems ISIS-AR and Type-Ned in 2024. Therefore, the data as shown here are most likely an underestimation of the numbers present in the Netherlands.

Conclusions

- In 2024, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 15% of these isolates, information was reported on tests for carbapenemase production; of these, 17% produced carbapenemase. The proportion of carbapenem-resistant *P. aeruginosa* in ICUs returned to pre-COVID-19 levels in 2022 and remained stable in 2023 and 2024, after two years during the COVID-19 pandemic in which this proportion was remarkably lower.
- In 2024, two percent of the total number of *P. aeruginosa* isolates in ISIS-AR was MDR and 68% of these MDR isolates were carbapenem-resistant.
- Twenty-two percent of the CPPA isolates in the enhanced CPPA surveillance in 2024 were from samples of Ukrainian patients.
- The predominant (41%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was *bla*$_{\text{VIM-2}}$. In contrast, in 2022-2023 the dominant carbapenemase encoding allele in CPPA was *bla*$_{\text{NDM-1}}$.*bla*$_{\text{GES-5}}$, *bla*$_{\text{VIM-11}}$ and *bla*$_{\text{IMP-13}}$ are regarded rare carbapenemases in the Netherlands.
- Ukrainian patients carried CPPA with *bla*$_{\text{IMP-1}}$ or *bla*$_{\text{NDM-1}}$, thereby contributing to the changing molecular epidemiology of CPPA in the Netherlands since 2022.
- A total of 83% of the carbapenemase-producing *P. aeruginosa* had MICs for meropenem above the EUCAST defined clinical breakpoint (8 mg/L).

Conclusions (continued)

- The majority (88%) of the Ukrainian patients with CPPA had been hospitalized in a hospital abroad >24 hours, while this proportion was 31% among non-Ukrainian patients.
- Data from both ISIS-AR and the enhanced surveillance via Type-Ned could not give a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because laboratories did not always routinely perform tests for carbapenemase production, and/or submitted only a selection of the relevant isolates and data to one or both of the surveillance systems.

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5.2.4 *Carbapenem-resistant Acinetobacter baumannii-calcoaceticus complex*

Introduction
Acinetobacter baumannii is a common nosocomial pathogen. The *A. baumannii-calcoaceticus* complex entails the closely-related species *A. baumannii*, *A. calcoaceticus*, *A. nosocomialis*, *A. pittii*, *A. seifertii* and *A. lactucae* (also known as *A. dijkshoorniae*). While the incidence of multidrug-resistant (MDR) *A. baumannii* in the Netherlands is low, the emergence of MDR *A. baumannii-calcoaceticus* complex with intrinsic and acquired resistance mechanisms is a problem of global concern.[1] Therefore, in 2024 the World Health Organization ranked carbapenem-resistant *A. baumannii* as third organism in the 'priority: critical' group.[2]

Methods
Data on carbapenem-resistant *A. baumannii-calcoaceticus* complex (CRAB) were obtained from ISIS-AR and the national enhanced CRAB surveillance via Type-Ned.

Prevalence of carbapenem-resistant A. baumannii-calcoaceticus complex based on ISIS-AR
From the ISIS-AR database the first *A. baumannii-calcoaceticus* complex isolate per patient per year was extracted for the period 2020-2024. To avoid overestimation of the percentage carbapenem-resistant *A. baumannii-calcoaceticus* complex caused by active screening for highly resistant isolates, only data on diagnostic cultures (as categorized by the reporting laboratory) from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus were included in the analysis. Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (applying the R breakpoint of 8 mg/L or 15 mm zone diameter) and/or imipenem (R breakpoint of 4 mg/L or 21 mm zone diameter). The numbers were based on a selection of 39 laboratories (out of a total of 48 laboratories in the Netherlands), which provided complete data on the past five years (2020 to 2024).

Molecular characteristics of carbapenem-resistant and/or carbapenemase-producing Acinetobacter baumannii-calcoaceticus complex and patient related characteristics based on Type-Ned
In 2022, a pilot CRAB surveillance via Type-Ned was performed, and was subsequently implemented in the national surveillance for highly resistant microorganisms. All but one Dutch medical microbiology laboratories (n=47) participate, however, participation is voluntary, and not all laboratories will submit all eligible isolates. MMLs are requested to submit *A. baumannii-calcoaceticus* complex isolates to the RIVM with an MIC for meropenem of >8 mg/L and/or an MIC for imipenem >4 mg/L and/or with carbapenemase production and/or with a detected carbapenemase-encoding gene. A restriction is that the laboratory can only send the first isolate per person per year. The RIVM allows consecutive isolates from the same person if these are CRAB with other carbapenemase-encoding gene combinations when compared to the first isolate. The RIVM confirms the species by MALDI-ToF, determines the MIC for meropenem by Etest (bioMérieux), and detects carbapenemase

production by the carbapenem inactivation method (CIM).[3] The presence of carbapenemase-encoding genes are assessed by PCR (carba-PCR on *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{OXA-48-like}$ + *mcr-1*). An Acinetobacter-specific OXA-23-like, OXA-51-like and OXA-58-like PCR is not performed anymore. Next-generation sequencing (NGS) and nanopore sequencing is performed for all isolates that are CIM positive and have an MIC for meropenem of >8 mg/L as assessed by Etest. The data described in this chapter are based on the first unique CIM-positive *A. baumannii-calcoaceticus* complex species/carbapenemase-encoding allele combination per person with a person ID for 2024, and all of these isolates collected in 2024 were included.
Since Ukrainian patients who migrated due to the Ukraine/Russia war regularly carried highly resistant microorganisms[4], samples without a person ID from persons with country of origin Ukraine were included for further analysis if it was confirmed that it represented a unique person, based on sex, age and postal code. Other samples without a person ID (n=2) were excluded from further analysis, because in these cases duplication of CRAB cannot be determined.
Based on whole-genome multi-locus sequence typing (wgMLST), genetically closely related CRAB isolates are grouped in genetic clusters and assigned consecutive cluster numbers. A genetic cluster is defined per bacterial species and includes ≥2 CRAB isolates that differ ≤15 wgMLST alleles.[5] Assigning genetic cluster numbers in the CPE/CPPA surveillance started in 2020, but the genetic cluster numbers in the results of this report include all sequenced *A. baumannii-calcoaceticus* complex isolates available from (pilot) surveillance studies by the RIVM.[6] Except the first isolate, clusters of multiple isolates from the same patient, including over multiple years and/or submitted by different laboratories, were not counted. In addition to submitting an isolate, Dutch laboratories are also requested to fill in a clinical/epidemiological questionnaire on characteristics of the patient from whom the CRAB isolate was obtained.

Results

Prevalence of carbapenem-resistant A. baumannii-calcoaceticus complex
Based on the ISIS-AR database, 1% (18/1,501) of the diagnostic *A. baumannii-calcoaceticus* complex isolates were carbapenem-resistant in 2024 (Table 5.2.4.1). The percentage of carbapenem-resistant *A. baumannii-calcoaceticus* isolates from inpatient departments increased in the past five years and was generally higher among diagnostic samples from inpatient departments and intensive care units (ICUs) compared to other types of healthcare settings. The observed proportion of resistance fluctuated over the years 2020-2024, which may be influenced by the low absolute numbers of tested and resistant isolates (Figure 5.2.4.1).

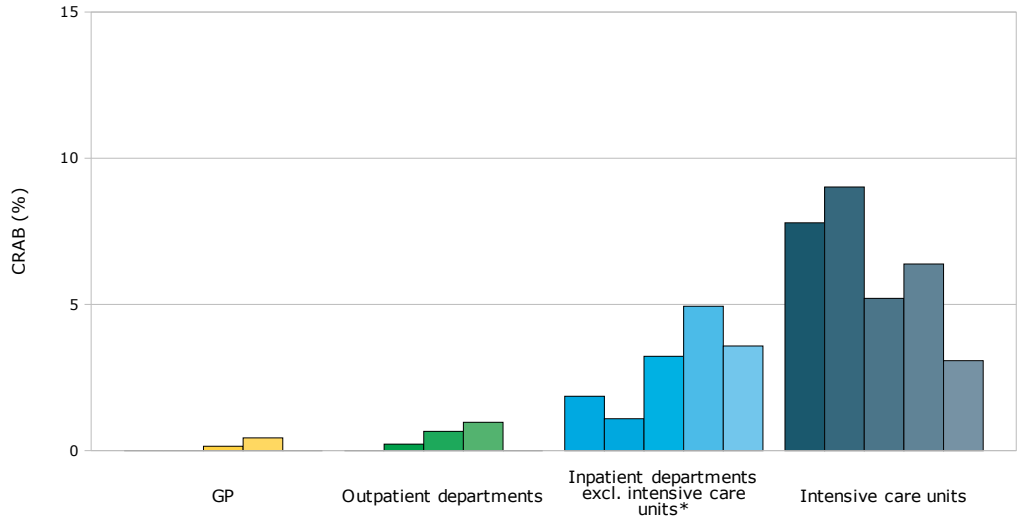
Table 5.2.4.1 *Carbapenem-resistant A. baumannii-calcoaceticus complex in the Netherlands in 2024, based on ISIS-AR data*

Sampling location	Tested isolates, N	Carbapenem-resistant *A. baumannii-calcoaceticus* complex, N (%)
General practitioners	608	0 (0.0)
Outpatient departments	381	0 (0.0)
Inpatient departments excl. intensive care units	447	16 (4) ↑
Intensive care units	65	2 (3)
Total	**1,501**	**18 (1.2)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *A. baumannii-calcoaceticus* complex isolate per patient was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (8 mg/L or 15 mm) and/or imipenem (4 mg/L or 21 mm).

Figure 5.2.4.1 *Percentages of carbapenem-resistant A. baumannii-calcoaceticus complex in the Netherlands (from left to right 2020 to 2024), based on ISIS-AR data*



Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years.
*Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
The first diagnostic *A. baumannii-calcoaceticus* complex isolate per patient per year was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2024 EUCAST breakpoints for meropenem (8 mg/L or 15 mm) and/or imipenem (4 mg/L or 21 mm).

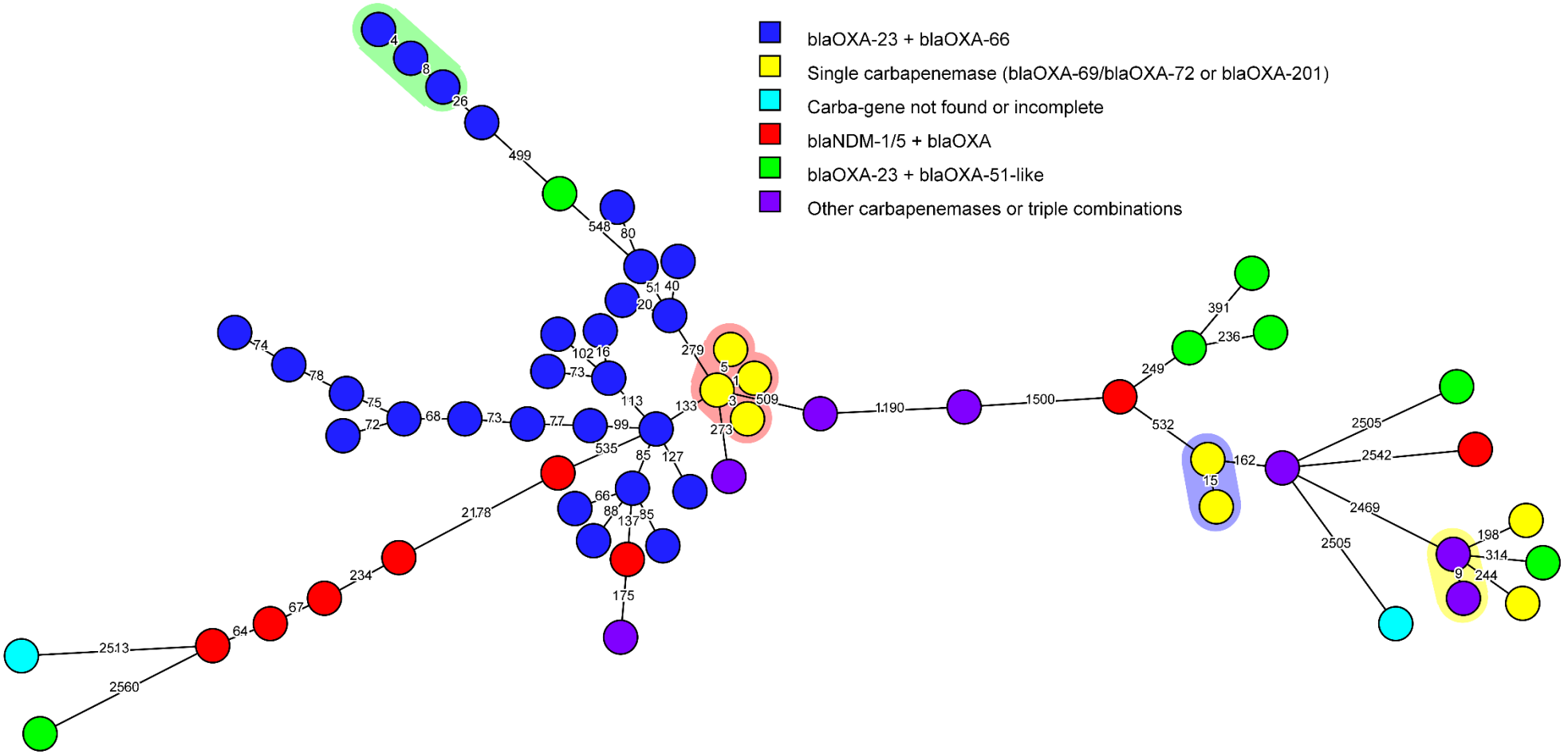
Molecular characteristics of carbapenem-resistant and/or carbapenemase-producing Acinetobacter baumannii-calcoaceticus complex (CRAB)

In the enhanced surveillance, the RIVM received 63 suspected carbapenem-resistant *A. baumannii-calcoaceticus* complex isolates from both diagnostic and screening samples collected in 2024 through Type-Ned, and all produced carbapenemase according to the CIM. Of the *A. baumannii-calcoaceticus* complex isolates, 97% (61/63) had an MIC for meropenem above the clinical EUCAST R breakpoint of 8 mg/L and are designated CRAB. The 63 *A. baumannii-calcoaceticus* complex isolates were from 63 patients and these isolates were submitted by 35 MMLs. Sixteen of the 63 isolates (25%) were obtained from Ukrainian patients. From 61/63 isolates whole-genome sequencing data was available and analysis revealed that 29 of the 61 *A. baumannii-calcoaceticus* complex isolates (48%) carried the *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$ carbapenemase gene combination, of which two harboured an additional *bla*$_{\text{OXA-72}}$ gene. Seven isolates carried *bla*$_{\text{OXA-23}}$ in combination with an OXA-51-like carbapenemase allele (*bla*$_{\text{OXA-69}}$, *bla*$_{\text{OXA-82}}$, *bla*$_{\text{OXA-90}}$, *bla*$_{\text{OXA-91}}$, or *bla*$_{\text{OXA-378}}$). Six isolates harboured *bla*$_{\text{NDM-1}}$ in combination with one (n=4, *bla*$_{\text{OXA-94}}$) or two other carbapenemase genes (n=2, *bla*$_{\text{OXA-23}}$/*bla*$_{\text{OXA-66}}$ or *bla*$_{\text{OXA-23}}$/*bla*$_{\text{OXA-69}}$). One isolate carried *bla*$_{\text{IMP-1}}$ and one isolate carried *bla*$_{\text{NDM-5}}$ in combination with *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$. Seven *A. baumannii-calcoaceticus* complex isolates carried *bla*$_{\text{OXA-72}}$ alone (n=2) or in combination with another carbapenemase gene (n=3, either *bla*$_{\text{OXA-66}}$, *bla*$_{\text{OXA-69}}$ or *bla*$_{\text{OXA-90}}$) or two carbapenemase genes (*bla*$_{\text{OXA-23}}$/*bla*$_{\text{OXA-66}}$). Fifteen *A. baumannii-calcoaceticus* complex isolates carried other more rare carbapenemase genes alone (n=9), or carbapenemase genes were not found or incomplete (n=2). Of the sixteen *A. baumannii-calcoaceticus* complex isolates from Ukrainian patients, eight harboured a *bla*$_{\text{OXA-23}}$ allele in combination with an *bla*$_{\text{OXA-51}}$-like carbapenemase allele (n=7 with *bla*$_{\text{OXA-66}}$, and n=1 with *bla*$_{\text{OXA-69}}$). No *bla*$_{\text{NDM}}$-type carbapenemases were detected in CRAB from Ukrainian patients.

The genetic relations of the 59/61 sequenced *A. baumannii-calcoaceticus* complex isolates were assessed by performing wgMLST (Figure 5.2.4.2). This revealed that most of the isolates were unrelated to other isolates. Four groups of 2, 2, 3 and 4 isolates respectively, differed in ≤15 wgMLST alleles and could therefore be regarded as genetic clusters.[5] Two of the four clusters were from Ukrainian patients, while the other two clusters were patients from the Netherlands. Furthermore, the isolates were diverse; 48 isolates were assigned to 30 distinct MLST types of which two MLST types, ST1816 (n=6) and ST195 (n=5), occurred most often. The carbapenemase genes and combinations hereof were randomly distributed among the isolates in the minimum spanning tree (Figure 5.2.4.2).

The following sample materials were reported: thirty-two *A. baumannii-calcoaceticus* complex were from screening swabs, ten were from wound samples, four from urine samples, two from pus, and the remainder from other isolation sites. The majority (57/63) of the *A. baumannii-calcoaceticus* complex were obtained from materials sent in by hospitals.

Figure 5.2.4.2 *wgMLST-based minimum spanning tree of 59/63 A. baumannii-calcoaceticus complex isolates from patients sampled in 2024, based on enhanced CRAB surveillance data. Each node represents an isolate, the numbers on the connecting lines indicate allelic distances between isolates. A colored halo indicates ≥2 isolates differing ≤15 wgMLST alleles*



Clinical/epidemiological characteristics of patients with carbapenem-resistant and/or carbapenemase-producing A. baumannii-calcoaceticus complex

Clinical/epidemiological questionnaire data in Type-Ned were available for 31 of the 63 persons with an *A. baumannii-calcoaceticus* complex isolate in 2024. Thirty samples were acquired within a hospital setting. Five (5/31, 16%) of these were acquired from patients admitted to the ICU at the moment of sampling and 25 (25/31, 81%) were acquired from patients admitted to a non-ICU hospital department. One sample was from a patient in a long-term care facility. Twenty-three patients were male and the median age was 69 years (range 20-92 years). Eight questionnaires concerned Ukrainian patients. For 2/8 (25%) of the Ukrainian patients, diagnostics of an infection was mentioned as the reason for taking the sample. Seven of these Ukrainian patients had been admitted in a Ukrainian hospital in the previous two months (six >24 hours, and one <24 hours). The Ukrainian patient who was not recently hospitalized abroad was already a known carrier of CRAB. Twenty-three questionnaires concerned non-Ukrainian patients. In these patients, 2/23 samples (9%) were taken for diagnostic purposes. Eighteen patients (18/23, 78%) had been admitted >24 hours in a hospital abroad in the previous two months, namely eight in Southern Europe, three in Western Asia, two in Eastern Africa and four patients each in different global regions. For one patient the country of recent hospital admission was unknown. For three patients, no risk factor for carrying CRAB was known. Five of the non-Ukrainian patients were admitted to the ICU at the moment of sampling, and eleven patients (including two of the ICU patients) had comorbidities.

Discussion

In 2024, in ISIS-AR, 1% of *A. baumannii-calcoaceticus* complex in diagnostic isolates were resistant to carbapenems. Since the absolute numbers in diagnostic samples in the Netherlands are low, the resistance percentages vary considerably over the years, and analysis of a trend in resistance levels over the past few years is difficult. Still, it is clear that the proportion of carbapenem-resistant *A. baumannii-calcoaceticus* complex in inpatient departments and ICUs are higher compared to resistance levels in isolates from general practitioners or outpatient departments. Although *A. baumannii-calcoaceticus* complex is regarded as a predominantly nosocomial pathogen, a large proportion of the diagnostic isolates in the ISIS-AR database were cultured in samples taken in a general practitioner's setting, which also include samples from long-term care facilities. Still, all carbapenem-resistant isolates were sampled in hospital settings.

The 2024 results of the enhanced CRAB surveillance submitted via Type-Ned revealed a genetically highly diverse, and highly resistant CRAB population in the Netherlands. Twenty-five percent of the *A. baumannii-calcoaceticus* complex isolates in the enhanced pilot surveillance in 2024 were from samples of Ukrainian patients, and none of these carried bla_{NDM}-like carbapenemases in contrast to isolates from non-Ukrainian patients. Unfortunately, it is not yet possible to get a complete overview through the past 5 years of carbapenem-resistant *A. baumannii-calcoaceticus* in the Netherlands, as the pilot surveillance started in 2022 and was implemented in the national surveillance in 2024. The absolute numbers of carbapenem-resistant *A. baumannii-calcoaceticus* complex isolates which were analyzed from the ISIS-AR database are not directly comparable to the absolute numbers included in the enhanced CRAB surveillance via Type-Ned, for a number of reasons. For the analysis of resistance percentages in ISIS-AR, only diagnostic samples are included, because the inclusion of screening samples would introduce bias towards higher resistance percentages as a result of selective testing methods for screening samples in laboratories. Furthermore, the selection of included laboratories differs between both surveillance systems and only a selection of the eligible isolates was submitted to Type-Ned in 2024. Therefore, the data as shown here might be an underestimation of the total CRAB proportion present in the Netherlands. The most important risk factor for patients to be infected or colonized with carbapenem-resistant *A. baumannii-calcoaceticus* complex is recent admission in a hospital abroad.[4]

Conclusions

- In 2024, in ISIS-AR, 1% of *A. baumannii-calcoaceticus* complex in diagnostic isolates were resistant to carbapenems, with the highest resistance proportions in samples from in-hospital patient departments and ICUs.
- The *A. baumannii-calcoaceticus* complex isolates in the Netherlands are diverse with a high variety of intrinsic and acquired carbapenemase encoding genes, and *A. baumannii-calcoaceticus* complex were from 30 distinct MLST types of which ST1816 (n=6) and ST195 (n=5) occurred most often.
- The predominant (48%, 29/61) carbapenemase-encoding gene combination in *A. baumannii-calcoaceticus* complex was *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$, and 11% (7/61) carried a *bla*$_{\text{NDM}}$-like carbapenemase.
- All Ukrainian patients with carbapenem-resistant *A. baumannii-calcoaceticus* complex had been recently hospitalized in a Ukrainian hospital or were known CRAB carriers. Additionally, the vast majority of the non-Ukrainian patients had been hospitalized in a hospital abroad recently and hospitalization abroad can therefore be considered as a risk factor.

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5.2.5 *Methicillin-resistant Staphylococcus aureus (MRSA)*

Introduction
The Netherlands remains a country with a low MRSA prevalence. This is most probably explained by the strict infection prevention rules (also known as "search and destroy" MRSA policy) in healthcare institutes and the constricted use of antibiotics. To gain insight in the prevalence of MRSA in the Netherlands, several surveillance systems are in place. The ISIS-AR database contains, among others, information regarding *S. aureus* culture and susceptibility testing results from routine practices in medical microbiology laboratories (MML). In addition, since 1989, a more enhanced molecular MRSA surveillance at a national level is performed by the RIVM which includes the submission and molecular typing of MRSA isolates. For this enhanced surveillance, the Type-Ned system is used to collect data in order to monitor the occurrence of MRSA, the molecular characteristics of circulating MRSA types and clinical/epidemiological characteristics of persons with MRSA.[1]

Methods
Prevalence of MRSA and antibiotic resistance among MRSA isolates, based on ISIS-AR
Data were selected from 39 laboratories that continuously reported complete data to the ISIS-AR database during 2020 to 2024. The first diagnostic *S. aureus* isolate per person per year was included from wound/pus only (from general practitioners (GPs)), or from blood, cerebrospinal fluid, urine, lower respiratory tract, or wound/pus (from outpatient departments, inpatient departments (excl. intensive care units, incl. emergency departments and intensive care units), cultured between 2020 to 2024. Prevalence of MRSA was calculated as the percentage of *S. aureus* isolates for which the MRSA confirmation test (presence of *mecA* or *mecC* gene or *pbp2*) was positive, or, if these tests were lacking, laboratory interpretation for cefoxitin was R, or, if no data on a cefoxitin test was available, laboratory interpretation for flucloxacillin/oxacillin was R. Additionally, the proportion of MRSA was calculated for *S. aureus* isolates from blood only. For the isolates that were identified as MRSA, resistance levels and time trends were calculated for a set of relevant antibiotics by setting of care. For more details on isolate selection and calculation of resistance levels within ISIS-AR, see section 5.1.1.1.

Molecular characteristics of MRSA and patient related characteristics based on Type-Ned
For the enhanced MRSA surveillance, Dutch laboratories are requested to submit MRSA isolates via the Type-Ned system which are obtained from patients during routine practices. Molecular typing is performed by multiple-locus variable number of tandem repeat analysis (MLVA), in which detection of the *mecA, mecC* and *lukF-PV* gene (encoding *pbp2* and Panton-Valentine leucocidin, respectively) are additionally incorporated. MLVA types that differ on one locus in MLVA profile were combined into MLVA-complexes. Since 2020, one isolate per person within a three-year period is eligible to be submitted. Isolates in the database were categorised as either diagnostic (isolated from samples of infection-related materials, i.e., blood, cerebrospinal fluid, sputum, pus, urine or wound) or screening (isolated from human MRSA-screening

materials, i.e. swabs from throat, nose, skin, ear, perineum and/or rectum). MRSA of MLVA-complex MC0398 were previously predominantly found in people with livestock contact and therefore labelled as livestock-associated MRSA (LA-MRSA).
The first MRSA isolate per person sampled in the period 2020 to 2024 was included, with the exception that only the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person. Samples without a person ID were only included for further analysis if it was confirmed to represent a person not included yet, based on MLVA type, sex, age and postal code, or based on MLVA type, sex, and age if the postal code was unknown. Samples from non-human origin, isolates from Caribbean laboratories and isolates lacking a *mecA* or *mecC* gene were also excluded from molecular analysis.
A semi-random selection of MRSA isolates is analysed through whole-genome sequencing (WGS). It concerns a random selection of 40 isolates per month that meet the following criteria: one isolate per person (per three years), per MLVA type, per laboratory. Isolates were not sequenced in case no person ID was available (e.g. asylum seekers and neonates) or when the surveillance questionnaire was not filled out. All liquor and blood-derived isolates that are not part of the initial selection are additionally included. Sequencing data were used for multi-locus sequence typing (MLST) using SeqSphere software and antimicrobial resistance genes were identified using the ResFinder database.
A clinical/epidemiological questionnaire on person characteristics is requested to be completed for each submitted MRSA isolate, except for persons who are part of a contact tracing investigation. Questionnaires related to isolates from employees in a healthcare facility that were screened as part of a local screening programme were excluded. Clinical/epidemiological data from the persons with included isolates are described for 2024 and compared with the previous four years, for all isolates combined and after stratification into diagnostic and screening isolates.

Results

Prevalence of MRSA and antibiotic resistance among MRSA isolates, based on ISIS-AR

In ISIS-AR, the overall proportion of diagnostic *S. aureus* isolates in 2024 that was identified as MRSA was 3% (n=1,862/62,773). A higher proportion of 4% was seen for cultures requested by GPs (wound/pus only) and for intensive care units, while a proportion of 2% was observed for outpatient departments (Table 5.2.5.1). In blood isolates only, the prevalence of MRSA in 2024 was 2% (n=81/4,089). Figure 5.2.5.1 shows the trends in MRSA prevalence from 2020 to 2024 in diagnostic isolates, by setting of care. The prevalence of MRSA increased slightly among cultures requested by GPs (wound/pus only) (from 3% in 2020 to 4% in 2024), among cultures of patients from inpatient departments (excl. ICU) (from 2% in 2020 to 3% in 2024) and among cultures of ICU patients (from 3% to 4%). The prevalence of MRSA for isolates from outpatient departments remained stable at 2%.

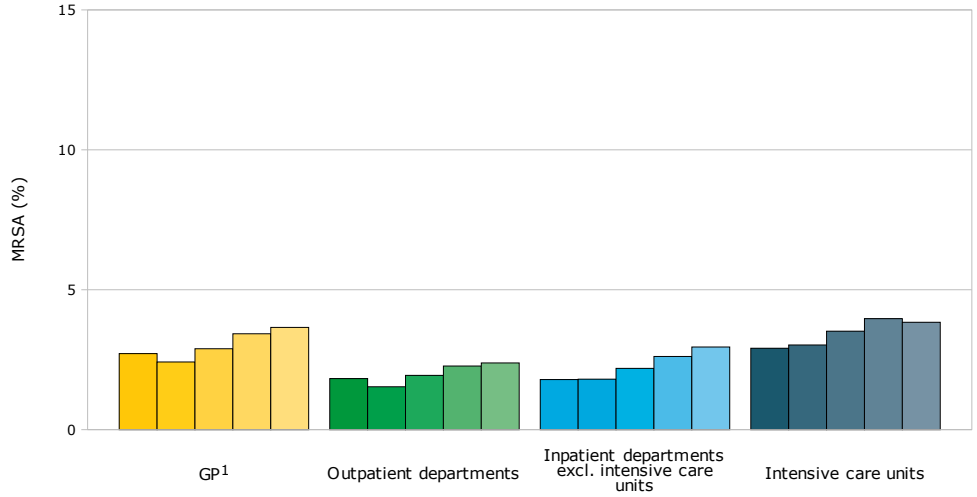
Table 5.2.5.1 *Methicillin-resistant S. aureus (MRSA) in the Netherlands in 2024, based on ISIS-AR data*

Healthcare setting	Tested isolates, N	MRSA, N(%)
GP[1]	18,800	687 (4)
Outpatient departments	24,493	584 (2)
Inpatient departments excl. intensive care units	17,708	523 (3)
Intensive care units	1,772	68 (4)
Total	**62,773**	**1,862 (3)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

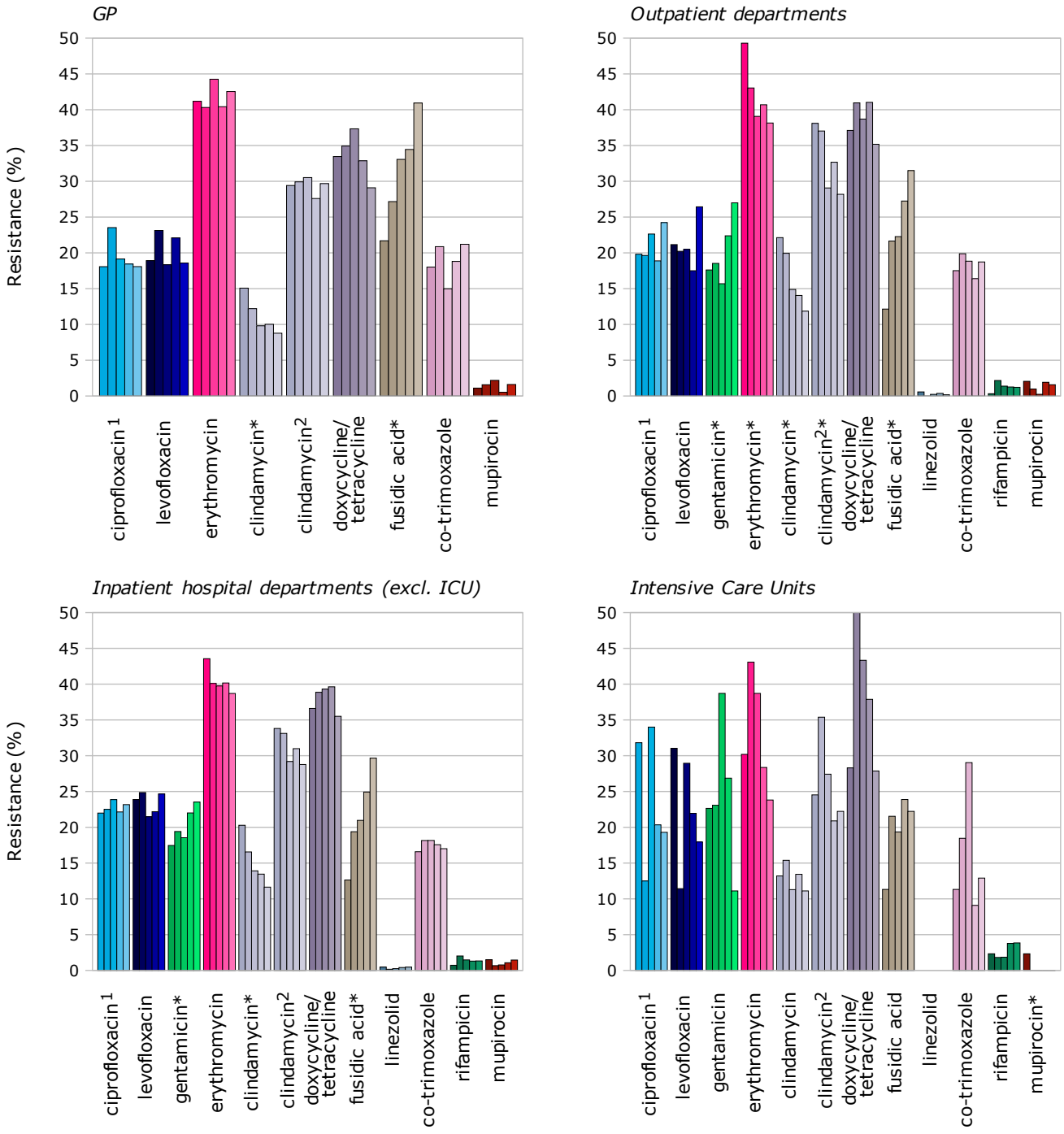
Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic *S. aureus* isolate per patient was selected.
The prevalence of MRSA isolates was based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2). If these tests were lacking, prevalence was based on re-interpretation of test-values for cefoxitin according to EUCAST 2024 or, if no data on a cefoxitin test was available, for oxacillin.
[1] From GP patients only wound or pus isolates were selected.

Figure 5.2.5.1 *Trends in methicillin-resistant S. aureus (MRSA) in the Netherlands (from left to right 2020 to 2024), based on ISIS-AR data*



Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years.
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
The first diagnostic *S. aureus* isolate per patient per year was selected.
The prevalence of MRSA isolates was based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2). If these tests were lacking, prevalence was based on re-interpretation of test-values for cefoxitin according to EUCAST 2024 or, if no data on a cefoxitin test was available, for oxacillin.
[1] From GP patients only wound or pus isolates were selected.

Figure 5.2.5.2 *Trends in antibiotic resistance (from left to right 2020 to 2024) among diagnostic MRSA isolates in the Netherlands, based on ISIS-AR data*



* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
[1] Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.
[2] Including inducible resistance. For the method used to estimate clindamycin resistance including inducible resistance, see section 5.1.1.1.
Note: the number of isolates from the ICU tested for each antibiotic is <100 for each year.

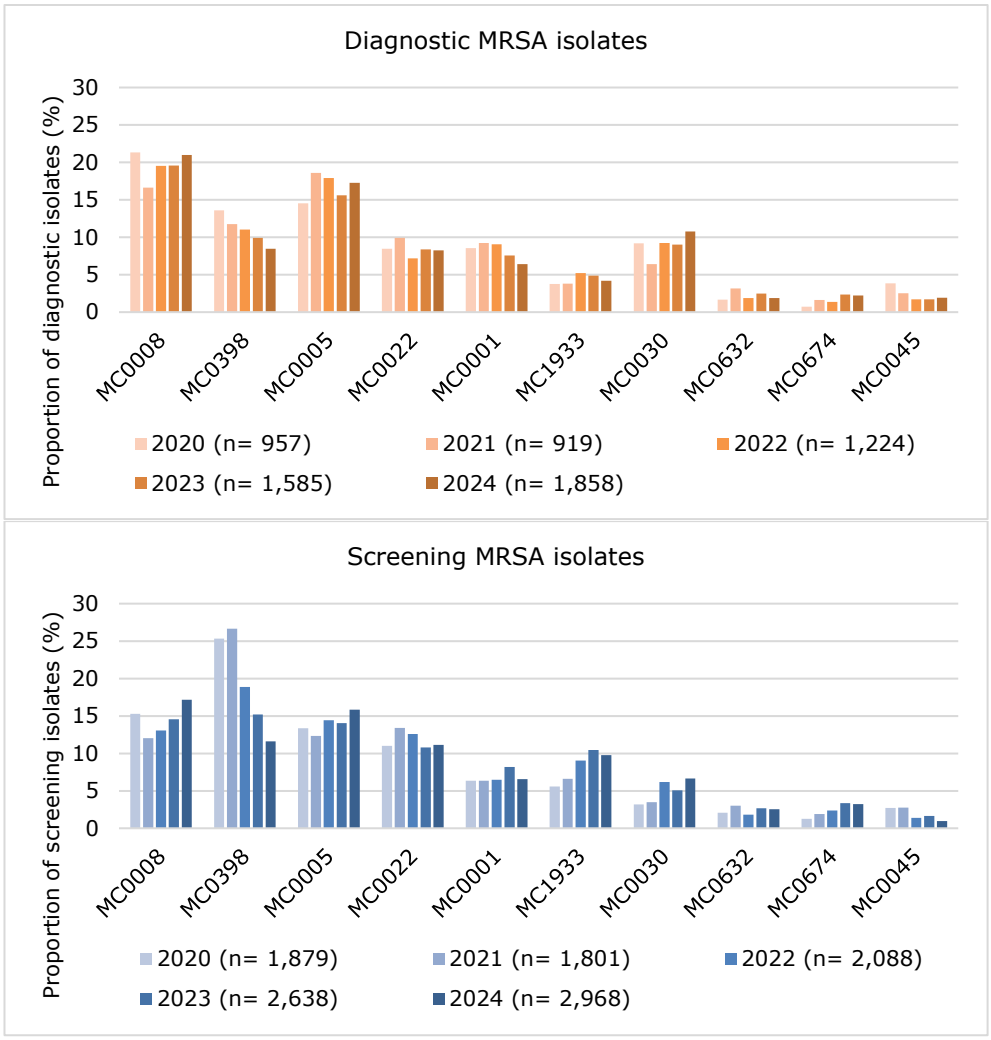
Antibiotic resistance among MRSA isolates based on ISIS-AR
Five-year trends in resistance among MRSA isolates for a set of relevant antibiotics are shown in figure 5.2.5.2. In cultures requested by GPs resistance levels for clindamycin (excl. inducible resistance) decreased from 15% in 2020 to 9% in 2024 and in cultures from outpatient departments from 22% in 2020 to 12% in 2024 and inpatient departments (excl. ICU) from 20% in 2020 to 12% in 2024. Resistance levels for gentamicin in isolates from the outpatient departments increased from 18% in 2020 to 27% in 2024 and in isolates from inpatient departments (excl. ICU) from 17% in 2020 to 24% in 2024. Fusidic acid resistance levels increased from 22% in 2020 to 41% in 2024 in cultures requested by GPs, from 12% to 31% among isolates from outpatient departments and from 13% to 30% among isolates from inpatient hospital departments (excl. ICU).

Molecular characteristics of MRSA based on Type-Ned
In 2024, the RIVM received 5,733 *S. aureus* isolates that were *mecA* or *mecC* gene positive and 4,839 isolates fulfilled the selection criteria specified in the methods section. The absolute number of MRSA isolates included in the analysis in 2024 is higher compared to previous four years (minimum: 2,736 isolates in 2021, maximum: 4,236 isolates in 2023) and also higher compared to the annual numbers in the pre-COVID-19 years. It is important to mention that during the COVID-19 pandemic that started in 2020 there were significantly lower numbers of submitted MRSA isolates compared to previous years.
The majority of isolates were cultured from samples submitted to the MML by hospitals (n= 3,053; 63%), followed by GPs (n= 1,342; 28%), municipal health services (n= 185; 4%) and long-term care facilities (LTCFs) (n= 144; 3%). Based on the sample material, 61% (n= 2,968) of the isolates were isolated from screening samples. A total of 1,858 isolates (38%) were considered diagnostic isolates, of which the majority were cultured from wound material or pus/punctate/biopsy (1,476/1,858; 79%) and 51 isolates were cultured from blood (3%). For 13 isolates, the material was unknown. The above-mentioned percentages are largely comparable to previous years. The absolute numbers of both diagnostic and screening isolates were lower compared to pre-pandemic during the COVID-pandemic and have clearly increased each year since 2022 (from 1,224 in 2022 to 1,858 in 2024 for diagnostic isolates and from 2,088 in 2022 to 2,968 in 2024 for screening isolates).
In 2024, 16 methicillin-resistant *Staphylococcus argenteus* (MRSArg) isolates were submitted, of which 15 (94%) were screening isolates.

For 2024, the MRSA population consisted of 1,183 different MLVA-types. The majority (1,107 MLVA types; 4,600 isolates) could be grouped into 24 MLVA-complexes (MCs). For the remaining 76 MLVA-types (239 isolates) no MC could be assigned. The most frequently identified MCs were MC0008 (n= 901 isolates; 19%), MC0005 (n= 796; 16%), MC0398 (LA-MRSA; n= 503; 10%) and MC0022 (486; 10%). Like in the previous years, the proportion of MC008 was higher in diagnostic (21%) than in screening isolates (17%), whereas the proportion of MC0398 (LA-MRSA) was higher in screening isolates (12%) than in diagnostic isolates (8%). During the 2020-2024 surveillance period, there has been a decreasing trend in the proportion of MC0398 in both diagnostic and

screening isolates (Figure 5.2.5.3). Furthermore, MC1933, MC0008, MC005 and MC0030 are increasing among screening isolates, MC0045 is decreasing among screening isolates and MC0001 is decreasing among diagnostic isolates.

Figure 5.2.5.3 *Trends in the ten most frequently identified MLVA-complexes of MRSA in the Netherlands (2020 to 2024) among diagnostic and screening isolates, based on enhanced MRSA surveillance data*



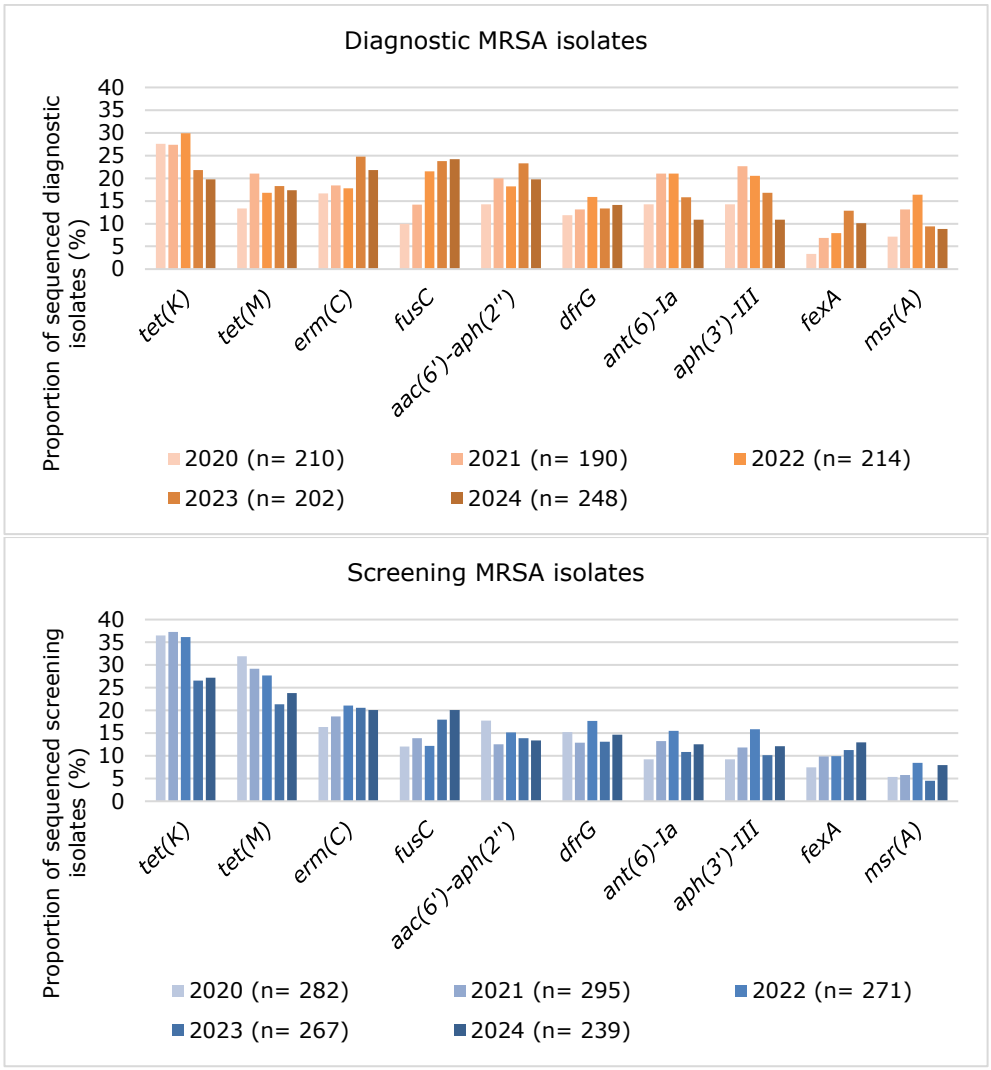
The graph displays the proportions of MLVA-complexes per sampling year.
The first MRSA isolate per person sampled in the period 2020 to 2024 was selected, with the exception that the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person.

Of the 4,839 isolates submitted in 2024, 26 (0.5%) contained the *mecC* gene and no *mecA* gene, of which 15 were diagnostic isolates. Overall, 27% of the isolates (n= 1,314) were Panton-Valentine Leukocidin (PVL) positive. Except for 2021 (21%), the proportions of PVL-positive isolates were comparable in previous years. Of the 1,314 PVL-positive isolates, 559 (43%) were screening isolates and 752 (57%) were diagnostic isolates. Like in previous years, the proportion of PVL-positive isolates was higher in diagnostic isolates (752/1,858 isolates; 40%) than in screening isolates (559/2,968 isolates; 19%). In 2024, 27% of diagnostic LA-MRSA (MC0398) isolates (43/157) were PVL-positive,

which is higher compared to 2020-2022 (4-21%) and slightly lower compared to 2023 (32%). The percentage of PVL-positives among non-LA-MRSA diagnostic isolates was 42% (709/1,701) in 2024. Importantly, PVL-positive LA-MRSA isolates are often not associated with livestock contact. The majority of PVL-positive LA-MRSA isolates belonged to MLVA types MT0569 (19/43; 44%) and MT2306 (6/43; 14%). Only 7% of screening LA-MRSA isolates (25/345) were PVL-positive (compared to 20% in non-LA-MRSA), which is comparable to previous years.

In 2024, 487 (10%) of the MRSA isolates included for molecular analysis were also analysed through whole-genome sequencing. The isolates belonged to 60 different multi-locus sequence types (STs), of which ST5 was most frequently identified (n= 70; 14%), followed by ST398 (n= 57; 12%), ST8 (n= 46; 9%) and ST22 (n= 46; 9%) and ST6 (n=42; 9%). In the period 2020-2024, the most frequently identified ST among sequenced PVL+ LA-MRSA was ST1232 (91%; 31/34). Among *S. argenteus*, the majority (12/16; 75%) belonged to ST2250.
Besides *mecA*, the antimicrobial resistance (AMR) genes *blaZ* (encoding beta-lactamase; 94%) and *tet(K)* (encoding tetracycline resistance ribosomal protection protein; 30%) were most frequently identified in the genomes of MRSA isolates obtained between 2020 and 2024. The other nine most frequently identified AMR genes in MRSA isolates putatively encode for resistance against tetracyclin (*tet(M)*), erythromycin (*erm(C)*), fusidic acid (*fusC*), aminoglycosides (*aac(6')-aph(2'')*, *ant(6)-Ia*, *aph(3')-III*), trimethoprim (*dfrG*), amphenicol (*fexA)* and macrolide, Streptogramin B resistance (*msr(A)*). There is a trend of increasing proportion of isolates containing the *fusC* or *fexA* gene in both diagnostic and screening isolates (Figure 5.2.5.4).[1,2] In addition, the proportion of diagnostic isolates containing the *aac(6')-aph(2'')* gene is increasing. A decrease is observed in the proportion of screening isolates containing the *tet(M)* gene and the proportion of both diagnostic and screening isolates with the *tet(K)* gene (Figure 5.2.5.4).

Figure 5.2.5.4 *Trends in the ten most frequently identified antimicrobial resistance genes in MRSA in the Netherlands (2020 to 2024), based on enhanced MRSA surveillance data*



The graph displays the proportion of isolates in which the depicted antimicrobial resistance genes were identified per sampling year.
The *mecA* and *mecC* genes are not included since their presence was part of the isolate inclusion criteria. Furthermore, the *blaZ* gene was not included, since this was present in almost all isolates.
MRSA isolates that were sequenced and fulfilled the inclusion criteria for molecular analysis are included.

MRSA clusters of special interest

In 2024, MRSA was the second most frequent micro-organism in hospital outbreaks (11/33) reported to the Early warning and response meeting for Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR). For HRMO outbreaks in LTCFs it was the only reported micro-organism (n=17, see Chapter 5.2.7). In addition, MRSA was mentioned two times in the weekly report of the general Early warning and response meeting in 2024.

- October 2024. A follow-up was reported on MRSA outbreaks on neonatology wards, reported to the SO-ZI/AMR. This concerns multiple MLVA types.
- November 2024. An increase of fusidic acid resistant MRSA was reported. This concerned multiple MLVA types, including the impetigo-

associated MT4627, that started localised in a region in the east of the country in 2019 and slowly spread in the south-western part of the country in the following years.
Another remarkable cluster was with MRSA MT0240, PVL-positive, among students in the west of the country, that was first seen in 2023, and expanded in 2024. As MT0240 is a rather common MLVA type of MRSA, a genetic cluster of two or more MT240 isolates can only be noticed after WGS.

Clinical/epidemiological characteristics of patients with MRSA based on Type-Ned
In 2024, the persons with an MRSA isolate submitted to Type-Ned, included in the analysis (n=4,839) had a median age of 36 (range 0 - 102) years and 2,734 (56%) were male. Based on information provided at sample submission, diagnostic purposes were the reason for sampling in 48% of the persons (2,318/4,839), which is comparable to the 48-50% of the two last years but much higher than before. Screening was the reason for sampling in 49% (2,356/4,839): in 40% (1,939/4,839) screening was performed because of presumed increased risk for MRSA carriage including active surveillance, and in 9% (417/4,839) because the person was part of a contact tracing investigation. For 3% (n=165) the reason of sampling was unknown.
For 65 of the 4,839 (1%) persons it was recorded in the questionnaire that they were employees in a healthcare facility that were tested as part of a local screening programme, and for 1,590 persons, including contacts in a contact tracing investigation, no additional data were available. Therefore, additional epidemiological questionnaire data for 2024 were available for 64% (n=3,086) of the persons, 5 percentage points less than last year.
In table 5.2.5.2 a selection of the clinical/epidemiological data of included persons is summarized. Seventy-two percent (2,222/3,086) were sampled in the hospital, of which 44% in outpatient departments, 28% in inpatient departments, 4% during their stay in the intensive care unit and 24% on other departments. In the group of persons that were sampled for screening/active surveillance, the large majority (95%) met the SRI[3] risk category 1, 2, or 3[2], whereas in diagnostic isolates this proportion was - as expected - much lower (44%). This percentage is comparable to 2021-2023 (44-46%). Work-related exposure to livestock animals was reported for 2% of persons with diagnostic samples and 13% of persons with samples that were taken for screening/active surveillance. The latter varied between 17 and 25% in the years before. The proportion of persons for which this is unknown however increases, up to 54% in 2024. The main group of livestock animals to which this group was exposed were pigs (79%), and from 92% of persons with a livestock-related profession an LA-MRSA was sampled. Among persons with diagnostic samples with LA-MRSA, the proportion with work related exposure to livestock animals was 14% (n=14/98) in 2024, compared to 22-36% in 2020-2023. In persons with LA-MRSA that were sampled for screening/active surveillance this percentage was 70% (n=127/181).
The proportion of persons for whom hospitalization abroad for at least 24 hours during the previous two months was recorded was 125/1,739 (7%), comparable to 2022-2023 (8%), after a drop in 2020-2021 due to travel restrictions during the COVID-19 pandemic (5-6%). The main geographic regions of previous hospitalizations in 2024 were Southern

Europe (16%), Western Asia, including Turkey (15%), and Western Europe (13%). Turkey was the country most frequently reported (n=13/126, 10%), followed by Egypt and Morocco (7 and 6% respectively).
In 547/1,805 (30%) persons with underlying illness reported (underlying illness unknown in 1,281 persons), skin disorders (185/547, 34%) and diabetes (168/547, 31%) were the most mentioned underlying conditions.

Table 5.2.5.2 *Epidemiological data of 3,086 MRSA positive persons (excluding employees of healthcare facilities) with an isolate in the enhanced MRSA surveillance system, with a sampling date in 2024*

	Diagnostic and screening combined[a]		Diagnostic		Screening / active surveillance	
Characteristic	**Data available (N)**	**n (%)**	**Data available (N)**	**n (%)**	**Data available (N)**	**n (%)**
Sampling location (hospital only)						
Outpatient departments	2,222	977 (44)	1,097	495 (45)	1,099	468 (43)
Inpatient departments (excluding Intensive Care Units)	2,222	612 (28)	1,097	292 (27)	1,099	319 (29)
Intensive Care Units	2,222	98 (4)	1,097	45 (4)	1,099	51 (5)
Other/unknown	2,222	535 (24)	1,097	265 (24)	1,099	261 (24)
Risk factors						
Meeting SRI risk category 1,2, or 3[b,c]	2,608	1,833 (70)	1,277	565 (44)	1,295	1,233 (95)
Work-related exposure to livestock animals	2,126	155 (7)	1,099	19 (2)	1,006	134 (13)
Pigs	155	116 (75)	19	8 (42)	134	106 (79)
Cattle	155	33 (21)	19	9 (47)	134	24 (18)
Other/unknown	155	6 (4)	19	2 (11)	134	4 (3)
Hospitalization abroad >24 hours during the previous two months	1,739	125 (7)	955	28 (3)	770	95 (12)
Western Europe	125	16 (13)	28	3 (11)	95	13 (14)
Western Asia (including Turkey)	125	19 (15)	28	3 (11)	95	16 (17)
Southern Europe	125	20 (16)	28	4 (14)	95	16 (17)
Other/unknown country	125	70 (56)	28	18 (64)	95	50 (53)
Living in asylum centre	2,854	770 (27)	1,448	104 (7)	1,359	659 (48)

[a] Including persons for whom the reason for sampling was unknown.
[b] This question did not appear in all questionnaires and is therefore not completed for all MRSA positive persons.
[c] SRI risk category 1: the person is known to be MRSA positive; risk category 2: person at high-risk for MRSA carriage; risk category 3: person at low-risk for MRSA carriage; risk category 4: person not suspected of MRSA carriage.

Discussion

In the ISIS-AR data, the proportion MRSA among diagnostic *S. aureus* isolates is 3%. MRSA isolates submitted via GPs, outpatient and inpatient departments show an increase in phenotypic fusidic acid resistance. Among sequenced diagnostic MRSA isolates in 2024 the fusidic acid resistance gene *fusC* is indeed the most common antimicrobial resistance gene (24%), besides beta-lactam resistance genes *mecA* and *blaZ*. The proportion of isolates with the *fusC* gene is increasing in both diagnostic and screening isolates. Fusidic acid is frequently used in case of skin infections, such as impetigo. In case of impetigo due to fusidic acid-resistant MRSA, both the first and second advised treatment for general practitioners are not effective.[3]

The high proportion of LA-MRSA MC0398 isolates is probably partially attributable to active screening of MRSA carriage in persons with professional exposure to livestock. Despite active screening, LA-MRSA is no longer the predominant MRSA clade and in the period 2020-2024 the absolute numbers of LA-MRSA screening isolates are decreasing. This could be (partially) the result of the decrease in livestock farms with pigs and veal calves and decrease in total no of broilers and pigs.[4]

With the interpretation of the surveillance results several factors should be taken into account. Within the ISIS-AR and Type-Ned surveillance, routine culture results or isolates from MMLs are collected. However, this can introduce overestimation of resistance percentages due to selective sampling by GPs, with cultures taken generally only in case of clinical therapy failure, which occurs to a lesser extent in hospital departments. Blood samples for culturing are taken routinely in case of suspected bloodstream infection or meningitis, and, therefore, isolates from blood cultures are considered to be the least biased to calculate resistance percentages. MRSA screening isolates originate from selective cultures for MRSA that do not detect methicillin sensitive isolates and cannot be used to calculate the percentage of MRSA among all *S. aureus*. Besides (changes in) selective sampling and culturing, (changes in) screening practices could also affect the numbers/percentages and trends in time. Therefore, we only included diagnostic isolates to assess MRSA prevalence and diagnostic and screening isolates are shown separately for Type-Ned data. In addition, misclassification of screening and diagnostic isolates might have occurred in the molecular results since distinction between screening and diagnostic isolates is solely based on the material of origin. Furthermore, only 10% of the MRSA isolates are included in the analysis of STs and resistance genes based on a semi-random selection of isolates for sequencing. In this selection, persons with no person ID available are excluded (e.g. asylum seekers and neonates). Laboratories with large numbers of MRSA and common MLVA-types are underrepresented and blood and liquor isolates are overrepresented.

Conclusions

- The overall proportion of routinely collected diagnostic *S. aureus* isolates that were MRSA positive in 2020-2024 was stable at a low level of 3%. A higher proportion of 4% was seen for cultures requested by GPs (wound or pus only) and for intensive care units.
- A strong increase was observed in phenotypic fusidic acid resistance among diagnostic MRSA isolates. This corresponds to a clear increase in the fusidic acid resistance gene *fusC* in both screening and diagnostic MRSA isolates.
- The absolute number of MRSA isolates submitted for the enhanced MRSA surveillance is higher compared to pre-COVID-19 pandemic levels, both among diagnostic and screening isolates.
- The most frequently identified MLVA-complexes in 2024 were MC0008 (19%), MC0005 (16%), MC0398 (LA-MRSA; 10%) and MC0022 (10%). Gradual shifts in the prevalence of MCs occurred throughout the years.
- In 2024, 40% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 19% of the screening isolates were PVL-positive.
- The top 10 most occurring antimicrobial resistance genes among MRSA isolates putatively encode for tetracycline, erythromycin, fusidic acid, aminoglycosides, trimethoprim, amphenicol and macrolide/streptogramin B resistance.
- The majority of persons with samples that were taken for screening/active surveillance, met SRI-risk category 1,2 or 3² (95%). The percentage for whom work-related exposure to livestock animals was the risk factor decreased from 26-27% in 2021-2022 and 17% in 2023 to 13% in 2024.
- Hospitalization abroad for at least 24 hours during the previous two months was recorded in 7% of the MRSA positive persons. The main geographic regions of recent hospitalizations abroad of MRSA positive persons were Southern Europe (16%) and Western Asia, including Turkey (15%).

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5.2.6 Vancomycin-resistant Enterococci

Introduction
In the last few years, a considerable number of Dutch hospitals have been confronted with outbreaks of vancomycin-resistant *Enterococcus faecium* (VRE_{fm}). There is no national surveillance program with centrally organised characterisation of VRE-strains in The Netherlands. Here we give an overview of available data that describe the epidemiology of VRE in The Netherlands.

Methods
VRE_{fm} outbreaks are reported through the Early warning and response meeting for Healthcare associated Infections and Antimicrobial Resistance (SO-ZI/AMR, see section 5.2.7). In addition, based on the national surveillance system of antimicrobial resistance, ISIS-AR, the proportion of vancomycin resistance in *E. faecium* isolates among patients in various healthcare settings in the Netherlands was determined. The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests (molecular detection of *vanA*/*B*), or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2024, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin. Both diagnostic isolates (isolates cultured from clinical material) and screening isolates (predominantly rectal swabs) were included. Numbers are based on data from 39 laboratories in the Netherlands that continuously reported to the ISIS-AR database in the past five years. The first diagnostic or screening *E. faecium* isolate per patient was selected.

Results
In 2024, 14 outbreaks with VRE_{fm} in the Netherlands were reported to the SO-ZI/AMR (see section 5.2.7), which were all in hospitals. This number of outbreaks is higher than the number of outbreaks that were reported since 2020 (range 5-9 outbreaks yearly in 2020-2023). It was comparable to the numbers in the years before 2019, when 10-15 outbreaks per year were reported. In total, since the start of SO-ZI/AMR in April 2012, 151 outbreaks with VRE_{fm} have been reported. The contribution of VRE_{fm} outbreaks to the total number of reported outbreaks in hospitals was substantial in the previous years, with a proportion varying between 20 and 32% of all reported outbreaks in SO-ZI/AMR yearly.
The percentage of diagnostic VRE_{fm} isolates of the total number of *E. faecium* isolates from general practitioners and (outpatient and inpatient) hospital departments in 2024 in the Netherlands based on ISIS-AR is shown in table 5.2.6.1. Figure 5.2.6.1 shows the trends in vancomycin resistance in diagnostic *E. faecium* isolates over the years. The proportion of diagnostic isolates with VRE_{fm} was persistently low, although slight increases can be seen at intensive care units.
The absolute numbers of VRE_{fm} isolates from screening samples of inpatient hospital departments (including intensive care units), from 39 laboratories continuously reporting to ISIS-AR show a range of 80-155 positive isolates per year, with the lowest number in 2020 and the highest number in 2024 (Table 5.2.6.2).

Table 5.2.6.1 *Vancomycin-resistant E. faecium (VRE_{fm}) in diagnostic isolates in the Netherlands in 2024, based on ISIS-AR data*

Type of department	Tested isolates, N	VRE, N (%)
General practitioner	523	1 (0.2)
Outpatient departments	609	3 (0.5)
Inpatient departments excl. intensive care units	3,052	17 (0.6)
Intensive care units	695	8 (1.2) ↑
Total	**4,879**	**29 (0.6)**

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

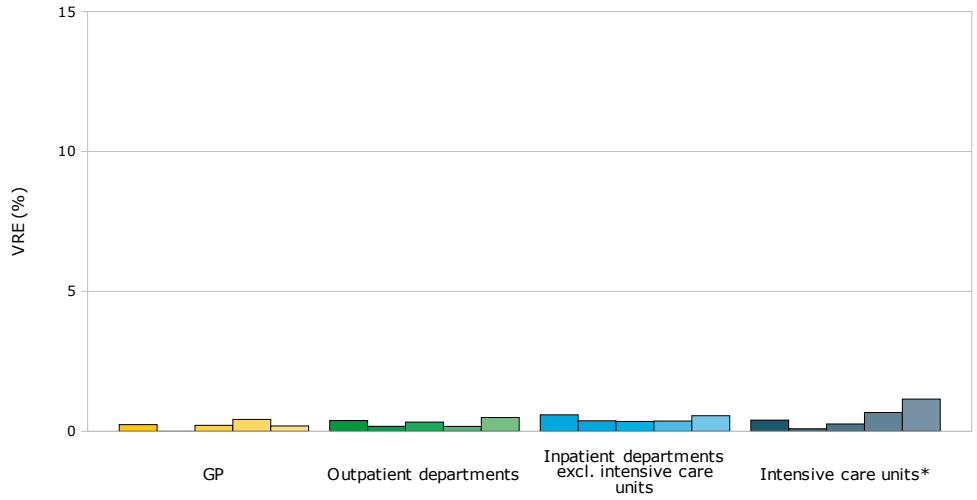
Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *E. faecium* isolate per patient was selected.
The prevalence of (VRE_{fm}) isolates was based on positivity of VRE confirmatory tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2024, with (VRE_{fm}) being defined as resistant to amoxicillin/ampicillin and vancomycin.

Table 5.2.6.2 *Absolute numbers of vancomycin-resistant E. faecium (VRE_{fm}) isolates in the Netherlands, 2020-2024, based on ISIS-AR data*

	GP and outpatient departments			Inpatient departments including intensive care units			Total		
Year	D	S	Total	D	S	Total	D	S	Total
2020	3	47	50	22	82	104	25	129	154
2021	1	46	47	13	80	93	14	126	140
2022	3	50	53	13	137	150	16	187	203
2023	3	38	41	15	113	128	18	151	169
2024	4	60	64	25	153	178	29	213	242

Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *E. faecium* isolate per patient was selected.
GP: general practitioners, D: diagnostic, S: screening
The prevalence of (VRE_{fm}) isolates was based on positivity of VRE confirmatory tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2024, with (VRE_{fm}) being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 5.2.6.1 *Trends in percentage of diagnostic VRE$_{fm}$ isolates of the total number of diagnostic E. faecium isolates in the Netherlands (from left to right 2020 to 2024), based on ISIS-AR data*



Numbers are based on a selection of 39 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic *E. faecium* isolate per patient per year was selected.
* Trend is significant and microbiologically relevant (for details see section 5.1.1.1).
The prevalence of (VRE$_{fm}$) isolates was based on positivity of VRE confirmatory tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2024, with (VRE$_{fm}$) being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

The number of reported VRE$_{fm}$ outbreaks in 2024 was higher than the numbers in 2020-2023, but comparable to the years before 2019. The decrease since 2020 could have been related to the COVID-19 pandemic, and a change in infection prevention measures. Although the number of screening samples that were tested is unknown, the absolute number of positive screening isolates continued to increase to pre-COVID levels (220 for 2018 and 159 for 2019).
Currently, there are no centrally collected data on molecular typing of VRE$_{fm}$ isolates in the Netherlands, even though the WHO listed VRE$_{fm}$ in the high-risk category.[1] Thus, there are no reliable data available on the molecular epidemiology of VRE$_{fm}$ in Dutch hospitals.

Conclusions

- The number of reported hospital outbreaks with VRE$_{fm}$ in 2024 was comparable to the numbers before 2019, but lower than in the past four years, a trend that is probably related to the COVID-19 pandemic.
- The proportion of VRE$_{fm}$ in infection-related isolates with *E. faecium* in various healthcare settings is still low (below 1%), exception ICU: 1.2%.
- The absolute number of positive screening VRE$_{fm}$ isolates continued to increase to the pre-COVID-19-period. The absolute number of diagnostic isolates is still low, but seems to show an increase as well.

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5.2.7 *Early warning and response meeting for Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR)*

Introduction

In 2012, the Early warning and response meeting for Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded. The initial purpose of the SO-ZI/AMR is to mitigate large-scale outbreaks of AMR in healthcare institutes and to prevent spread to other health care facilities through early warning and reporting.[1] Since 2015 long-term care facilities (LTCFs) are also requested to report outbreaks of highly-resistant microorganisms (HRMO). Since then, the name of the early warning and response meeting was changed to Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR).[2]
The SO-ZI/AMR consists of experts in the field of clinical microbiology, infection prevention, elderly care and public health and meets once a month. The SO-ZI/AMR assesses the risk of the outbreak to public health, monitors the course of the outbreak and facilitates – on request of the hospital or LTCF – in the acquisition of external expertise. An overview of active outbreaks is reported to professionals involved in infection prevention and control (IPC) on a monthly basis. Outbreaks of special concern are reported with more detailed information in the weekly report of the general Early warning and response meeting.[3]
Notifications are voluntary, though not without obligation. All hospitals and LTCFs have committed to participate in SO-ZI/AMR via their umbrella organizations (NVZ, NFU, Actiz).[4-6] Moreover in 2017, a financial compensation rule has been introduced by the NZA (Nederlandse Zorgautoriteit) to compensate for detection and control of HRMO outbreaks in LTCFs by national health care authorities, provided that these outbreaks are reported to the SO-ZI/AMR.[7]

Methods

Notifications and monthly updates of outbreaks are submitted through the web-based application system Osiris. Outbreaks can be reported by infection control practitioners, medical microbiologists, or elderly care physicians. During monthly meetings of the SO-ZI/AMR, each outbreak is categorized in one of four phases with 0 as lowest, III as highest risk for public health due to uncontrolled spread. In the monthly meetings, the SO-ZI/AMR (re-)assesses the phase of each notified outbreak which has not yet been (re-)classified as phase 0, based on the latest information, updated by the notifiers in Osiris. Once an outbreak is contained, it is re-classified as phase 0. Otherwise, the categories are: Phase I: transmission is under control, all necessary information on the situation is available, active monitoring is in place; phase II: active transmission cannot be ruled out, complete overview on the outbreak has not yet been achieved (active contact screening and/or source tracing and control is still ongoing); phase III: transmission is ongoing in spite of IPC measures, which are thus incomplete or not sufficiently effective.

Results

Table 5.2.7.1 provides an overview of the outbreaks (n=50) reported in 2024. These were reported by 41 different healthcare institutions: 33 outbreaks in hospitals and 17 in LTCFs (including 10 LTCF for elderly people, 1 rehabilitation centre, 2 LTCF for people with intellectual

disabilities, 1 LTCF for psychiatric care, 1 LTCF for youth care, and 2 home care organisations). Most outbreaks (n=47) ended before July 1 2025 and 3 continued after July 1 2025. The median number of patients (5) involved in outbreaks in hospitals was similar to the previous five years, except 2021 when this number was higher (12). In LTCFs the median number of patients involved was 4. The maximum number of involved patients was much higher in hospitals compared to LTCF (141 vs 15).
The number of reported outbreaks in hospitals with vancomycin-resistant *Enterococcus faecium* (VRE) (n=14) was higher than the number of VRE outbreaks reported yearly since 2020 (range 5-9 outbreaks yearly in 2020-2023). Five hospital outbreaks were with carbapenemase-producing (CP) gram-negatives (2 outbreaks with CP Enterobacterales and 3 with CP *P. aeruginosa*). The number of MRSA outbreaks in hospitals (n=11) was lower than in 2023, when an extraordinary high number of 20 MRSA hospital outbreaks were reported, but higher than the years before. Still in 2024, comparable to 2023, approximately half (6/11) of the MRSA outbreaks were related to pediatric or neonatal wards. Two outbreaks in separate hospitals were related to each other, because of an MRSA-positive healthcare worker who had been working in both hospitals. Another MRSA outbreak on a neonatal ward was related to an earlier outbreak notified in 2023, from which a yet unnoticed MRSA-positive baby was transferred from a neonatal intensive care unit in a large hospital to a smaller hospital, leading to introduction of MRSA in the second.
In LTCFs only MRSA outbreaks were reported in 2024, which was also the most reported HRMO in previous years. The total number of 17 MRSA outbreaks in LTCFs was substantially higher than earlier years since 2020, but as high as in the pre-COVID-19 period, with 17 MRSA outbreaks in LTCF reported in 2019 as well.

Overall, three outbreaks were classified as phase III. Two of these concerned extensive, long-lasting VRE outbreaks in hospitals. The first was reported to SO-ZI/AMR in August 2024 and involved over 140 *VanA* VRE-positive patients. The outbreak was not over yet at the moment of data analysis of this report (July, 2025), and the duration was at least eleven months. The second VRE hospital outbreak (also *VanA*) which was classified as phase III was notified to SO-ZI/AMR in December 2024. The total number of patients involved in this outbreak was over 120 and the duration was more than eight months. Finally a long-lasting outbreak of MRSA in an LTCF, a residential care home for elderly people, was assessed in phase III. The outbreak was notified to SO-ZI/AMR in November 2024, but had started in August of the same year. The MRSA outbreak strain was *mecA* positive and grouped into MLVA complex (MC)0008 and assigned MLVA type (MT)0008. In total, six MRSA-positive clients and four healthcare workers were involved.

Table 5.2.7.1 *Characteristics of outbreaks reported to the SO-ZI/AMR in 2024*

	Hospitals n=33	LTCFs[4] n=17	Total 2024 n=50
	n	n	n
Microorganism (resistance mechanism)[1]			
Staphylococcus aureus (MRSA)	11	17	28
Enterococcus faecium (VRE)	14		14
Pseudomonas aeruginosa (CPPA)	3		3
Enterobacterales (CPE) (various species)	2		2
Klebsiella pneumoniae (ESBL)	3		3
Highest level phase[2]			
phase I	4	5	9
phase II	27	11	38
phase III	2	1	3
Median number of patients[3] (range)	5 (1-141)	4 (1-15)	4 (1-141)
Duration outbreak			
<1 month	1	1	2
1-6 months	27	14	41
>6 months	5	2	7

n: number of outbreaks, LTCF: long-term care facilities
[1] MRSA= =methicillin-resistant *Staphylococcus aureus*; VRE=vancomycin-resistant *Enterococcus faecium*; CPPA=carbapenemase-producing *Pseudomonas aeruginosa*; CPE=carbapenemase-producing Enterobacterales; ESBL=extended-spectrum β-lactamase producing
[2] Outbreaks are categorized in one of four phases with 0 as lowest, III as highest risk. Once an outbreak is contained it is reclassified as phase 0. Phase I: transmission is under control, all necessary information on the situation is available, active monitoring is in place; phase II: active transmission cannot be ruled out, a complete overview on the outbreak has not yet been achieved (active contact screening and/or source tracing and control is still ongoing); phase III: transmission is ongoing in spite of infection control measures, which are thus incomplete or not sufficiently effective
[3] In three outbreaks, one patient and one or more health care workers were involved
[4] Including 10 LTCF for elderly people, 1 rehabilitation centre, 2 LTCF for people with intellectual disabilities, 1 LTCF for psychiatric care, 1 LTCF for youth care, and 2 home care organisations

Discussion

The total number of 50 outbreaks in 2024 was higher than in 2020-2023 (n=34, 27,36 and 44 in those years respectively), and more comparable to 2017-2019, when around 60 outbreaks were reported each year. This trend was similar for both hospitals and LTCF, showing that the attention to HRMO prevalence has raised again after the potentially waned vigilance during the COVID-19-pandemic and the years onwards. In LTCF, increased attention for IPC, and the financial compensation rule[7] may have increased the number of notifications and outbreak investigations, which is illustrated by the quickly contained MRSA outbreaks with only a small number of clients involved.
The long-lasting outbreaks assigned phase III by SO-ZI/AMR in 2024, emphasize the need for continued attention for IPC and well organized IPC infrastructures.

Conclusions

- In total, 50 outbreaks were reported to the SO-ZI/AMR in 2024, which is higher compared to 2020-2023, but comparable to the pre-COVID-19 years.
- The number of VRE outbreaks in hospitals was notably higher compared to previous years at n=14.
- Approximately half of MRSA outbreaks in hospitals are related to pediatric or neonatal wards.
- In LTCFs, all outbreaks concerned MRSA.
- Most outbreaks were classified as maximum as phase II and three outbreaks were classified as phase III, which concerned two long lasting VRE hospital outbreaks with high numbers of patients involved and an MRSA outbreak in an LTCF.

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5.2.8 *Neisseria meningitidis*

Introduction
Neisseria meningitidis can cause rare but life-threatening invasive meningococcal disease (IMD). Globally, there is concern about a rise in penicillin non-susceptible meningococcal isolates. Ongoing surveillance is required to assess development of antibiotic resistance among IMD isolates.

Methods
Isolates cultured from IMD patients are submitted for serogrouping and antibiotic susceptibility testing to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM, Amsterdam UMC) by all Dutch microbiological laboratories. Coverage is estimated to be 95% of all IMD cases.
The MIC for penicillin was determined by Etest using Mueller-Hinton Fastidious Agar (MHF) plates and incubation at 36°C under 5% CO2 for 18-24 h. The 2024 EUCAST criteria for penicillin resistance were applied (susceptible ≤0.25 mg/L; resistant >0.25 mg/L). In case of phenotypic resistance to penicillin, susceptibility to ceftriaxone was also assessed by Etest using MHF plates and incubation at 36°C under 5% CO2 for 18-24h. Isolates were classified as susceptible when MIC <0.125 mg/L, in line with EUCAST guidelines.

Results
From 2015-2024, 1,250 IMD cases were registered based on culture-positive blood or cerebrospinal fluid (CSF) sample, and culture-negative PCR-positive CSF or blood sample. For 23 culture-negative CSF PCR-positive cases, a co-submitted blood isolate was available to assess penicillin resistance. In total, 1,052 *N. meningitidis* isolates were available for the study period; 305 isolates from meningitis patients (based on CSF-positive culture or a PCR-positive CSF-sample plus blood isolate) and 747 isolates from patients with a positive blood culture only (non-meningitis IMD patients). The number of meningococcal isolates per year in this period ranged between 25 isolates (2021) and 186 isolates (2018).
In 2024, 33 (97%) of the 34 received CSF or blood isolates from meningitis patients were susceptible to penicillin, and one isolate was resistant (Table 5.2.8.1). For IMD blood isolates, 75 out of 77 (97%) isolates were penicillin susceptible and 2 (3%) were resistant (Table 5.2.8.2). Overall, in the past 10 years, only 10 (1.0%) out of 1,052 isolates displayed phenotypic penicillin resistance (Tables 5.2.8.1 and 5.2.8.2), but all these isolates were susceptible to ceftriaxone.

Discussion
Penicillin resistant isolates have been increasingly detected across the globe. In contrast, penicillin resistance is rare among meningococcal isolates from IMD patients in the Netherlands in the past decade.

Conclusions
- Penicillin resistance in *N. meningitidis* isolates is rare in the Netherlands.
- Ceftriaxone resistance was not observed.

Table 5.2.8.1 *Penicillin susceptibility of N. meningitidis strains isolated from CSF or blood (in combination with PCR-positive CSF) from meningitis patients, 2015-2024*

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2015	33	**100**	0	**0**	33
2016	37	**100**	0	**0**	37
2017	46	**100**	0	**0**	46
2018	54	**98**	1	**2**	55
2019	33	**100**	0	**0**	33
2020	14	**93**	1	**7**	15
2021	7	**100**	0	**0**	7
2022	18	**100**	0	**0**	18
2023	27	**100**	0	**0**	27
2024	33	**97**	1	**3**	34

* MIC values in mg/L

Table 5.2.8.2 *Penicillin susceptibility of N. meningitidis blood isolates from non-meningitis IMD patients, 2015-2024*

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2015	51	**100**	0	**0**	51
2016	100	**100**	0	**0**	100
2017	128	**99**	1	**1**	129
2018	129	**99**	2	**1**	131
2019	102	**100**	0	**0**	102
2020	39	**100**	0	**0**	39
2021	18	**100**	0	**0**	18
2022	38	**100**	0	**0**	38
2023	60	**97**	2	**3**	62
2024	75	**97**	2	**3**	77

* MIC values in mg/L

5.2.9 *Neisseria gonorrhoeae*

Introduction
Neisseria gonorrhoeae is a species of Gram-negative bacteria which can cause gonorrhoea after sexual transmission. Gonorrhoea is the second most common bacterial sexually transmitted infection (STI) in the Netherlands. Third generation cephalosporins, such as ceftriaxone and cefixime, are the current first-line treatment for gonorrhoea in most countries. In the Netherlands, cefotaxime was the first-line therapy for gonorrhoea from 2003-2006, and ceftriaxone from 2006 onwards. In the past, *N. gonorrhoeae* has developed antimicrobial resistance to all drugs used for treatment of gonorrhoea. While resistance to ceftriaxone has been reported in Europe only incidentally, resistance levels in the Asian-Pacific region surpass 5% in several countries.[1]

Methods
The national Gonococcal Resistance to Antimicrobials Surveillance (GRAS) programme started in 2006, collecting epidemiological data on gonorrhoea and resistance patterns of isolated strains obtained via Sexual Health Centres (SHC) across the Netherlands. In 2024, 15 out of the 24 SHC participated in GRAS, which together accounted for 81% of SHC gonorrhoea diagnoses. Diagnosis of gonorrhoea is made by PCR on patients' materials. For GRAS, additional culture and susceptibility testing using Etest, are performed. The aim is to perform culture and susceptibility testing for all gonorrhoea patients in these SHC, but due to logistical and financial restrictions in practice there is a culture performed for around 75% of PCR-positive patients. Isolates are tested for susceptibility to ciprofloxacin, cefotaxime, ceftriaxone, and azithromycin. Resistance levels are calculated using the EUCAST breakpoints for resistance.[2]

Results
The yearly national number of gonorrhoea diagnoses reported by SHC was around 6,000 from 2015 to 2020, and increased since then to 13,952 diagnoses in 2024. Between 2015 and 2024, the number of SHC participating in GRAS ranged from 10 in 2015 to 17 in 2018, but was mostly around 15. The percentage of diagnoses including a susceptibility test result was stable around 33% in the past 10 years (31.6% in 2024, Figure 5.2.8.1). In 2024, 75% of isolates were from men who have sex with men (MSM), 13% from women and 10% from heterosexual men. Gonococcal resistance to ciprofloxacin was around 30% between 2015 and 2018 but increased since then and was 59.2% in 2024. Resistance to cefotaxime has been slowly decreasing since 2018 and was 0.1% in 2024. For azithromycin, resistance increased from 3.2% in 2015 to 30.6% in 2023, but decreased in 2024 to 22.4%. No resistance was reported to ceftriaxone (Figure 5.2.8.2).

Figure 5.2.8.1 *Number of reported gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test result at Sexual Health Centres, 2015-2024*

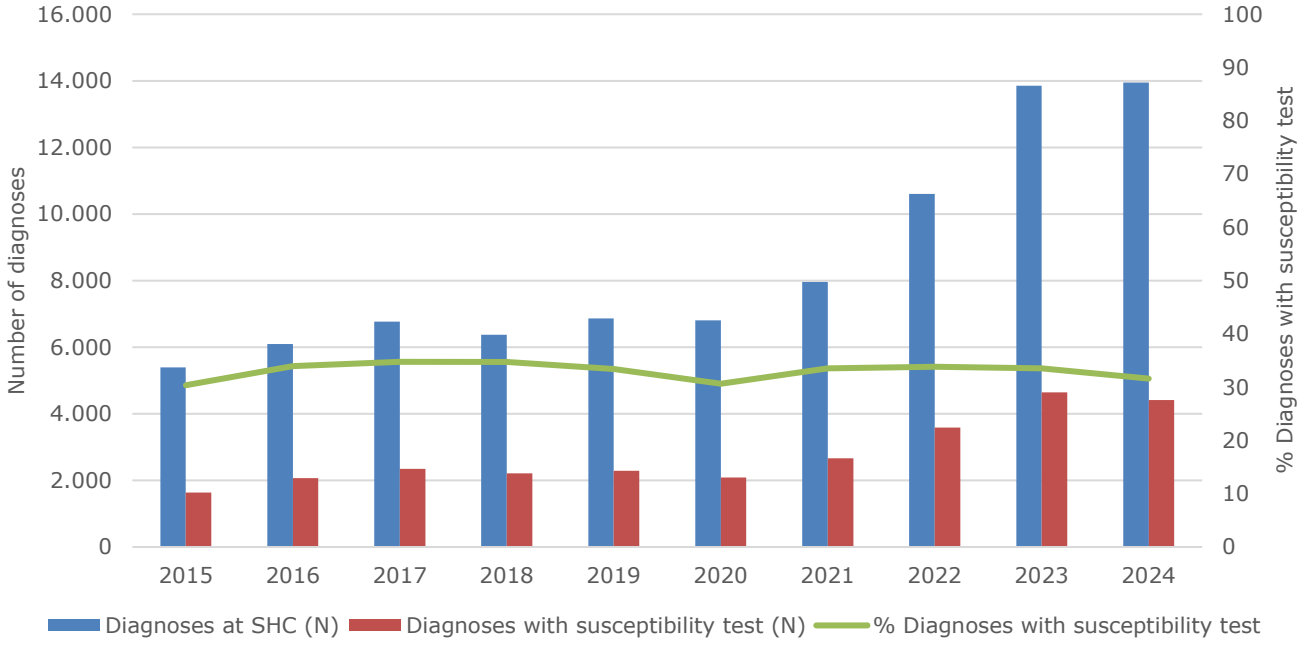
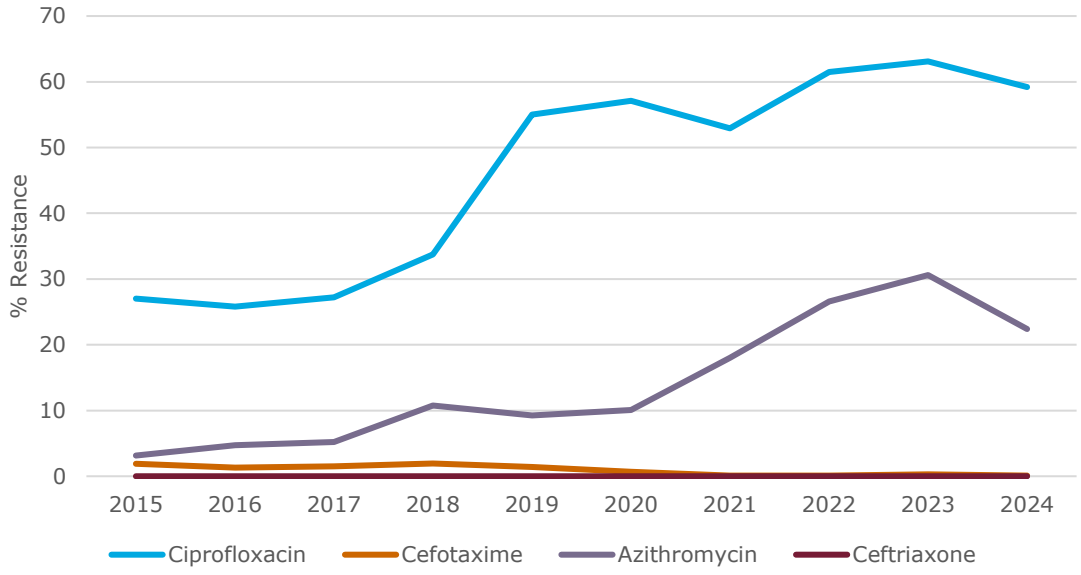


Figure 5.2.8.2 *Trends in antimicrobial resistance among Neisseria gonorrhoeae (following EUCAST breakpoints) in the Netherlands, 2015-2024*

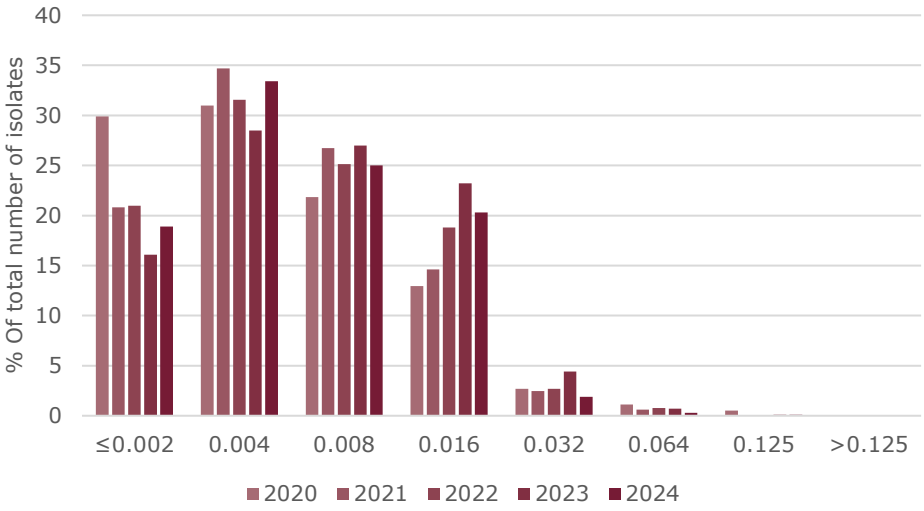


No resistance to ceftriaxone has been reported.

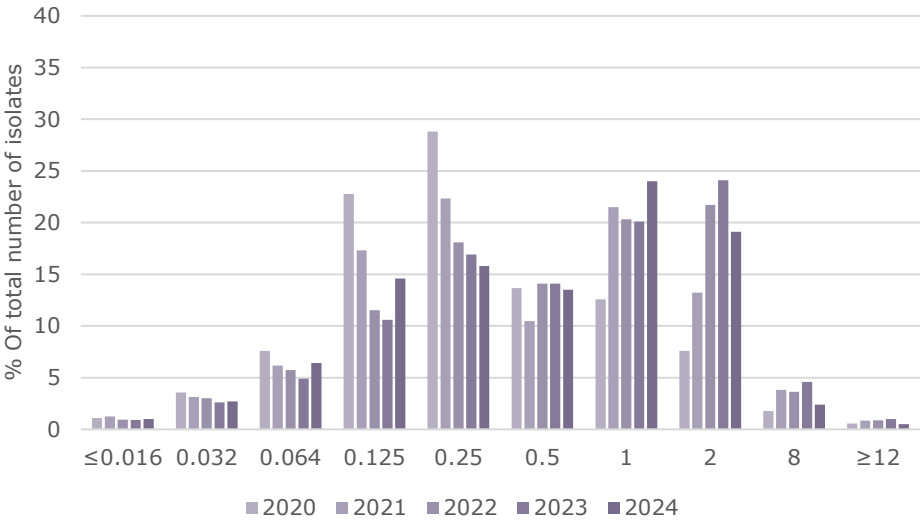
In the MIC distribution of ceftriaxone a shift is observed since 2019 where the proportion of isolates with an MIC ≤ 0.002 mg/L decreased and the proportion of isolates with slightly higher MIC values (MIC 0.008-0.032 mg/L) increased (Figure 5.2.8.3a). For azithromycin a shift towards higher MICs is also observed over time. (Figure 5.2.8.3b).

Figure 5.2.8.3 *MIC distributions of ceftriaxone and azithromycin for Neisseria gonorrhoeae, 2020-2024*

A: MIC distribution for ceftriaxone. Following EUCAST breakpoints, an MIC of >0.125 mg/L is considered resistant



B: MIC distribution for azithromycin. Following EUCAST breakpoints, an MIC of 1 mg/L is considered the epidemiological cut-off value for resistance



Discussion

As in previous years, in 2024 for one-third (31.6%) of all gonorrhoea diagnoses at the SHC susceptibility test results were available. Susceptibility test results are not available for all gonorrhoea cases because some SHC do not participate in GRAS, and within participating SHC only ~75% of PCR confirmed cases are cultured, of which ~50% are culture negative, making susceptibility testing impossible. In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500 or 1000 mg). Thus far, no ceftriaxone resistance has been reported despite the shift towards higher MIC

values. Yet, trends of increasing resistance have been observed for multiple antimicrobial agents monitored in GRAS. Resistance to ciprofloxacin more than doubled since 2016 and MIC values for azithromycin also increased in the past years. These findings are remarkable, because both ciprofloxacin and azithromycin are not first-choice treatments for gonorrhoea, according to guidelines in the Netherlands. However, both antibiotics are prescribed for a range of other infectious diseases, which might contribute to the decreasing gonococcal susceptibility. In 2024, the proportion of isolates resistant to azithromycin decreased. The cause for this decrease is yet unknown. Potentially, the change in treatment guidelines for chlamydia infections from azithromycin to doxycycline and the decrease in number of chlamydia diagnoses in 2024 could have contributed to reduced exposure to azithromycin of SHC clients, given that *Chlamydia trachomatis* and *N. gonorrhoeae* co-infections are common.[3] Alternatively, the import and/or changing transmission dynamics of specific *N. gonorrhoeae* lineages could contribute to changes in trends of resistance in the Netherlands as well.
The increasing resistance trends call for a continued effort to monitor the prevalence and emergence of antimicrobial resistance in gonococci, especially since also the number of gonorrhoea infections is rising.

Conclusions

- Thanks to the GRAS program, around 33% of gonorrhoea diagnoses from SHC include susceptibility testing results each year.
- No resistance to ceftriaxone, the current first-line treatment for gonorrhoea, has been reported. However, the MIC distribution has shifted towards higher MICs since 2020.
- Resistance to ciprofloxacin and azithromycin continues to be high.

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5.2.10 Mycobacterium tuberculosis complex

Introduction
Of all infectious diseases, tuberculosis (TB) remains one of the deadliest infectious diseases worldwide. Although the incidence is slowly declining globally, it has been estimated that a significant proportion of the global population is still latently infected by its main causative agent; *Mycobacterium tuberculosis*. In the Netherlands we have reached the elimination phase in the native population; more than 80% of TB cases currently diagnosed are acquired outside the Netherlands and/or in foreign-born persons.

Worldwide, there is a concern about the development and spread of resistant *M. tuberculosis*, which hampers the adequate treatment of tuberculosis. After an initial diagnosis of *M. tuberculosis* complex in a medical microbiological laboratory (MML), cultured isolates are always sent to the RIVM for confirmation of species identification, susceptibility prediction/testing and whole genome sequencing (WGS) typing of *M. tuberculosis* complex isolates in the Netherlands. These results are incorporated in the national TB surveillance and are used to guide tuberculosis therapy of individual patients, as well as to support cluster investigations. The national RIVM TB laboratory participates in the proficiency studies of the WHO for international TB reference laboratories to monitor the quality of the resistance testing.

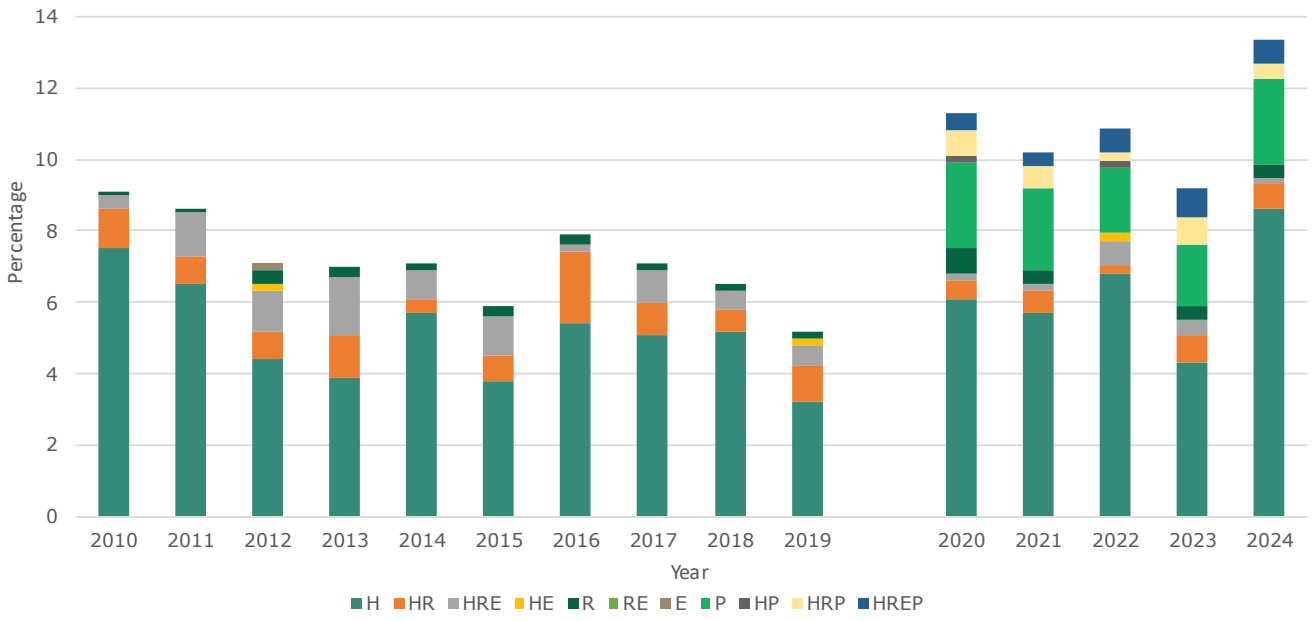
Methods
Around 30 MMLs in the Netherlands are involved in the diagnosis of TB and all send any *M. tuberculosis* complex isolate to the RIVM. These isolates are immediately subjected to WGS for susceptibility prediction and fingerprinting to identify potential epidemiological clusters. Fingerprinting data is used to support TB source contact tracing performed by Municipal Health Services. Before 2020, susceptibility testing was based on a phenotyping with Mycobacteria Growth Indicator Tubes (MGIT, BD USA). From 2020, all *M. tuberculosis* complex isolates are initially screened by WGS for the presence of resistance mutations confidently associated with a raised MIC to any of the first line drugs.[1] In the absence of such mutations, isolates are determined to be susceptible to standard first line therapy and no further testing is performed. If resistance mutations are detected, phenotypic resistance testing is performed to confirm the genotypic findings and to screen for additional undetected resistance.[1,2,3,4] As injectable drugs are no longer part of the TB treatment regimen, since 2020 we no longer routinely predict resistance against streptomycin. From 2020 onwards, we also monitor resistance to pyrazinamide (PZA), for which the combination of the results of WGS and phenotypic testing (a composite reference standard) yield more reliable predictions than phenotypic testing alone. Furthermore, problems with the supply of commercial phenotypic PZA assays (from 2024) have further increased the use and importance of WGS data for the prediction of PZA susceptibility. Comparisons of molecular and phenotypic resistance testing have been described by Jajou et al[2] and Walker et al[3]. These studies form the basis of the current testing algorithm.[4]

Results
After a fall in the number of cases between 2019 and 2022 and an increase of 11.5% in 2023, in 2024 the number of notified TB cases increased again by 8.3%; from 709 patients in 2023 to 768 patients in 2024.

In 2024, of the 768 notified cases, 536 (70%) were culture confirmed. Isolates from all these confirmed cases were received at the RIVM and subjected to WGS.
Approximately 87% of these isolates were assumed to be sensitive for first line antimycobacterial drugs as no relevant resistance mutations were detected by WGS. In 13% (72/536) of the isolates resistance to one or more antimycobacterial drugs was detected. This mainly concerned isoniazid (INH) resistance (Figure 5.2.10.1). In total 11 multi-drug resistant (MDR)-TB cases (defined as resistant to at least both isoniazid and rifampicin) were detected and 2 TB cases resistant to at least rifampicin (RR).
These observations were initially based on the detection of resistance mutations in WGS data from isolates for which resistance to at least one agent was predicted (and subsequently confirmed by phenotypic testing).
MDR and RR TB cases represented 2.4% (13/536) of the culture positive cases diagnosed in 2024, which is comparable to the combined MDR and RR resistance in 2023 (3.1%).

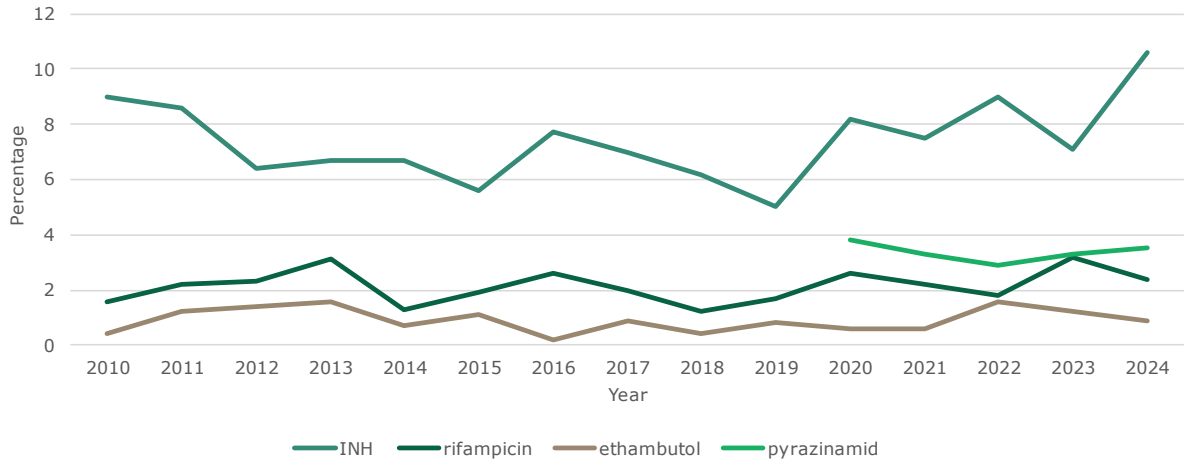
Discussion
In 2024, 13.4% (72/536) of the isolates tested in the Netherlands revealed some form of resistance, compared to 9.1% (47/517) in 2023. From 2024 we started monitoring the prevalence of fluoroquinolone resistance mutations in isolates sensitive to the first line drugs as these agents may be added to first line therapy. From 2024 a prediction of the fluoroquinolone susceptibility has been included in the standard report. The trend in fluoroquinolone resistance will be analyzed and reported in future years.
The number of MDR isolates, in the Netherlands, decreased slightly to 2.4% of all isolates, a total of 13 MDR/RR-TB cases. The extended hospitalization, complicated MDR-TB treatment, and contagious nature of this disease continues to justify special attention for these resistant infections.
Worldwide, resistance is an important aspect in TB control. As the majority of TB cases in The Netherlands are diagnosed in patients originating from high prevalence areas, it remains important to continue the structural surveillance to support the efforts to prevent and ultimately eliminate local transmission in particular of resistant strains.

Figure 5.2.10.1 *Percentage (combined) antibiotic resistance for M. tuberculosis complex isolates 2010-2024*



H=Isoniazid / INH, R=Rifampicin, E=Ethambutol and P=Pyrazinamide.
From 2020 the primary screen for resistance was based on WGS rather than phenotypic testing[4]. Prior to 2020 resistance to P was not monitored as the reliability is much higher for this drug when WGS-based gene mutations are used to support drug susceptibility or resistance.

Figure 5.2.10.2 *Percentage antibiotic resistance for M. tuberculosis complex isolates 2010-2024*



Conclusions

- Resistance to the first line antimycobacterial drugs remained almost stable over the last years, except in 2024 the percentage of INH resistance was higher compared to previous years. This is due to the growth of two INH resistant WGS clusters.
- Although the number of MDR-TB cases was fairly stable in recent years (average of 10/12 cases per year), in 2024 there was a slight decrease in the proportion of MDR cases to 13 (2.4% of culture confirmed cases) compared to 2023. This trend is carefully monitored.
- The increase of the number of TB cases in the Netherlands is still ongoing, in 2023 an increase of 11.5% and in 2024 an increase of 8.3% compared to the previous year. This may be in part a regression to the mean after the sharp decline in TB notifications in 2020-2022, which was presumably related to the COVID-19 pandemic.

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5.2.11 Helicobacter pylori

Introduction
Helicobacter pylori is a Gram-negative curved bacterium that resides only on the gastric epithelium. Primary colonization often occurs during childhood and can last a lifetime. The global prevalence of *H. pylori* carriage is estimated to range between 20%-30% in Northern and Central European countries, to over 70% in parts of Asia, Africa and Southern Europe.[1] *H. pylori* is an important factor in the etiology of a wide range of gastric disorders including peptic ulcer disease, chronic gastritis, Mucosa-Associated Lymphoid Tissue (MALT) lymphoma, and gastric cancer.[2] In the past decades, this highly prevalent infection has been treated with various antimicrobial regimens, and concerns about antimicrobial resistance in this pathogen are rising, also in the Netherlands.[3,4] In this section we describe for a selection of frequently used agents the prevalence and trends in antimicrobial resistance in *H. pylori* in the Netherlands during the period 2020-2024.

Methods
Data were selected from the ISIS-AR database. Isolates sampled from gastro-intestinal mucosa, pus, and normally sterile tissue or liquid were all included because we could not distinguish gastric specimens specifically. Further data selection as well as the calculation of resistance levels and time trends were executed using the same methods as those described in section 5.1.1.1.

Results
In total, 2841 *H. pylori* isolates were included, with 517 isolates being sampled in 2024. In table 5.2.11.1 resistance levels for 2024 are presented. Five-year trends in resistance are shown in figure 5.2.11.1.

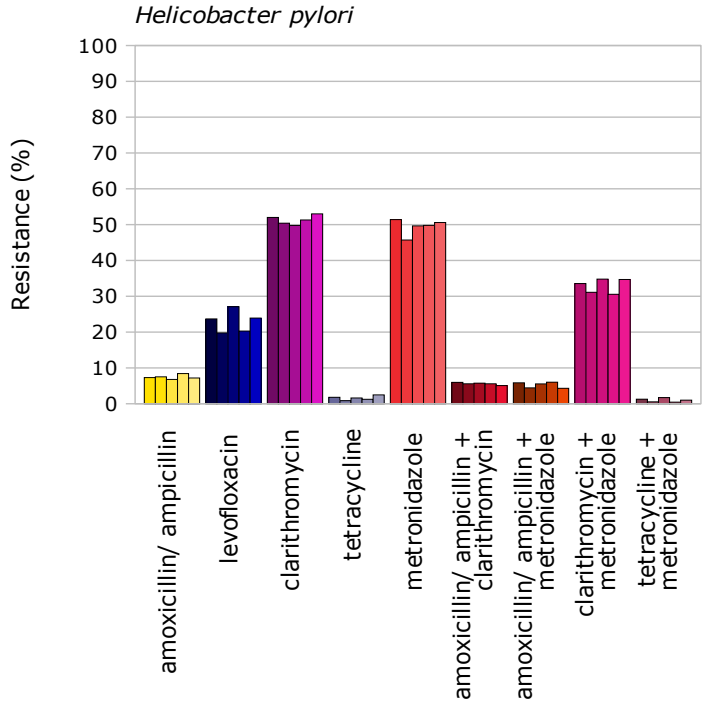
Discussion
For the culture of *H. pylori* and subsequent phenotypical antimicrobial susceptibility testing, a biopsy from the gastric epithelium is required. However, usually an *H. pylori* infection is primarily diagnosed using non-invasive methods such as a stool antigen test or a urea breath test. Only when empirical treatment was unsuccessful, a biopsy is likely to be performed. Therefore, the results may be biased towards higher resistance levels compared to resistance levels in the total population with an *H. pylori* infection. Nevertheless, because the degree of bias is assumed to be constant over the years, we expect the calculated time trends to be a valid estimate of the changes through the years.

Table 5.2.11.1 *Resistance levels (%) among 517 isolates sampled from gastro-intestinal mucosa, pus and normally sterile tissue or liquid of H. pylori, ISIS-AR 2024*

	H. pylori
Antibiotic	
amoxicillin/ampicillin	7
levofloxacin	24
clarithromycin	53
tetracycline	2
metronidazole	51
Empiric therapy combinations	
amoxicillin/ampicillin + clarithromycin	5
amoxicillin/ampicillin + metronidazole	4
clarithromycin + metronidazole	35
tetracycline + metronidazole	1

10 ↑	Significant and microbiologically relevant increasing trend since 2020.
10 ↓	Significant and microbiologically relevant decreasing trend since 2020.
10°	Trend not calculated because data from the years before 2024 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
-	Resistance not calculated.
	For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 5.1.1.1.

Figure 5.2.11.1 *Trends in antibiotic resistance (from left to right 2020 to 2024) among gastro-intestinal mucosa, pus and normally sterile tissue or liquid isolates of H. pylori in ISIS-AR*



Warning: The Y-axis of this figure differs from the standard format. The Y-axis is scaled up to 100%.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 5.1.1.1).

Conclusions

- Since 2020, we have added data for *H. pylori* to NethMap. The results may be biased towards higher resistance levels due to sampling policies.
- Resistance levels for the several **antibiotic combination therapies** for *H. pylori* infections remained stable over the last five years.
- Resistance to **amoxicillin/ampicillin** traditionally has been low and was 7% this year. Resistance to **doxycycline/tetracycline** remained low (2%). Resistance to **claritromycin** (53%) and **metronidazole** (51%) remain high.
- For the treatment of *H. pylori* infections, first choice combination treatment consists of **amoxicillin and clarithromycin**, of which combined resistance was 5% in 2024. If treatment fails, a combination of **tetracycline plus metronidazole** or **amoxicillin plus metronidazole** is recommended. Both have combined resistance levels of less than 4%.

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5.2.12 *Clostridioides difficile*

Introduction

Clostridioides difficile is a spore-forming intestinal bacterium found in humans and animals but can also be isolated from the environment. Asymptomatic colonization with toxin-producing strains may progress to clinical infection with diarrhoea, severe colitis and/or a life-threatening toxic megacolon. After recognition of *C. difficile* ribotype (RT) 027 outbreaks in 2005, surveillance programs were initiated worldwide. In the Netherlands, sentinel surveillance started in May 2009 to monitor the incidence and characteristics of *C. difficile* infections (CDI). The Dutch surveillance is coordinated by the national CDI Expertise Center, a collaboration between the Center for Infectious Disease Control (CIb) of RIVM and Leiden University Medical Centre (LUMC). For *C. difficile* historically ribotyping was used for molecular epidemiology. After validation of core genome multi-locus sequence typing (cgMLST) for routine *C. difficile* typing,[1] cgMLST is routinely performed as of 2023. For backwards comparison, ribotyping is currently performed in parallel. Current recommended first-line therapeutics are fidaxomicin and vancomycin, and metronidazole in conditional cases.[2] A recent report from the US suggests that reduced fidaxomicin susceptibility after treatment with fidaxomicin is not as rare as previously thought (>5% in a cohort of 108 cases).[3] Late 2023, Public Health England reported a new RT, RT955, that has caused two large clusters in UK hospitals and is resistant to metronidazole, fluoroquinolones, and erythromycin.[4] RT955 is genetically closely related to the hypervirulent RT027, and also belongs to sequence type (ST) 1. Subsequently, this RT 955 has also been detected in Poland and a closely related variant in Serbia.

Methods

From 2022 onward, the CDI sentinel surveillance is conducted continuously in 5 centers (3 academic, 2 general hospitals) geographically spread over the Netherlands. Inclusion criteria for surveillance are: hospitalized patients with a clinical picture compatible with CDI and a positive CDI diagnostic test (toxin enzyme-immunoassay, or toxigenic *C. difficile* polymerase chain reaction (PCR)), or presence of pseudomembranous colitis. Of the included cases, *C. difficile* isolates or fecal samples were submitted to the Expertise Center for *C. difficile* culture and typing. The web-based system OSIRIS was used to capture epidemiological data such as location of onset, disease severity, disease course (ICU admission, and / or surgery) and CDI-attributable mortality. Severe CDI is defined as meeting one or more of the following criteria: (i) bloody diarrhoea, (ii) pseudomembranous colitis, (iii) diarrhoea with dehydration, (iv) diarrhoea with hypoalbuminemia <20g/L, (v) temperature >38.0°C with leukocytosis >15 x 10^9/L. In 2024, a question on anti-CDI treatment was added to the epidemiologic data. To calculate CDI incidence, the number of included CDI cases was divided by the number of clinical patient days in the centers participating in the sentinel surveillance program. In addition to the sentinel surveillance, the *ad hoc* typing service is offered for all microbiology laboratories in the Netherlands for typing *C. difficile* isolates of patients with severe disease or isolates from a suspected outbreak based on an epidemiological link. We here present circulating

RTs over the years, and for 2023 and 2024 also the cgMLST typing results.[1]

Results

In 2024, there were 290 cases (median age 70 years, IQR 57-78) from 5 hospitals that met inclusion criteria for the CDI sentinel surveillance, of which 217 *C. difficile* isolates could be cultured and typed.

Key epidemiological characteristics over time are shown in table 5.2.12.1 CDI incidence was relatively stable with 3.4/10,000 patient days (95% CI 2.4-4.8, n=290 cases) in 2024, compared to 3.2/10,000 patient days (95%CI 2.2-4.5, n=291 cases) in 2022 and 2.8/10,000 patient days (95% CI 1.9-4.0, n=249 cases) in 2023. CDI-attributable mortality was 2% (n=6, 95% CI 0.3-3.9), within range of the previous 10 years. The increase of percentage of patients with severe disease which was noted in the previous years, was not sustained and decreased from 39.9% (95%CI 33.8-46.0) in 2023 to 27% (95% CI 21.4-32.4) in 2024. The proportion of cases with a complicated course, i.e. with 30-day mortality, needing ICU admission or surgery, was stable with 11% in 2024. The proportion of cases reported to be community onset cases was also stable with 44% (95% CI 38.3-50.6), thus the apparent increase noted in 2023 (proportion 56%, 95% CI 49.7-62.1) did not continue in 2024. Of the included sentinel surveillance cases, 285 had data on CDI treatment available. Of these, 42% were treated with vancomycin 10-14 days, 24% received fidaxomicin 10-14 days, 6.7% metronidazole, 3.9% vancomycin taper and pulse, and 2.8% extended fidaxomicin scheme.[2] 20% did not receive antibiotic CDI treatment.

One center that participated in the sentinel surveillance had detected 7 consecutive RT014 cases. Of these, cgMLST analysis identified a cluster of four genomically linked isolates (0-1 alleles difference). Application of whole genome SNP analysis and aditional sequencing of historical regional isolates did not identify a larger regional cluster or clonal dissemination. However, no definite epidemiological link by hospital infection control was identified that could confirm transmission within the hospital.

Besides, we received 19 samples from 7 centers for *ad hoc* typing of severe cases or suspected outbreaks. No outbreaks were confirmed. In one instance transmission of a PCR RT002, ST8 complex type 8048 strain from a mother (with severe CDI) to her three newborns was confirmed.[5] Two of the neonates developed diarrhoea which responded well to CDI treatment. CDI in neonates is rare and difficult to diagnose due to high colonization rates.

In 2024 the distribution of circulating RTs of the 217 typed *C. difficile* sentinel surveillance isolates was stable compared to previous years. See figure 5.2.12.1 for the proportions of the most common RTs in the current and past year(s) and figure 5.2.12.2 for circulating STs in 2024 (n=212). RT014/020 continued to be the most prevalent RT accounting for 18.4% of isolates. ST2 (n=22, 10.4%, includes RT014/020), ST8 (n=21, 9.9%, includes RT002), and ST11 (n=21, 9.9% includes RT078/126) continued to be the dominant sequence types. ST1, which includes RT027 and the newly identified RT955, was not detected in the five centers participating in the sentinel surveillance, nor in the samples submitted for *ad hoc* typing.

Table 5.2.12.1 *Key epidemiological characteristics of C. difficile surveillance over the years 2009-2024*

Surveillance period (May-May)	2009 - 2010	2010 - 2011	2011 - 2012	2012 - 2013	2013 - 2014	2014 - 2015	2015 - 2016	2016 - 2017	2017 - 2018	2018 - 2019	2019 - 2021[4]	2022	2023	2024
Incidence														
Per 10,000 patient-days	2.7	2.8	2.9	2.9	2.9	3.0	3.1	3.0	2.9	3.1	3.2	3.2	2.8	3.4
Location of onset														
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%	55%	53%	44%	56%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%	45%	47%	56%	44%
Course and outcome[1]														
Severe CDI[2]	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%	21%	30%	40%	27%
Uncomplicated course[3]	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%	89%	83%	87%	89%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%	2%	1%	4%	2%
PCR ribotype 027														
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%	1.2%	0.6%	0.2%	0.9%	0.0%	0.0%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	0	0	0	0	0	0	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1	0	0	0	0	0	0

[1] Data on complicated course and mortality from between the 2nd of November 2020 until the 10th of January 2021 were excluded due to technical issues with absence of some answer possibilities, indicating missingness at random.
[2] Severe CDI is defined as bloody diarrhoea and/or diarrhoea with hypovolaemia or hypoalbuminaemia (<20 g/L) and/or with fever (T >38.0°C) and leucocytosis (WBC count >15x10⁹/l), and/or with pseudomembranous colitis.
[3] Uncomplicated course is defined as not admitted to the intensive care unit as a consequence of the *C. difficile* infection, no need for surgery as a consequence of the *C. difficile* infection and no death within 30 days after sample date.
[4] Sentinel surveillance period May 2019 - January 2021.
[5] Data is based on 5 laboratories from 2022 onwards.

Figure 5.2.12.1 *Most common circulating ribotypes in C. difficile sentinel surveillance since 2009-2010*

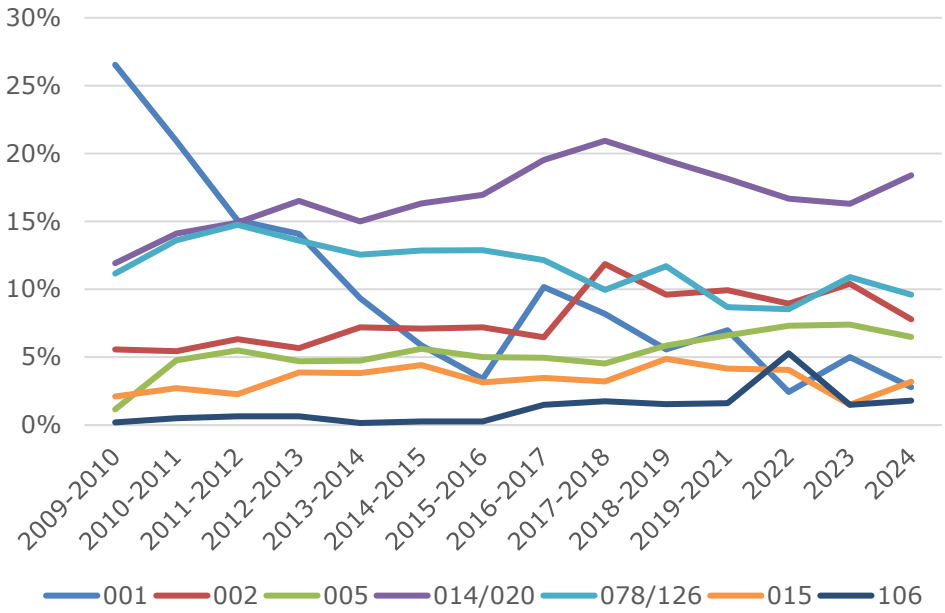
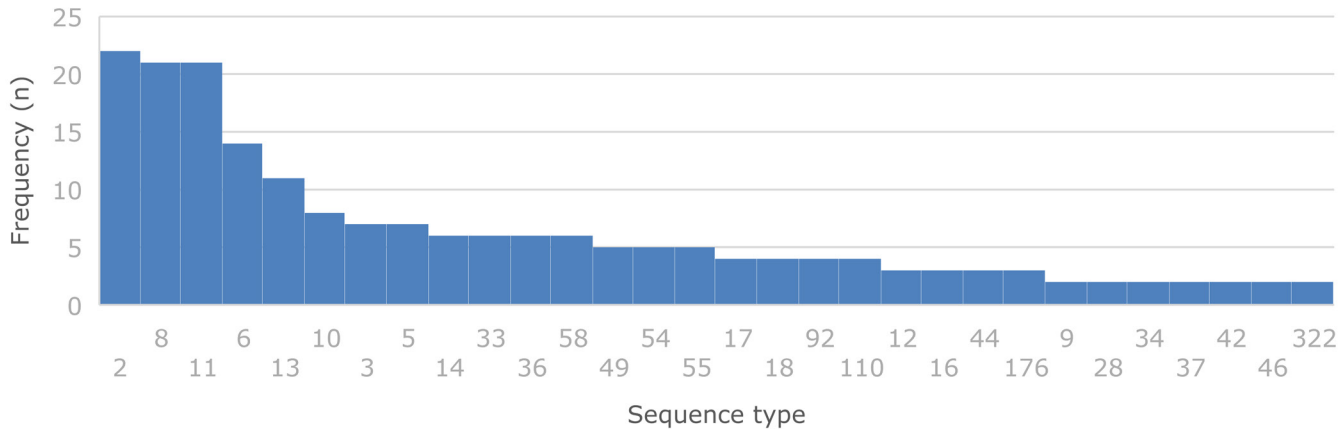


Figure 5.2.12.2 *Circulating sequence types (STs) in C. difficile sentinel surveillance in 2024 (n=212). Only STs shown that were detected more than once*



Discussion

The trend of more severe CDI cases first noted during the second COVID-19 wave in the Netherlands[6] and observed in 2023 did not continue. In fact, the proportion of severe CDI cases decreased compared to 2023. Also, the suggestion of increased CDI-attributable mortality was not sustained. Vancomycin and fidaxomicin were the most frequently used anti-CDI antibiotics, while use of metronidazole was rare. This suggests uptake of revised national (2023) and international treatment (2021) guidelines.[2] We did not encounter the hypervirulent RT027 nor the genetically related RT955 causing outbreaks in the UK. We continue to monitor circulating ribo- and sequence types and offer *ad hoc* typing for early recognition of new circulating strains and control of potential outbreaks. Reports on emergence of isolates with reduced susceptibility to the current firs-line antibiotic fidaxomicin have our attention.[3] Fidaxomicin resistance will be monitored and presented in future reports. The recently identified erythromycin resistance determinant *mrmA*[7] was also identified in Dutch *C. difficile* strains by manual search in sequenced genomes, as it is not yet present in commonly used databases. The prevalence of this resistance determinant will also be carefully monitored.

Conclusions

- Previously noted increase in severe CDI cases and community-onset CDI was not sustained.
- The hypervirulent RT027 and newly reported metronidazole resistant RT955 were not found in the Netherlands.
- No CDI outbreaks were detected in 2024.
- Vancomycin and fidaxomicin were the most frequently used anti-CDI antibiotics.
- Reports on emergence of fidaxomicin resistance warrant investigation in the Netherlands.

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5.2.13 Antibiotic susceptibility profile of clinically relevant anaerobic bacteria isolated from human clinical specimens

Introduction
Antibiotic resistance among clinically relevant anaerobic bacteria in the Netherlands is monitored by the UMCG. It is important to report changes in antibiotic resistance in this bacterial group, because in previous years, an increase in antibiotic resistance was observed. This includes resistance to antibiotics for which resistance was assumed to be absent in the past, like carbapenem and metronidazole resistance among the Bacteroidales.
In this chapter, the resistance rates for clinically relevant anaerobes are reported, using the most recent EUCAST breakpoints.

Methods
Bacterial isolates were retrieved from human clinical specimens which were obtained from different sites of infection, e.g. blood, orthopedic material, abscesses, and were considered to be clinically relevant. Isolates were identified using the MALDI-biotyper SMART (Bruker Daltonics, Bremen, Germany) with the V12 or V13 database. The subtyping-module was used to assess the presence of the *cfiA* gene in all encountered *Bacteroides fragilis* isolates.
The Minimal Inhibitory Concentration (MIC) for the different antibiotics was determined using gradient strips (BioMerieux, Marcy-l' Étoile, France). For Gram-negative anaerobes amoxicillin, amoxicillin-clavulanic acid, clindamycin and metronidazole were tested, but amoxicillin only for *Bacteroides*, *Phocaeicola*, *Porphyromonas* and *Prevotella* when the BD BBL Cefinase disk showed that they were not producing beta-lactamase. For these genera, with the exception of *Porphyromonas*, the MIC for meropenem was also assessed. For Gram-positive anaerobes, MICs for amoxicillin, clindamycin, and metronidazole were determined. The metronidazole resistance for cutibacteria, *Actinomyces* (including previous *Actinomyces* species) and bifidobacteria was not determined, since these genera are intrinsically resistant to this antibiotic.
Genera represented by less than ten isolates, were omitted from the analysis, even though they were reported in previous years. Resistance rates were calculated using the most current EUCAST breakpoints (v15.0, 2025). When no breakpoints are available, the "when there are no breakpoints" guidelines from EUCAST were used.

Results
A total of 791 anaerobic isolates were recovered from a variety of clinical samples retrieved from 524 patients. An overview of the MIC_{50}, MIC_{90} and percentage resistant isolates per genus is shown in table 5.2.13.1. 12% (15/122) of the *Bacteroides* spp. (including *Phocaeicola* spp.) were resistant to meropenem, of which 11 *B. fragilis* (MICs from 1.5 till >32 mg/L), 1 *Bacteroides uniformis* (MIC 12 mg/L), 2 *Bacteroides ovatus* (MICs 2 mg/L), and 1 *Phocaeicola vulgatus* (MIC 1.5 mg/L). The resistant Bacteroides isolates were derived from a variety of clinical samples, e.g. pus, bile, fluid. Three isolates, with MICs ranging from 1.5 to 2 mg/L, originated from samples related to an orthopedic infection. Another isolate with an MIC of >32 mg/L was derived from a blood culture.

Table 5.2.13.1 *MIC50, MIC90 and percentage resistance of clinically anaerobic isolates for the different antibiotics*

	amoxicillin			amoxi-clav			clindamycin		
	MIC50	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**
Gram-negative anaerobes									
Bacteroides spp. *(122-123)*[a,b]	nd[c]	nd	nd	0.19	24	20.3	3	>256	32.5
Fusobacterium spp. *(26-27)*	0.047	>256	11.1	<0.016	0.023	5.6	0.032	0.094	0
Prevotella spp. *(86-89)*	nd	nd	nd	<0.016	0.064	na[d]	0.016	>256	23.3
Veillonella spp. *(28-31)*	0.5	2	48.4	0.19	1.5	32.1	0.125	0.19	0
Gram-positive anaerobes									
Actinomyces spp. *(69)*	0.094	0.50	5.8	nd	nd	nd	0.19	>256	30.4
Anaerococcus spp. *(33)*	0.023	0.38	3.0	nd	nd	nd	0.094	>256	24.2
Clostridium spp. *(31-32)*[e]	0.38	1.0	22.6	nd	nd	nd	0.75	32	53.1
Clostridium perfringens (15)	0.032	0.047	6.7	nd	nd	nd	0.75	3	60.0
Cutibacterium spp. *(36)*[f]	0.125	0.25	0	nd	nd	nd	0.047	>256	19.4
Cutibacterium acnes (183)	0.064	0.19	3.3	nd	nd	nd	0.047	0.125	2.7
Finegoldia magna (72)	0.19	0.38	0	nd	nd	nd	1.5	>256	72.2
Parvimonas micra (24-25)	0.023	0.19	4	nd	nd	nd	0.19	0.75	12.0
Peptoniphilus spp. *(46)*	0.023	0.094	0	nd	nd	nd	1.0	>256	65.2
Peptostreptococcus spp. *(10)*	0.094	0.25	0	nd	nd	nd	0.023	0.75	20.0

[a] Not all isolates were tested for all antibiotics.
[b] *Bacteroides* spp.: includes *Phocaeicola* spp.
[c] Not determined.
[d] Not applicable, since no breakpoint is available
[e] *Clostridium* spp. : excluding *C. perfringens* and *C. difficile*, but including genera which were previously named *Clostridium*, e.g. *Hungatella*.
[f] *Cutibacterium* spp.: excluding *C. acnes*.

Table 5.2.13.1 (continued) *MIC50, MIC90 and percentage resistance of clinically anaerobic isolates for the different antibiotics*

	metronidazole			meropenem		
	MIC50	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**
Gram-negative anaerobes						
Bacteroides spp. (122-123)[a,b]	0.38	0.5	0.8	0.19	2	12.3
Fusobacterium spp. (26-27)	<0.019	0.064	0	nd	nd	nd
Prevotella spp. (86-89)	0.125	1.5	3.5	0.032	0.094	2.3
Veillonella spp. (28-31)	1.5	8	12.9	nd	nd	nd
Gram-positive anaerobes						
Actinomyces spp. (69)	nd	nd	nd	nd	nd	nd
Anaerococcus spp. (33)	0.125	0.75	0	nd	nd	nd
Clostridium spp. (31-32)[e]	0.25	1	6.5	nd	nd	nd
Clostridium perfringens (15)	0.5	1.5	0	nd	nd	nd
Cutibacterium spp. (36)[f]	nd	nd	nd	nd	nd	nd
Cutibacterium acnes (183)	nd	nd	nd	nd	nd	nd
Finegoldia magna (72)	0.19	0.38	0	nd	nd	nd
Parvimonas micra (24-25)	0.064	0.25	0	nd	nd	nd
Peptoniphilus spp. (46)	0.25	0.75	0	nd	nd	nd
Peptostreptococcus spp. (10)	0.047	0.19	0	nd	nd	nd

[a] Not all isolates were tested for all antibiotics.
[b] *Bacteroides* spp.: includes *Phocaeicola* spp.
[c] Not determined.
[d] Not applicable, since no breakpoint is available
[e] *Clostridium* spp. : excluding *C. perfringens* and *C. difficile*, but including genera which were previously named *Clostridium*, e.g. *Hungatella*.
[f] Cutibacterium *spp.: excluding* C. acnes*.*

According to the MALDI-TOF MS subtyping-module, 10 of the 12 meropenem-resistant *B. fragilis* isolates harboured a *cfiA* gene. The two *cfiA* negative *B. fragilis* isolates had the two lowest MICs among the meropenem resistant isolates: 1.5 and 3 mg/L. It was also noted that three meropenem susceptible *B. fragilis* isolates, MICs 0.094, 0.125 and 0.19 mg/L, harboured a *cfiA* gene in their genome.
Furthermore, 2 *Prevotella* isolates (a *Prevotella melaninogenica* and a *Prevotella denticola* isolate) were considered to be meropenem-resistant with MICs of 0.38 mg/L.
One *B. ovatus* isolate was resistant to metronidazole, with an MIC of 8 mg/L, and 3 *Prevotella* isolates, two *Prevotella bivia* isolates (MICs 8 and 24 mg/L) and a *Prevotella timonensis* isolate (MIC 128 mg/L). None of the metronidazole resistant *Bacteroides* and *Prevotella* isolates were resistant to meropenem. It should be noted that also 4 isolates identified as *Veillonella* spp. were resistant to metronidazole, with MICs ranging from 8 to 16 mg/L.
Among the (former) clostridia two isolates were metronidazole resistant, a *Clostridium innocuum* isolate with an MIC of 8 mg/L and a *Clostridium symbiosum* isolate with an MIC of >256 mg/L.
High rates of clindamycin resistance were observed for *Peptoniphilus* spp. and *Finegoldia magna*. Previous year the resistance rate was

approximately 25%. In 2024 these were 65.2% and 75.5%, respectively, the highest rates so far.

Discussion

A worrisome increase in meropenem resistance among *Bacteroides* spp. is observed. The resistance rate increased from 6% in 2023 to 14% in 2024. Three susceptible *B. fragilis* isolates harbored a *cfiA* gene. This gene is more actively transcribed when there is an insertion (IS) element present upstream of the gene. The fact that these isolates had low MICs indicate that no IS-element is present. However, resistance can easily be triggered when such an element inserts upstream. Low rates of meropenem resistant *Prevotella* isolates were encountered, in contrast to previous years, due to the fact that the breakpoint was lowered considerably.

As in previous years, some metronidazole resistant *Bacteroides* and/or *Prevotella* isolates were encountered. These resistance rates seem to remain stable. However, it should be noted that an increase in metronidazole resistance is observed among *Veillonella* isolates, from 2.7% to 12.9%. The first resistant isolates were encountered among the isolates from 2022 and 2023, in each year one. Among the 31 isolates of 2024, 4 resistant isolates were encountered. This trend and the increase in carbapenem resistance among *Bacteroides* species should be monitored during the next years.

Conclusions

- Compared to previous years, carbapenem resistance among *Bacteroides* spp. increased.
- Metronidazole resistance rates among *Bacteroides* and *Prevotella* remain stable.
- There are indications that metronidazole resistance in *Veillonella* spp. increases.
- High resistance rates for clindamycin were observed among *Peptoniphilus* spp. and *F. magna*.

5.2.14 *Aspergillus fumigatus and Candida (Candidozyma) auris*

Introduction
Antifungal resistance is an increasing concern among human pathogenic fungi. Since 2013, resistance surveillance has been conducted in the Netherlands for the saprobic mold *Aspergillus fumigatus* by screening clinical *A. fumigatus* isolates for resistance to medical azoles. Azole resistance has emerged primarily driven by resistance selection through the use of azole fungicides in our agricultural/horticultural environment. In *A. fumigatus*, resistance is mainly due to isolates harboring TR_{34}/L98H or TR_{46}/Y121F/T289A mutations in the *Cyp51A* gene, which confer a pan-azole resistant phenotype. To determine resistance levels and trends in the distribution of these resistance mutations of *Aspergillus* in the hospital setting, a sentinel surveillance is performed.

Recently, the emergence of antifungal drug resistance in various (*Candida*) yeasts has become a significant concern, particularly with the rise of multidrug-resistant *Candida auris* (*Candidozyma auris*). Since its first identification in 2009, *C. auris* has rapidly emerged as a global health threat, distinguished by its frequent resistance to fluconazole and its ability to cause difficult-to-control outbreaks in healthcare settings. A study conducted by the European Centre for Disease Prevention and Control (ECDC) reported a rising number of *C. auris* cases across European member states in 2020-2021[1]. To gain insight in the spread and epidemiology of *C. auris* medical microbiology laboratories are encouraged to submit *C. auris* isolates to the national mycology reference laboratory for species identification, resistance testing, and whole genome sequencing.

Methods
In five University Medical Centers (UMCs) and five teaching hospitals all clinical *A. fumigatus* isolates are screened for triazole resistance using a four-well agar plate (VIPcheck™, MediaProducts, Groningen, the Netherlands). Three wells contain agars supplemented with itraconazole, voriconazole and posaconazole, and one well acts as growth control. Growth on one of the triazole containing wells is highly indicative for resistance and these isolates are sent to the mycology reference laboratory (Radboudumc, Nijmegen and RIVM, Bilthoven) for MIC-testing (according to the EUCAST microbroth dilution method and recommended clinical breakpoints) and sequence-analysis of the *Cyp51A* gene. The resistance frequency is based on the number of patients with *A. fumigatus* screened and is determined for all participating centers and compared with previous years.

For *C. auris*, all laboratories are requested to submit the first *C. auris* isolate from each confirmed colonized or infected patient to the mycology reference laboratory. Information regarding travel history, presence of invasive candidiasis, infection control measures and possible secondary cases is collected. Species identification is confirmed using MALDI-TOF MS, and the EUCAST microdilution reference method was used for antifungal susceptibility testing. Clinical breakpoints were recently published by EUCAST for amphotericin B, anidulafungin and micafungin. For fluconazole a paucity of true wild-type, non-outbreak isolates precluded the determination of an ECOFF and treatment with

fluconazole is not supported by EUCAST even when fluconazole MICs are low.[2] Illumina whole-genome sequencing (WGS) was performed to determine clades, assess phylogenetic clustering, and identify antifungal resistance markers.

Results
In 2024 *A. fumigatus* isolates from 1,433 culture-positive patients were screened for triazole resistance, including 766 (range 73 to 328 per center) patients from UMCs and 667 (range 37 to 170 per center) patients from teaching hospitals. Overall 103 patients harbored an azole-resistant *A. fumigatus* isolate corresponding with an overall resistance frequency of 7.2%, with a resistance frequency of 9.4% (72 of 766 patients) in UMCs and 4.6% (31 of 667 patients) in teaching hospitals (Table 5.2.14.1). A total of 119 azole-resistant isolates (including multiple isolates from individual patients) were analyzed for the presence of resistance mutations in the *Cyp51A*-gene. A tandem repeat (TR_{34} or TR_{46}) resistance mutation was detected in 81 isolates (68.1%), of which 53 TR_{34} and 28 TR_{46}.

The first *C. auris* case was identified in the Netherlands in March 2018, and since then 26 cases with *C. auris* were reported by March 2025 (Figure 5.2.14.1). The cases were reported by 22 different hospitals, and two patients had invasive *C. auris* infection (fungemia), while the remaining patients were colonized. All cases had recently traveled to a foreign country with a high incidence of *C. auris*, and all but three had been admitted to a hospital. All hospitalized patients were admitted in isolation and no transmission or outbreaks were reported in the Netherlands, which was supported by phylogenetic analyses.[3] All *C. auris* isolates showed high MICs for fluconazole, and one isolate also showed resistance to the echinocandins. WGS showed that the isolates belonged to clades I and III.[3]

Discussion
Azole resistance among *A. fumigatus* remains stable in the Netherlands with similar rates in 2024 compared to previous years. The resistance rates are lower in the teaching hospitals, while those in the UMCs show considerable variation ranging between 4.7% and 20%. Resistance in *A. fumigatus* remains to be dominated by isolates harboring TR-mediated mutations (mainly TR_{34}), which is consistent with environmental resistance selection.
C. auris is only sporadically detected in patients and all *C. auris* are imported mainly by transfers of patients from hospitals in endemic countries. Patients are admitted to Dutch hospitals in isolation following the HRMO infection control practice guideline (https://www.sri-richtlijnen.nl), which since the 2024 revision now includes *C. auris*. This approach seems to have prevented secondary cases in the Netherlands. All isolates were resistant to fluconazole and one isolate showed high MICs of echinocandins, which is the first choice treatment recommended for invasive candidiasis. The increasing rates of *C. auris* globally and in Europe as well, support the need for systematic surveillance in the Netherlands, which is currently investigated in an ongoing research project (CAUTION).[4]

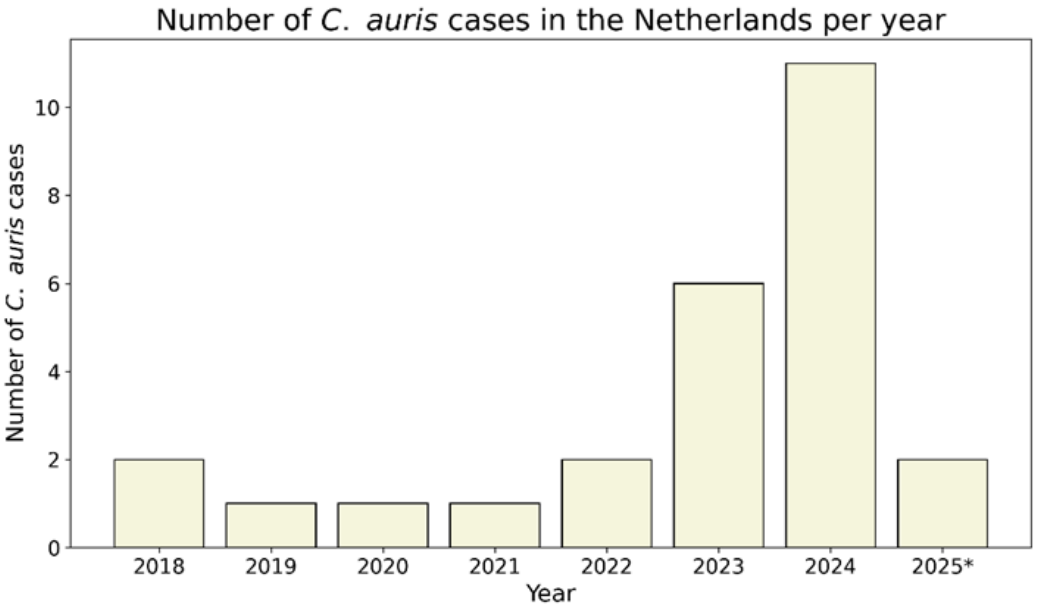
Table 5.2.14.1 *Triazole resistance proportion in unselected clinical A. fumigatus isolates in 5 University Medical Centers and 5 teaching hospitals, 2018-2024*

	2018		2019		2020		2021		2022		2023		2024	
	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)
UMCs														
ErasmusMC	129	17 (13.2)	102	18 (17.6)	108	12 (11.1)	142	17 (12)	119	7 (7.6)	114	10 (8.8)	141	9 (6.4)
LUMC	120	25 (20.8)	90	14 (15.6)	83	8 (9.6)	103	7 (6.8)	81	12 (14.8)	65	9 (13.8)	73	3 (4.7)
Radboudumc	196	23 (11.7)	230	23 (10)	193	20 (10.4)	205	25 (12.2)	175	18 (10.3)	172	13 (7.6)	328	25 (7.6)
UMCG	238	34 (14.3)	230	27 (11.7)	181	31 (17.1)	209	28 (13.4)	206	27 (13.1)	188	25 (13.8)	109	12 (11.0)
AmsterdamUMC	81	13 (16)	51	6 (11.8)	172[a]	16 (9.3)	173	20 (11.6)	175	16 (9.1)	110	18 (16)	115	23 (20.0)
Total UMCs	**764**	**112 (14.7)**	**703**	**88 (12.5)**	**737**	**87 (11.8)**	**832**	**97 (11.7)**	**756**	**80 10.6)**	**649**	**75 (11.6)**	**766**	**72 (9.4)**
Teaching hospitals														
Medisch Spectrum Twente	88	5 (5.7)	90	2 (2.2)	95	2 (2.1)	182	8 (4.4)	98	2 (2.0)	140	5 (3.6)	167	4 (2.4)
St Anthonius Hospital	265	28 (10.6)	177	10 (5.7)	193	15 (7.8)	151	12 (7.9)	211	15 (7.1)	155	11 (7.1)	170	10 (5.9)
PAMM*	81	4 (4.9)	147	8 (5.4)	150	3 (2)	129	6 (4.7)	141	4 (2.8)	114	1 (0.9)	37	1 (2.7)
CWZ	155	11 (7.1)	90	6 (6.7)	163	7 (4.3)	120	8 (6.7)	99	6 (6.1)	92	4 (4.4)	140	8 (5.7)
Isala	195	13 (6.7)	222	18 (8.1)	183	10 (5.5)	222	20 (9)	237	11 (4.6)	204	6 (3.0)	153	8 (5.2)
Total teaching hospitals	**784**	**50 (7.8)**	**726**	**42 (6.1)**	**784**	**37 (4.7)**	**804**	**54 (6.7)**	**786**	**38 (4.8)**	**705**	**27 (3.8)**	**667**	**31 (4.6)**

[a] Includes both VUmc and AMC, since 2020 AmsterdamUMC

* In 2023 PAMM serviced Máxima Medisch Centrum, Catharina Hospital, Anna Hospital, Elkerliek Hospital and St. Jans Gasthuis Weert Hospital

Figure 5.2.14.1 *Number of C. auris cases per year in the Netherlands*



* For 2025 only cases detected until April are shown.

Conclusions

- In 2024 the triazole resistance frequency in *A. fumigatus* was 9.4% in UMCs and 5.5% in teaching hospitals, which is stable in comparison with previous years.
- In three of five UMCs and all teaching hospitals the azole resistance frequency was below 10%.
- Overall, 81.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism, of which 65.4% harbored TR_{34} and 34.6% TR_{46}.
- Since 2018 26 cases of *C. auris* were detected in the Netherlands all of which were imported through stay or hospital admission in endemic countries.
- Since 2022 the yearly number of *C. auris* cases has increased.
- Until now, there has been no evidence for local transmission or outbreaks of *C. auris* within healthcare settings, which is likely due to stringent infection control practices.

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5.2.15 General antiviral resistance

The current antimicrobial resistance (AMR) awareness and monitoring activities along with the preventive/mitigation measures still focus largely on antibacterial resistance, notorious for the death of thousands worldwide, each year. Importantly, resistance to drugs and therapies occurs in a vast array of other pathogens as well, from viruses and parasites to fungi and others.
Like other resistance phenomena, antiviral drug pressure confers survival advantage to subpopulations of viral isolates relatively less susceptible to the drug effect, driving the emergence of resistant viral isolates. Spontaneous mutations due to drug exposure can also occur and alter the virus genetic stability, pathogenicity and transmissibility. High viral replicative loads, elevated viral mutation rates and prolonged drug exposure (specifically inadequate antiviral therapy or inappropriate dosages) also favour the selection of resistance viral isolates, rendering antiviral drugs ineffective.
On the clinical aspect, the concern around antiviral resistance is to limit its significance and manifestations, along with reducing the possibility of mutants' development and transmission, particularly to immunocompromised and vulnerable populations.
Noteworthy, efforts to analyse antiviral treatment failure should rely on systematic antiviral resistance monitoring activities for all relevant treatable viral infections, along with enhanced characterisation of both antiviral susceptibility and underlying drivers for the emergence of resistance events.
In the Netherlands, antiviral resistance monitoring is carried out by several clinical virology laboratories and working groups.
Since 2009, NethMap has been entailing data on the systematic monitoring of antiviral resistance for Influenza viruses' treatments. Herein, the update on Influenza antiviral resistance data is accompanied by a resumed chapter on antiviral resistance in HIV, extracted from the SHM annual report[1].
Besides these, there are ongoing studies on antiviral susceptibility monitoring accounting other viral infections, such as HBV, HCV, Herpes virus, Varicella Zoster virus (VZV), Cytomegalovirus (CMV), Mpox, Respiratory Syncytial virus (RSV) and SARS-CoV-2.
There have been few documented instances of antiviral resistance observed among the aforementioned viral pathogens. Consequently, these cases have not been the focus of attention within this report. Nonetheless, the continuous development and increased use of direct-acting antiviral agents as therapeutic options used in clinical practice is, simultaneously, associated to the growing possibility of antiviral resistance occurrence.
Noteworthy is the programmatic implementation of nirsevimab (Beyfortus) for RSV-immunisation of neonates until 1 year of age, as part of the National Immunisation Program in the 4th quarter of 2025. This requires follow up on the effectiveness of the intervention, including antiviral susceptibility.

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5.2.15.1 Influenza virus

Introduction
When vaccination against influenza is unavailable or proves ineffective due to antigenic mismatch with circulating influenza viruses, influenza antiviral drugs may be used for (post-exposure) prophylaxis as well as treatment of influenza cases with (expected) severe course of disease. In the Netherlands, the following antiviral drugs are approved for use: 1) the M2 ion channel blockers (M2B) amantadine and rimantadine, 2) the neuraminidase inhibitors (NAI) oseltamivir and zanamivir, and 3) the acidic endonuclease inhibitor baloxavir marboxil (BXM). M2B are active against type A viruses only, whereas NAI and BXM are effective against both influenza type A and B viruses. M2B and BXM inhibit viral replication by preventing uncoating of the virus or viral genome replication in the host cell, respectively. In contrast, NAI inhibit the release of progeny virions from the cell, thereby limiting the infection of surrounding cells. Since 2010, seasonal influenza type A viruses have become fully resistant to M2B. Surveillance of antiviral susceptibility in seasonal human influenza viruses has been conducted in the Netherlands for NAIs since the 2005/2006 season and for BXM susceptibility since the 2019/2020 season.[1,2]

Methods
Monitoring of influenza antiviral susceptibility is incorporated into the integrated clinical and virological primary care surveillance of acute respiratory infections (ARI), which utilizes data and specimens obtained by the Nivel Netherlands Institute for Health Services Research Primary Care Database sentinel general practitioner (GP) network. The specimens are analysed at the National Institute for Public Health and the Environment (RIVM) location of the Dutch National Influenza Centre (NIC). In addition, a subset of influenza virus-positive specimens identified in clinical diagnostic laboratories is submitted for further characterization to the Erasmus Medical Centre location of the Dutch NIC. This includes assessment of antiviral susceptibility. For viruses obtained from both sources, patient information regarding antiviral treatment, travel history, and immune competence status in the 14 days preceding specimen collection is collected. Furthermore, influenza viruses obtained through the community-based participatory surveillance of ARI (Infectieradar) are also included in the assessment of antiviral susceptibility at RIVM. However, for these viruses, no patient data concerning antiviral treatment, travel history, or immune competence status in the 14 days preceding specimen collection is collected.

Antiviral susceptibility is monitored using whole genome Nanopore sequencing to detect known amino acid changes associated with reduced susceptibility to both NAIs and BXM. For a subset of influenza viruses, susceptibility to NAIs is assessed using an enzyme inhibition assay, which determines the drug's 50% inhibitory concentration (IC_{50}). This assay is used to confirm the impact of known amino acid changes associated with reduced antiviral susceptibility and to identify novel markers.

The utilisation of antiviral drugs is monitored using data from the Foundation for Pharmaceutical Statistics (SFK), which collects information from more than 98% of community pharmacies in the Netherlands. These pharmacies serve 16.5 million people, representing 91.3% of the Dutch population.

Results

Table 5.2.15.1.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2020/2021 respiratory season. From the 2020/2021 season through the 2022/2023 season, in each season either no or only a limited number of viruses with reduced susceptibility to NAIs and BXM were detected. When reduced susceptibility was detected and the antiviral treatment status was known, these cases were frequently associated with prior antiviral drug use. However, information on antiviral treatment was often unavailable. During the 2023/2024 season, A(H1N1)pdm09 viruses emerged that carry the double amino acid substitution NA-I223V;S247N phenotypically resulting in mildly reduced inhibition by oseltamivir. In the 2024/2025 season, A(H1N1)pdm09 viruses with this double amino acid substitution were no longer detected. Still, A(H1N1)pdm09 with NA-S247N with elevated IC_{50} but below the threshold for reduced inhibition were detected. In the 2024/2025 season, three A(H1N1)pdm09 viruses with the NA-H275Y substitution, which is associated with highly reduced inhibition (HRI) by oseltamivir, were identified. For only one of these cases, information on antiviral treatment was available. The virus was detected during oseltamivir therapy and phenotypically confirmed HRI by oseltamivir. Additionally, in the 2024/2025 season, one influenza B/Victoria lineage virus with the NA-M464T substitution was detected previously associated only with reduced inhibition by peramivir but phenotypically showed reduced inhibition by zanamivir as well.

Figure 5.2.15.1.1 shows the utilisation of oseltamivir since 2020 in the Netherlands, as reported by the SFK. Community pharmacy dispensations of oseltamivir peak in parallel with periods of increased influenza virus circulation. According to SFK for the Netherlands, zanamivir has not been dispensed since 2020, and BXM has not been dispensed at all since its EU authorization in early 2021.

Table 5.2.15.1.1 *(Highly) reduced inhibition/susceptibility of influenza viruses by neuraminidase inhibitors (NAIs) and baloxavir marboxil (BXM) in the Netherlands, 2020/2021 - 2024/2025 respiratory seasons*[1]

Season	A(H1N1)pdm09		A(H3N2)		B	
	NAI	BXM	NAI	BXM	NAI	BXM
2020/2021[2]	ND	ND	0/20	ND	0/1	ND
2021/2022	1/432 (<1%)[3]	0/285	3/1772 (<1%)[4]	2/1158 (<1%)[5]	0/62	0/41
2022/2023	3/647 (<1%)[6]	0/505	0/367	0/321	0/436[7]	0/424
2023/2024	33/890 (3.7%)[8]	0/678	0/509	0/483	0/49	0/34
2024/2025[9]	3/632 (<1%)[10]	0/415	0/673[11]	0/631	1/325 (<1%)[12]	0/287

[1] Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year through week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; BXM = baloxavir marboxil; ND = not done.

[2] During the winter period 2020/2021 no influenza viruses were detected. Only very late in the season after COVID-19 measures were partly lifted in summer 2021 few influenza viruses were detected and analysed for antiviral susceptibility.

[3] One virus with NA-H275Y associated with highly reduced inhibition by oseltamivir and normal inhibition by zanamivir was detected; additional data on treatment status of the patient unknown.

[4] Three viruses with NA-N329R associated with reduced inhibition by zanamivir and normal inhibition by oseltamivir were detected; by phenotypic testing two were indeed reduced inhibited by zanamvir with fold-change just over the threshold for reduced inhibition and one was normal inhibited by both oseltamivir and zanamivir. All three viruses came from the same submitter. Influenza antiviral treatment history of all three patients was unknown.

[5] Two viruses showed the amino acid substitution PA-E23G, previously associated with mild reduced susceptibility to baloxavir marboxil. By phenotypic testing at the WHO CCs for influenza in Tokyo and Atlanta of one virus, the virus was clearly reduced susceptible for baloxavir marboxil. One patient was hospitalized. The status of the other patient was unknown. For both patients no antiviral exposure data were available.

[6] One virus with NA-I223V and NA-S247N (double mutant) was detected. All but one double mutant viruses detected in the 2023/2024 season and phenotypically tested showed reduced inhibition by oseltamivir (median fold-change 12.3; range 10.4-16.1) and normal inhibition by zanamivir. The sequences of one clinical specimen contained NA-D199G/D(91%G) and NA-H275Y/H(8%Y), previously associated with 17-fold reduced and 221 to 1637-fold highly reduced inhibition by oseltamivir, respectively. However, the virus isolate used for phenotypic testing contained NA-D199G/D(24%G) and NA-H275Y/H(75%Y) and therefore the phenotypic outcome is mainly driven by NA-H275Y. The patient did not receive influenza antiviral treatment prior to specimen collection. One virus with NA-H275Y was detected, previously associated with highly reduced inhibition by oseltamivir and normal inhibition by zanamivir. Additional data on treatment status of the patient was unknown.

[7] A cluster of B/Victoria viruses emerged almost exclusively in The Netherlands with NA-K360E previously associated with highly reduced inhibition by peramivir. However, by phenotypic testing of 9 of the 89 viruses detected they appeared normal inhibited by peramivir, likely due to additional compensating amino acid substitutions A395V and L396F/S in the close vicinity of the 360 position in the 3D structure of the neuraminidase.

[8] Thirty viruses with NA-I223V and NA-S247N (double mutant) were detected that caused mild reduced inhibition by oseltamivir (median fold-change 12.3; range 10.4-16.1) and normal inhibition by zanamivir by phenotypic test. One virus with NA-I233T previously associated with reduced inhibition by oseltamivir (9-15 fold) and normal inhibition by zanamivir was detected. Additional data on treatment status of the patient unknown. The virus could not be isolated for phenotypic testing. Two viruses with NA-H257Y previously associated with highly reduced inhibition by oseltamivir but normal inhibition by zanamivir were detected. Of one patient the treatment status was known; the virus with NA-H275Y was retrieved during oseltamivir therapy and confirmed highly reduced inhibited by oseltamivir and normal inhibited by zanamivir in phenotypic test. The other virus could not be isolated for phenotypic testing.

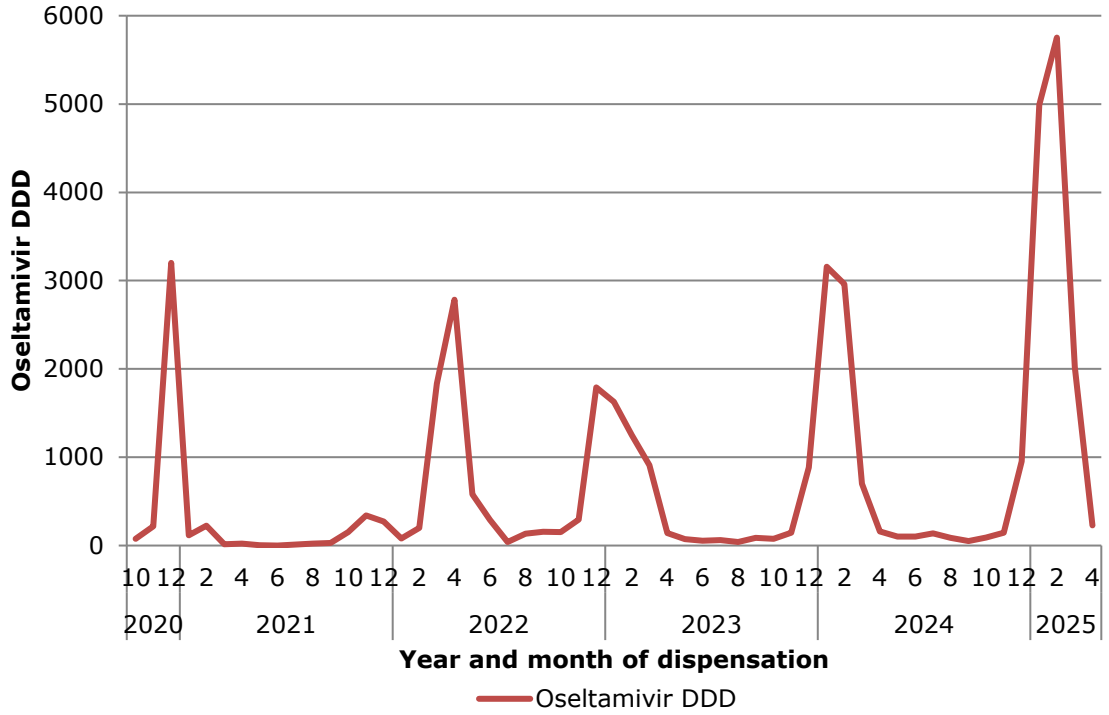
[9] Preliminary data up to week 20/2025.

[10] Three viruses with NA-H257Y previously associated with highly reduced inhibition by oseltamivir but normal inhibition by zanamivir were detected. Of one patient the treatment status was known; the virus with NA-H275Y was retrieved during oseltamivir therapy and confirmed highly reduced inhibited by oseltamivir and normal inhibited by zanamivir in phenotypic test. The other viruses could not be isolated for phenotypic testing.

[11] Three viruses with NA-S331R previosuly associated with normal to reduced inhibition by oseltamivir and zanamivir. One virus could be isolated and appeared normal inhibited by oseltamivir and zanamivir by phenotypic test.

[12] One virus with NA-M464T previously associated with reduced inhibition by peramivir was detected and appeared also reduced inhibited by zanamivir (5.1-fold) but normal inhibited by oseltamvir by phenotypic test. The patient was not treated with antiviral drugs.

Figure 5.2.15.1.1 *Community pharmacy dispensations of oseltamivir in the Netherlands, 2020/2021 - 2024/2025 respiratory seasons. Shown are the Defined Daily Doses (DDD) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands*



Discussion

In the Netherlands, the proportion of influenza viruses with reduced susceptibility to NAIs remained very low over the period 2020/2021 through the 2024/2025 season, similar to the global situation through the 2023/2024 season.[3] The double mutant A(H1N1)pdm09 virus, which exhibited mildly reduced inhibition by oseltamivir and emerged during the 2023/2024 season, was not detected in the Netherlands in the 2024/2025 season. Furthermore, this genotype was also absent in sequences available from the GISAID influenza virus sequence database for clinical specimens collected worldwide since October 2024. In the 2024/2025 season, influenza viruses with reduced susceptibility to NAIs were only sporadically detected in the Netherlands. No naturally occurring BXM-reduced susceptible viruses were detected in the Netherlands, consistent with their very low global prevalence.[3] Despite the low prevalence of influenza viruses with reduced antiviral susceptibility observed in recent seasons, ongoing surveillance remains essential to promptly detect the emergence of reduced antiviral susceptibility and safeguard effective treatment options.

Conclusions

- Sporadically, neuraminidase inhibitor (NAI) reduced inhibited influenza viruses were detected in the Netherlands in the 2024/2025 season.
- No baloxavir marboxil (BXM) reduced susceptible viruses were detected in the Netherlands in the 2024/2025 season.
- Community pharmacy dispensations of oseltamivir remain low with sharp increases during every influenza epidemic.
- Community pharmacies have not dispensed zanamivir since late 2020 and baloxavir marboxil (BXM) since its 2021 registration.

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5.2.15.2 Antiviral Drug Resistance data - HIV Summary as part of the national repository with reference to the HIV monitoring rapport 2024, Stichting HIV Monitoring[1]

Introduction

There are 2 types of Human Immunodeficiency virus (HIV) - HIV-1 and HIV-2 - which are subdivided in groups (M, N, O, P for HIV-1; A to H for HIV-2), subtypes (A-D, F-H, J-K for HIV-1 group M), and sub-subtypes (A1-A7 for subtype A, F1 and F2 for subtype F in HIV-1 group M). Circulating recombinant forms (CRF), and unique recombinant forms have also been identified in a number of infections. From its first isolation in 1983, virological diagnostic and monitoring techniques have greatly improved. Innovative treatment developments lowered the morbidity and mortality associated with HIV. In the Netherlands, effective antiretroviral therapy (ART) is available since 1996. Currently, 96% of people who started ART have suppressed HIV-1 RNA. In recent years worldwide the emergence of HIV drug resistance strains has increased.[2] This rise in antiretroviral drug resistance is caused by alterations in the virus genetic structure. A full characterisation of circulating HIV strains with drug resistance is crucial to prevent HIV transmission and HIV-associated morbidity and mortality.[3]

HIV resistance can be categorised as:

1. *Transmitted drug resistance* resulting from infection with HIV strains harbouring resistance associated mutations (RAM)
2. *Acquired resistance* which can occur during treatment for instance because of decreased drug exposure to ART or when treatment adherence to pre-exposure prophylaxis (PrEP) is decreased and that person acquires HIV. Subsequently, more RAM can be selected ultimately resulting in treatment failure.

Dutch guidelines, in alignment with other countries, recommend testing for viral resistance in all individuals newly diagnosed with HIV entering care. Testing for viral resistance at entry to care has been recommended since 2023 but is not performed in all Dutch HIV treatment centers. Resistance testing is also recommended at the time of suspected virological failure during antiretroviral treatment. These tests help to determine the most appropriate therapeutic scheme while attempting to minimize the emergence and spread of HIV drug resistance.

Methods

By obtaining resistance analysis results at baseline, individual patient treatment can be optimized in both adults and children, as recommended by WHO. These antiretroviral resistance (AVR) studies are annually published in the HIV Monitoring report (SHM).[1] Genotypic resistance tests assess reverse transcriptase (RT), protease and/or integrase RAM. These data are then analysed using the Stanford HIVdb which infers antiretroviral drug susceptibility from resistance scores.[4] The 2022 International Antiviral Society-USA (IAS-USA) HIV drug resistance mutation list is used to score major RAMs.[5]

Results

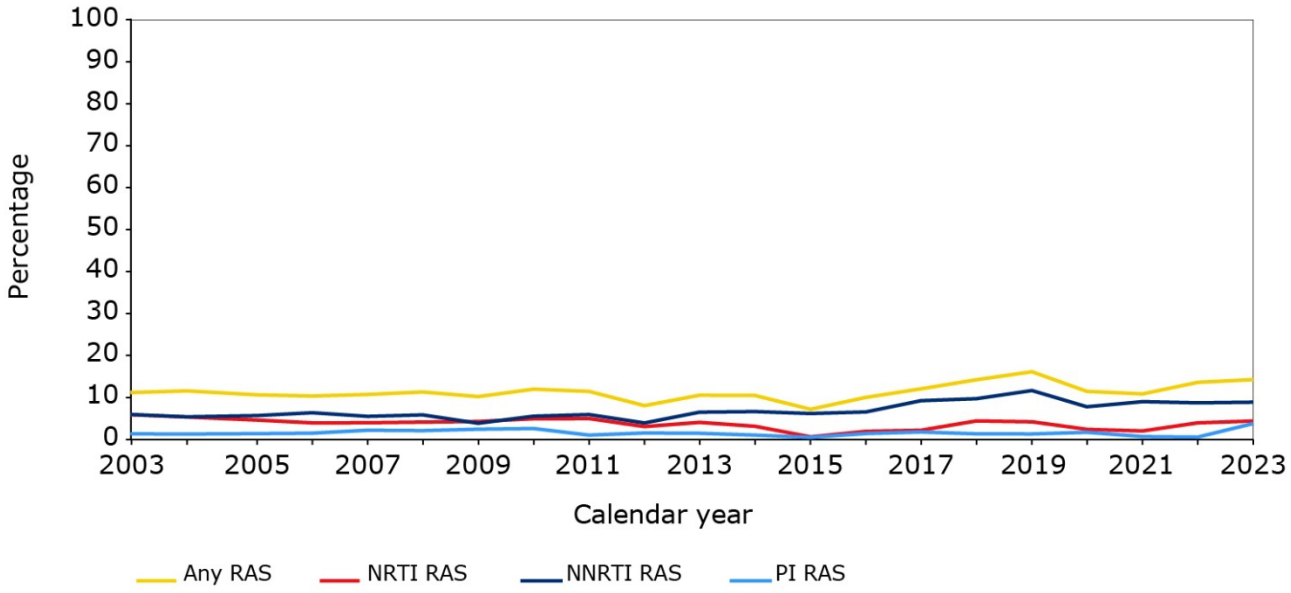
Between January 2014 and December 2023, 25,939 people with HIV were in care and on ART in the Netherlands. AVR is mainly studied when pre-treatment sequences are available, as after the therapy is started, HIV-1 is suppressed in the majority of individuals in care.

Transmitted drug resistance

Transmitted drug resistance is considered when RAM can be detected prior to the initiation of ART. From 2003-2023, 9,523 HIV-1 sequences were obtained from 9,195 ART-naïve individuals. In patients with multiple sequences, only the first was selected. Analysis was performed on 9,508 reverse transcriptase sequences available from 9,183 individuals; 8,945 protease sequences were available from 8,632 individuals; 588 integrase sequences were available from 587 individuals. In total, at least one or more major resistance-associated mutations were found in 1030 (11.2%) of the individuals, including 370 (4.0%) with nucleotide/nucleoside reverse transcriptase inhibitor (NRTI)-associated resistance mutations, 590 (6.4%) with non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated resistance mutations, and 162 (1.8%) with protease inhibitor (PI)-associated resistance mutations. The prevalence of transmitted drug resistance was average and remained stable between 2003 and 2023 as depicted in figure 5.2.15.2.1. High-level resistance to at least one antiretroviral drug was found in 301 (3.3%) individuals screened for transmitted drug resistance; 53 (0.6%) to at least one NRTI; 226 (2.5%) to at least one NNRTI; and 37 (0.4%) to at least one PI.

Noteworthy, from 587 people that had an integrase sequence available prior to the time of entry into care (ART), only one major integrase RAM was detected (Y134Y/C). Overall, more than 96.8% of isolates seemed fully susceptible 2.8% (n=260) harboured high-level resistance in one drug class; 0.3% (n=29) in two drug classes; and less than 0.1% (n=5) to three drug classes (i.e. NRTIs, NNRTIs and PIs).

Figure 5.2.15.2.1 *Annual percentage of people with evidence of transmitted HIV drug resistance over time between 2003-2023*[1]



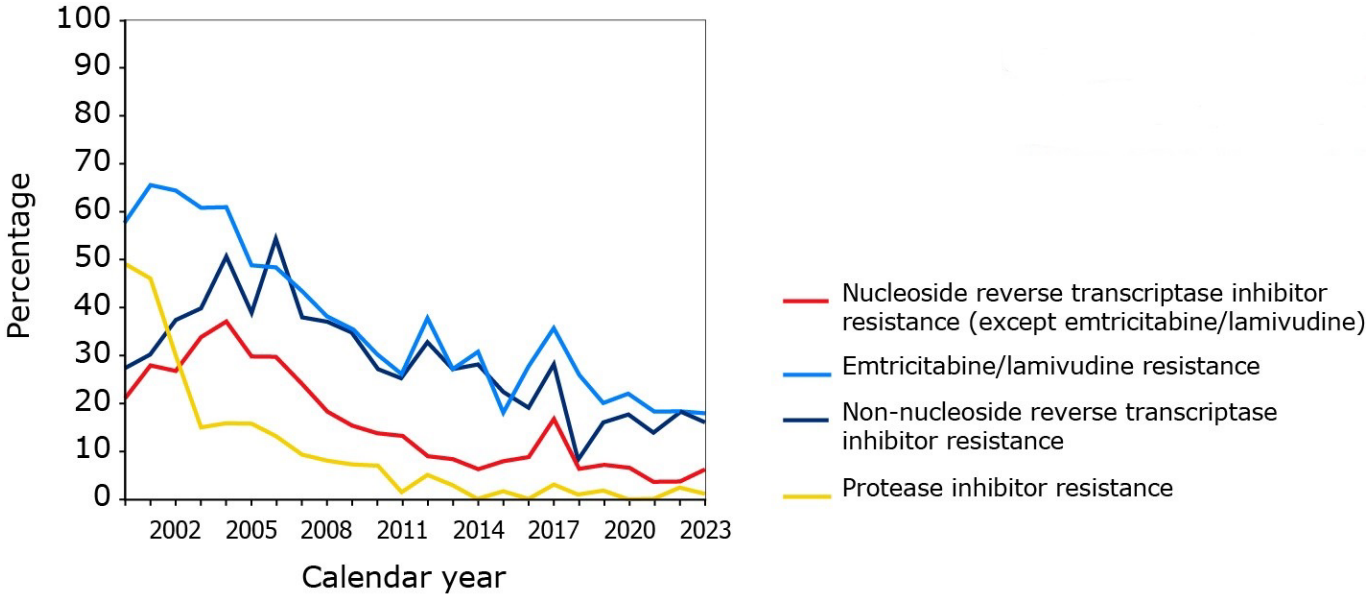
RAS: resistance associated substitutions

Acquired drug resistance

From 2000 to 2023, 5,147 HIV-1 sequences from 3,071 individuals who received cART for at least four months and whose HIV RNA was above 500 copies/ml, were analysed (Figure 5.2.15.2.2). In summary, 3,732 sequences were from 2,312 people who began treatment in the combination ART era, while the remainder had been previously exposed to mono- or dual NRTI therapy. 5,050 reverse transcriptase sequences were available from 3,039 individuals, 4,790 protease sequences from 2,880 individuals, and 716 integrase sequences from 547 individuals. The main HIV-1 subtype was B (66.6%), followed by recombinant form CRF_02AG (11.3%), and subtype C (6.0%).

A total of 3,012/5,147 (58.5%) sequences harboured high-level resistance to at least one antiretroviral drug. High-level NRTI resistance was detected in 3,040/5,050 (60.2%) sequences; of those, 2,584 (85.0%) harboured high-level resistance to the NRTIs emtricitabine or lamivudine. Notably, of the 1,872 individuals ever identified as harbouring the M184V or M184I NRTI-associated mutation who were still in care in 2023, 1,196 (63.7%) were still on ART containing lamivudine or emtricitabine, of whom 944 (78.9%) had undetectable HIV-RNA at their last visit.

Figure 5.2.15.2.2 *The annual percentage of sequences with evidence of high-level resistance by drug class and antiretroviral drug, obtained at the time of virological failure when receiving antiretroviral therapy (ART), among previously antiretroviral drug-naïve people. Results are shown by antiretroviral drug class: high-level resistance to at least one drug within class*



In addition, 1,808/5,050 (35.8%) harboured high-level resistance to at least one NNRTI, and 1,041/4,790 (21.7%) to at least one PI. The available 716 integrase sequences originated from 547 people who received integrase-containing ART for at least four months; 51 were pre-treated with monotherapy or dual NRTI therapy before initiating cART, and 496 were ARV-naïve before initiating cART. Most people had initiated ART years before; the median time between ART initiation and testing for integrase inhibitor resistance was 10.5 years (IQR 4.8-16.2). From the 547 individuals, 58 displayed at least one acquired major mutation

associated with integrase inhibitor resistance. The following major RAMs were detected: N155H/N, R263R/K, E92E/Q, Y143RC, T66T/I, Q148H/R, S147S/G.

PrEP related drug resistance
Genotypic resistance test results were available for 101 (80.8%) of the 125 individuals who reported having used PrEP prior to first entering HIV care. In 13 (12.9%) individuals reverse transcriptase (RT) RAM (M184V/I) were demonstrated suggesting intermittent or recent PrEP use. Two of these also harboured a K65R RT RAM (which is selected for by tenofovir and decreases susceptibility to tenofovir, abacavir, lamivudine and emtricitabine).

Discussion
In the Netherlands, the proportion of antiretroviral resistance shows low and decreasing trends among transmitted and acquired drug resistance groups. Data shows high rates of NRTI resistance among MSM that had used PrEP before acquiring HIV. Noteworthy, incomplete suppression of viral replication by ART may enable HIV mutations, potentially leading to ART failure. Continuous monitoring of RAMs in people with a new HIV diagnosis and in case of ART failure is crucial and will enable appropriate response to HIV drug resistance with comprehensive and effective HIV care, contribute to sustained viral suppression, and minimise the risk of HIV transmission. The presented data may not be representative of the entire population in HIV care in the Netherlands as some HIV treatment centres and laboratories did not share data with SHM.

Conclusions
- Overall, in 2023, viral suppression rates in patients with HIV receiving ART in the Netherlands are high and improving. Among those with ART failure, the annual percentage with acquired antiviral resistance remained low; similarly to other high-income settings.
- 3.3% of individuals screened for transmitted drug resistance and 58.5% of individuals tested for acquired drug resistance harboured high-level resistance to at least one antiretroviral drug.

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5.2.16 Shigella

Introduction
Shigellosis is an infection caused by *Shigella* bacteria. Humans are the only reservoir for the four *Shigella* spp. *S. sonnei, S. flexneri, S. boydii* and *S. dysenteriae*. All species can cause diarrheal illness, that can be bloody and prolonged. Severe illness (dysentery) is more commonly caused by *S. dysenteriae* serotype 1, and can be fatal. Rare post-infectious complications include reactive arthritis and hemolytic uremic syndrome (HUS). The primary route of transmission is fecal-oral, occurring either directly through person-to-person contact or indirectly via contaminated food or water. In the Netherlands, shigellosis is often associated with international travel and with sexual transmission in networks of men who have sex with men (MSM).

Antibiotic treatment is recommended for severe infections, as well as for individuals at increased risk of severe disease[1]. Antimicrobial resistance of Shigella spp. against commonly used antibiotics such as ciprofloxacin, third-generation cephalosporins, and to a lesser extent, azithromycin, is a problem that is increasing worldwide, and one that was preceded by resistance against ampicillin, chloramphenicol and co-trimoxazole. Multi-drug resistance is also occurring more frequently. In the Netherlands, azithromycin and ceftriaxone are currently recommended as respective oral and i.v. empirical treatment options for severe infections. The use of ciprofloxacin is only recommended for infections with proven sensitivity to this drug.

Methods
Phenotypic sensitivity data from 2020 to 2024 were extracted from the ISIS-AR-surveillance database. ISIS-AR's selection criteria are described in chapter 5.1.1. Additionally, travel history and transmission route data obtained from the Dutch registration system for notifiable infectious diseases (OSIRIS) were linked to the extracted ISIS-AR data, where possible. Antimicrobial resistance proportions were available for a broad selection of antibiotic drug classes, while confirmatory tests were available for the presence of CPE. Due to the limited amount of data on macrolide resistance, a reliable analysis of the level of resistance of *Salmonella* spp. against this class of antibiotics was not feasible.

Results
In 2024, *S. sonnei* (n=209; 51.4%) and *S. flexneri* (n=158; 38.8%) were the most frequently identified species, followed by *S. boydii* (n=15; 3.7%) and *S. dysenteriae* (n=6; 1.5%). Resistance proportions for amoxicillin/ampicillin, cotrimoxazole, and ciprofloxacin were very high across species, although inter-species differences exist (Table 5.2.16.1). The proportion of isolates resistant to third-generation cephalosporins was also high, and this was due to increasingly high percentages in both *S. sonnei* and *S. flexneri,* with cefotaxime/ceftriaxone resistance of 42% and 35%, respectively, in 2024. The difference between *S. sonnei* and *S. flexneri* appears to become smaller, as the resistance proportion for *S. flexneri* has been increasing more rapidly over the last 5 years (Figure 5.2.16.1 and 5.2.16.2). There were no positive CPE-confirmatory test results, and no meropenem/imipenem resistant isolates. MDR was more common

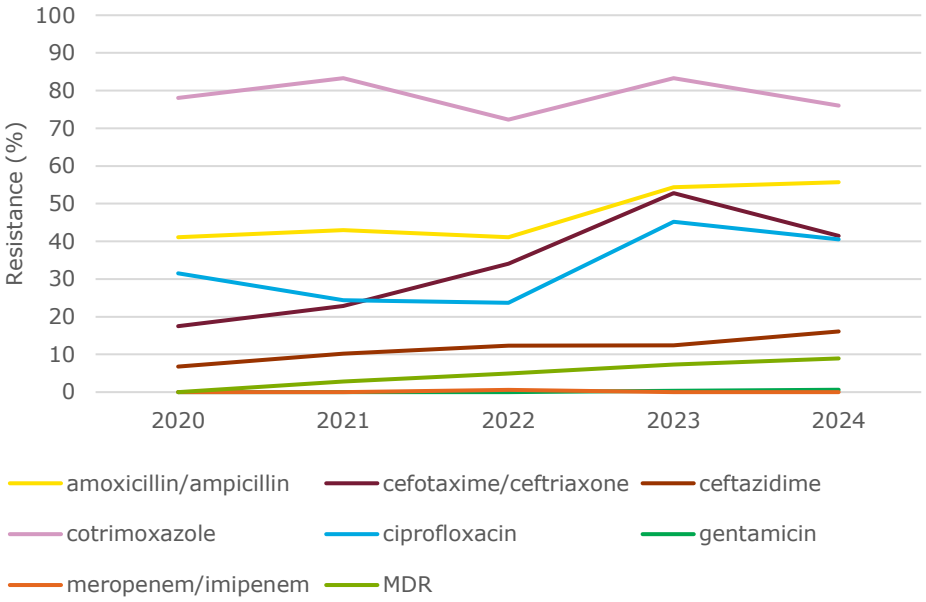
among *S. sonnei* isolates, and increased from 0% in 2020 to almost 9% in 2024. For patients with available OSIRIS data, 51% (156/304) acquired the infection abroad and 28% (91/323) had MSM contact in the week before onset of symptoms, resistance percentages for these groups are shown in table 5.2.16.1.

Table 5.2.16.1 *Resistance (%) of human Shigella isolates, total and stratified, in the Netherlands in 2024*

		Species		Persons at increased risk of infection	
	Total	***S. sonnei***	***S. flexneri***	**Infected abroad**	**MSM**
	n=407	**n=209**	**n=158**	**n=156**	**n=91**
amoxicillin/ ampicillin	72.3	55.7	93.2	52.7	90.9
cefotaxime/ ceftriaxone	38.8	41.5	35	31.8	40.7
ceftazidime	17.9	16.1	22.8	9.8	28.3
cotrimoxazole	52.2	76.0	25.0	61.5	47.2
ciprofloxacin	43.5	40.5	52.0	24.5	80.9
gentamicin	0.3	0.6	0	0.8	0
meropenem/ imipenem	0	0	0	0	0
CPE	0	0	0	0	0
MDR	5.4	8.9	2.0	5.6	5.1

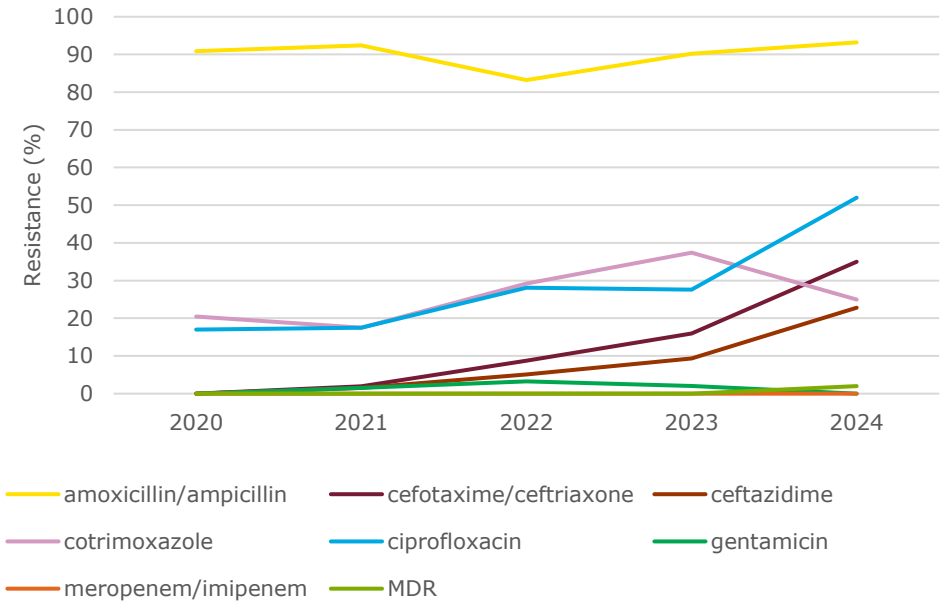
MSM, MSM contact in the week before onset of symptoms.
MDR, Multi-drug resistant: this includes resistance to ceftazidime, cefotaxime/ceftriaxone, ciprofloxacin, cotrimoxazole.

Figure 5.2.16.1 *Resistance trends (%) of human S. sonnei isolates, from 2020-2024*



MDR, Multi-drug resistant: this includes resistance to ceftazidime, cefotaxime/ceftriaxone, ciprofloxacin, cotrimoxazole.

Figure 5.2.16.2 *Resistance trends (%) of human S. flexneri isolates, from 2020-2024*



MDR, Multi-drug resistant: this includes resistance to ceftazidime, cefotaxime/ceftriaxone, ciprofloxacin, cotrimoxazole.

Conclusions

- In 2024, *S. sonnei* and *S. flexneri* were the most frequently identified species, comprising 51.4% and 38.8% of the isolates, respectively.
- For most antibiotics, resistance levels were higher among the MSM population compared to the overall population.
- Over the past five years, resistance to third generation cephalosporins and ciprofloxacin in *S. sonnei* isolates has been increasing. Multi-drug resistance has increased from 0% in 2020 to 9% in 2024.
- Over the past five years, there has been an upward trend in S. flexneri resistance to ciprofloxacin and third-generation cephalosporins.

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6 Antimicrobial resistance in zoonotic bacteria

This chapter describes susceptibility test results as determined in 2024 for the zoonotic pathogens *Salmonella enterica*, *Campylobacter* spp., Shiga-toxin producing and enteropathogenic *E. coli*, and *Yersinia* spp. For data extracted from the ISIS-AR database, the laboratory methods used and the selection of isolates are described in chapter 5.1.1. For all other data in this chapter, epidemiological cut-off values (www.eucast.org) were used for the interpretation of minimum inhibitory concentrations (MIC). Epidemiological cut-off (ECOFF) values are in most cases lower than clinical breakpoints; therefore, depending on the antibiotic in question, non-wild-type susceptible isolates (i.e. isolates displaying MICs above the ECOFFs) cannot automatically be classified as clinically resistant. For the purpose of this report, we designated all non-wild-type susceptible isolates as "resistant", and specified this per antibiotic if necessary.

6.1 Salmonella

Introduction

In this chapter, we report human, animal, and food-sample resistance data on non-typhoidal *Salmonella* (ntS: zoonotic bacterial pathogens), and to a lesser extent, about typhoidal *Salmonella*, pathogens with a reservoir that is much more restricted to humans. Human infections with ntS may lead to a form of gastrointestinal illness that is usually self-limiting, and that typically involves nausea, fever, diarrhea, and abdominal discomfort. Infections with typhoidal *Salmonella* (*S.* Typhi and *S.* Paratyphi A, B and C) gives rise to typhoid or paratyphoid fever, and these are acute more general illnesses that may lead to severe, and even life-threatening disease over the course of weeks. Typhoidal infections are not endemic in Europe. Unlike S. Paratyphi B *sensu stricto,* human paratyphoid infections with *S*. Paratyphi B, variant Java, stand out in the sense that they commonly infect poultry, while *not* typically causing typhoidal disease.

Carriage of ntS serotypes is widely distributed among domestic and wild animals. These animals are often asymptomatic carriers that shed ntS in their feces. Fecal-oral transmission to humans may occur through direct or indirect contact with infected animals or their feces. However, most infections arise from the consumption of contaminated animal products, such as meat or eggs.

Antibiotic treatment for human ntS infections is only recommended for individuals at increased risk of complications and in case of severe infection. For infections with fluoroquinolone-sensitive *Salmonella* strains in adults, ciprofloxacin is generally recommended as the treatment of choice. For infections with unknown sensitivity, either azithromycin (oral route) or a third generation cephalosporin is recommended.[1]

Methods

Isolates were obtained from human salmonellosis patients, healthy food-producing animals, food products of animal origin, and other food products. All isolates were subjected to phenotypic antimicrobial susceptibility testing, using MICs. Human *Salmonella* isolates were sent to the RIVM by clinical laboratories on a voluntary basis. Only the first isolate per patient, per serotype per year was included in this report (n = 1470). In addition to the testing of human isolates, 635 isolates from non-human sources were tested. These sources included broilers (n = 287), layers (n = 216), cattle (n = 57), pigs (n = 37), and a diversity of other sources, including vegetables, herbs and other animals (such as goats, horses, turkey) (n = 38). The non-human resistance data originated from a diversity of programs, including several surveillance programs and diagnostic activities for clinical infections in animals from the Animal Health Services in Deventer, isolates from food products (e.g. meat and products thereof), analyzed for antibiotic susceptibility by WFSR, the official food safety laboratory of the NVWA, and from poultry (mainly broilers and layer) tested by WBVR, in line with Decision (EU) 2020/1729.

Results

Overall resistance

MIC distributions of human and non-human *Salmonella enterica* isolates

MIC distributions and phenotypic resistance percentages of all 2105 human and non-human *Salmonella* isolates that were tested in 2024 are presented in table 6.1.1. When looking at all available human and non-human salmonella-isolates of 2024, the highest resistance proportions were those concerning fluoroquinolones (ciprofloxacin = 23%, nalidixic acid = 22%). High proportions were also found for sulfamethoxazole (20%), ampicillin (17%), tetracycline (17%), and trimethoprim (10%). Much lower proportions (<5%) were found for cefotaxime and ceftazidime, as well as for amikacin, azithromycin, chloramphenicol, gentamicin, and tigecycline resistance. There were no meropenem-resistant isolates. When focusing on the 10 most prevalent human and non-human serovars in 2024, high levels of ciprofloxacin resistance were observed for *S.* Chester (88%), *S.* Infantis (75%), and *S.* Enteritidis (24%) (Table 6.1.2).

Table 6.1.1 *MIC distribution (%) and resistance percentages (R%) for all Salmonella isolates tested for antibiotic susceptibility in 2024 (N=2105; 1470 human and 635 non-human isolates)*

Salmonella	MIC (%) distribution mg/L																		R%	95% CI
N = 2105	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**		
Ampicillin							23.4	56.1	3.9	0.2	0.1	0.1	16.2						16.6	15.1 - 18.3
Cefotaxime					95.8	2.0	0.1			2.1									2.2	1.6 - 2.9
Ceftazidime					63.2	30.2	4.3	0.3	0.2	0.2	1.6								2.0	1.5 - 2.7
Gentamicin						92.0	6.1	0.4		0.0	0.3	1.2							1.5	1.1 - 2.1
Tetracycline								80.4	2.2	0.1		0.3	16.9						17.2	15.7 - 18.9
Sulfamethoxazole										3.6	12.6	36.6	26.6	0.7	0.1	0.0	19.9		19.9	18.3 - 21.7
Trimethoprim					65.3	23.8	1.2	0.2	0.05	0.0		9.4							9.5	8.3 - 10.8
Ciprofloxacin	29.2	46.6	1.4	3.1	9.7	7.0	1.5	0.1	0.3	0.6	0.4								22.8	21.1 - 24.6
Nalidixic acid									71.0	6.8	2.2	3.6	0.2	16.2					22.2	20.5 - 24.1
Chloramphenicol										90.4	5.3	0.1	0.2	4.0					4.3	3.5 - 5.3
Azithromycin*								1.7	69.4	26.5	1.6	0.2	0.3	0.3					0.8	0.5 - 1.3
Colistin**							73.9	15.7	9.8	0.4		0.14							-	-
Meropenem		82.5	17.2	0.2															0.0	
Tigecycline***					74.7	18.2	6.3	0.8											0.8	0.5 - 1.3
Amikacin									99.8	0.2									0.2	0.1 - 0.5

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoints are identical.

* tentative set ECOFF during the EURL AMR WP meeting on 25 April 2015 in Lyngby (DK).

** Because of differences in natural susceptibility for colistin between serovars there is no general Salmonella ECOFF available for colistin. For this reason the percentage of resistance is not depicted.

*** Since 2019 the ECOFF is no longer available for Salmonella. The former defined ECOFF of EUCAST for tigecycline was used for monitoring purposes in 2018.

Table 6.1.2 *Resistance (%) of the top 10 most prevalent Salmonella serovars in human and non-human isolates in the Netherlands in 2024 (N tested)*

	Enteritidis (775)	Typhimurium (235)	Typhimurium monophasic (143)	Paratyphi B vr. Java (102)	Infantis (93)	Chester (41)	Dublin (39)	Virchow (39)	Mbandaka (37)	Typhi (34)
ampicillin	7.1	24.7	69.9	18.6	18.3	26.8	2.6	0.0	0.0	17.6
cefotaxime	0.4	0.4	0.0	0.0	2.2	2.4	2.6	0.0	0.0	5.9
ceftazidime	0.3	0.0	0.0	0.0	2.2	2.4	0.0	0.0	0.0	5.9
gentamicin	0.0	6.4	2.8	0.0	2.2	2.4	0.0	0.0	2.7	0.0
tetracycline	3.7	39.1	55.9	1.0	67.7	29.3	2.6	0.0	0.0	2.9
sulfamethoxazole	2.8	40.4	59.4	50.0	69.9	12.2	15.4	0.0	0.0	17.6
trimethoprim	0.1	7.2	11.2	68.6	43.0	9.8	2.6	0.0	0.0	17.6
ciprofloxacin	24.0	4.7	9.8	22.5	75.3	87.8	10.3	5.1	5.4	64.7
nalidixic acid	24.0	5.1	9.1	22.5	76.3	87.8	10.3	5.1	0.0	64.7
chloramphenicol	0.5	13.6	6.3	0.0	2.2	9.8	12.8	0.0	0.0	14.7
azithromycin	0.6	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
tigecycline	0.1	0.4	0.7	0.0	5.4	0.0	0.0	0.0	0.0	0.0
amikacin	0.1	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Resistance of (monophasic) S. Typhimurium and S. Enteritidis in humans and animals

S. Typhimurium

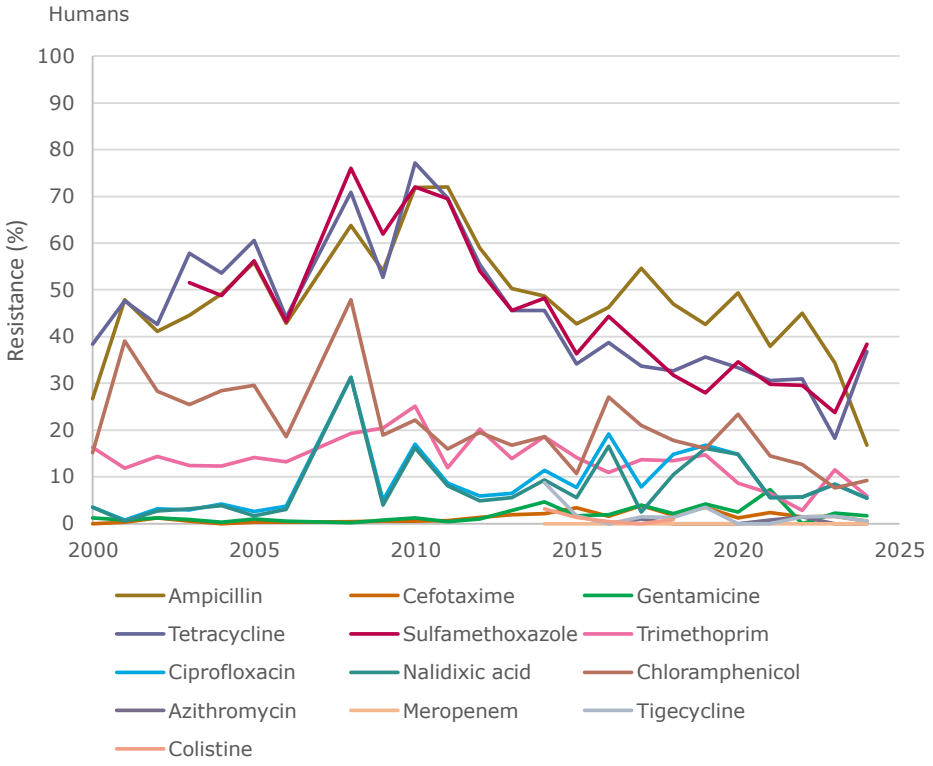
Pigs and cattle serve as important animal reservoirs for *S.* Typhimurium and its monophasic variant. Table 6.1.3 shows the resistance patterns of these strains among human and non-human isolates. For human isolates, resistance percentages were high for ampicillin, sulfamethoxazole, and tetracycline, especially among monophasic *S.* Typhimurium. Resistance to the clinically important third-generation cephalosporins was rare, and it was only detected among human *S.* Typhimurium isolates (cefotaxime: 0.5%). Long-term resistance trends of human *S.* Typhimurium isolates are shown in figure 6.1.1. After a steady decrease of resistance percentages of almost all classes of drugs since the peak in 2010, we now see an increase in resistance to sulfamethoxazole and tetracycline in 2024; from 24% and 18% in 2023, to 38% and 37% in 2024, respectively. Resistance levels among non-human isolates were high for ampicillin, tetracycline and sulfamethoxazole, especially among those of pigs and cattle. Among *S.* Typhimurium isolates from cattle and pigs, chloramphenicol resistance was common.

Table 6.1.3 *Resistance percentages of S. Typhimurium and S. Typhimurium monophasic (N tested) isolated from humans and non-human sources in 2024*

	***S.* Typhimurium (235)**				***S.* Typhimurium monophasic (143)**		
	Humans (185)	**Cattle (12)**	**Pigs (10)**	**Other non-human sources (28)[a]**	**Humans (125)**	**Pigs (7)**	**Other non-human sources (11)[b]**
ampicillin	16.8	83.3	90.0	28.6	68.8	71.4	81.8
cefotaxime	0.5	0.0	0.0	0.0	0.0	0.0	0.0
ceftazidime	0.0	0.0	0.0	0.0	0.0	0.0	0.0
gentamicin	1.6	66.7	10.0	10.7	2.4	0.0	9.1
tetracycline	36.8	66.7	70.0	32.1	56.8	57.1	45.5
sulfamethoxazole	38.4	66.7	70.0	32.1	59.2	71.4	54.5
trimethoprim	6.0	8.3	30.0	7.1	12.0	0.0	9.1
ciprofloxacin	5.4	0.0	0.0	3.6	10.4	0.0	9.1
nalidixic acid	5.4	8.3	0.0	3.6	10.4	0.0	0.0
chloramphenicol	9.2	66.7	40.0	10.7	5.6	14.3	9.1
azithromycin	0.0	0.0	0.0	0.0	0.0	14.3	0.0
meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0
tigecycline	0.5	0.0	0.0	0.0	0.8	0.0	0.0
amikacin	0.0	0.0	0.0	0.0	0.0	0.0	9.1

[a] Non-human sources include horses (13), layers (9), broilers (5), and ducks (1).
[b] Non-human sources include broilers (5), cattle (3), goats (1), turkeys (1), and unspecified non-human sources (1).

Figure 6.1.1 *Resistance (%) trends for human S. Typhimurium 2000-2024*



S. Enteritidis

In the Netherlands, layers and their eggs are the main reservoir of *S.* Enteritidis. For human *S.* Enteritidis isolates in 2024, resistance against fluoroquinolones was high (ciprofloxacin and nalidixic acid: 31.3%; Table 6.1.4). This percentage occurs against the background of an upward trend over the last 10 years, including a peak percentage of 38% in 2022. Although a these high percentages cannot be fully explained, we suppose that it may be partly attributable to a substantially higher rate of ciprofloxacin resistance among strains isolated from travellers. Third-generation cephalosporins resistance among human *S.* Enteritidis isolates was rare, and no meropenem resistant isolates were found.

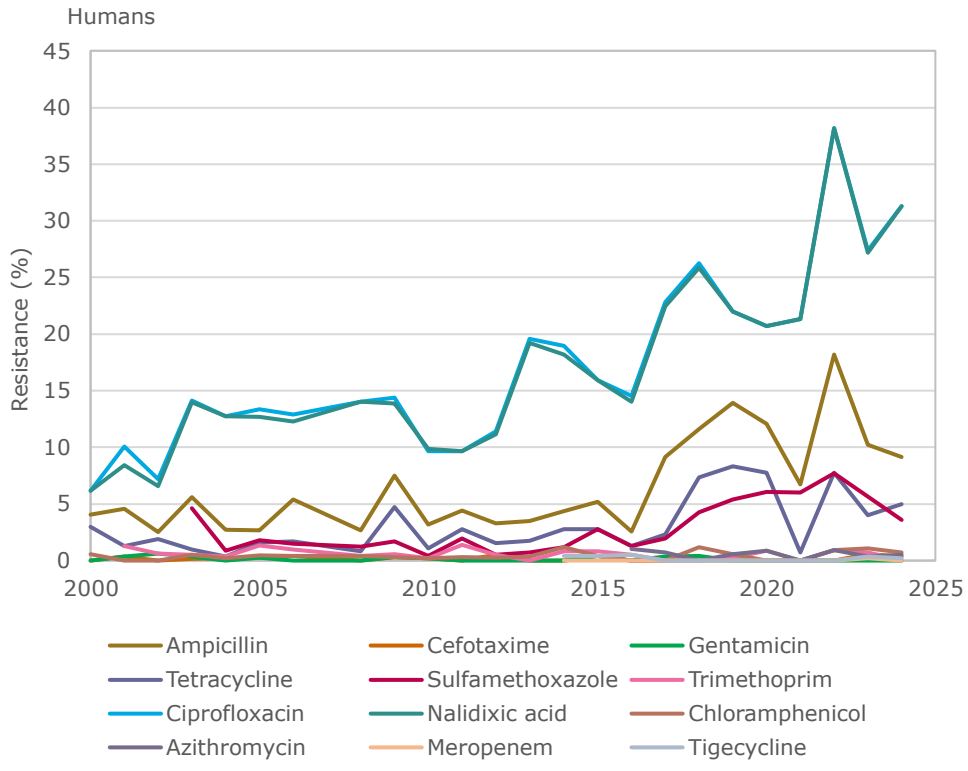
Fluoroquinolone-resistance levels among isolates from layers and broilers were much lower (1.8% and 4.8%, respectively) than among isolates from humans. It has to be stressed that these non-human resistance data should be interpreted with caution as the number of isolates is relatively small. Long-year trends for human *S.* Enteritidis isolates are shown in figure 6.1.2.

Table 6.1.4 *Resistance percentages of S. Enteritidis (N tested) isolated from humans and non-human sources in 2024*

	***S.* Enteritidis (775)**			
	Humans (581)	**Layers (167)**	**Broilers (21)**	**Other non-human sources (6)[a]**
ampicillin	9.1	0.6	0.0	16.7
cefotaxime	0.3	0.0	0.0	16.7
ceftazidime	0.3	0.0	0.0	0.0
gentamicin	0.0	0.0	0.0	0.0
tetracycline	5.0	0.0	0.0	0.0
sulfamethoxazole	3.6	0.0	4.8	0.0
trimethoprim	0.0	0.0	4.8	0.0
ciprofloxacin	31.3	1.8	4.8	0.0
nalidixic acid	31.3	1.8	4.8	0.0
chloramphenicol	0.7	0.0	0.0	0.0
azithromycin	0.5	0.0	0.0	33.3
meropenem	0.0	0.0	0.0	0.0
tigecycline	0.2	0.0	0.0	0.0
amikacin	0.0	0.0	0.0	16.7

[a] Other non-human sources include horses (2), pigs (2), and unspecified non-human sources (2).

Figure 6.1.2 *Resistance (%) trends for human S. Enteritidis, 2000-2024*



ESBL/pAmpC-producing and multidrug resistant Salmonella enterica in humans and animals
The global emergence of human infections with multidrug-resistant *Salmonella* strains including both fluoroquinolone resistance and resistance against beta-lactams, including cephalosporins, through the production of extended-spectrum beta-lactamases (ESBL) and plasmid-mediated AmpC (pAmpC), results in severe limitations of antimicrobial treatment of human infections. In 2024, the percentage of all human and non-human genotypically confirmed ESBL/pAmpC-producing *Salmonella* isolates was 1% (21/2105). This percentage is comparable to the ones from previous years (0.9% in 2023 (20/2139), 1.2% in 2022 (14/1156), 0.8% in 2021 (10/1264)). In the group of 21 ESBL-producing *Salmonella* isolates, 20 were obtained from humans, and 19/21 (90%) were also resistant to ciprofloxacin.

Resistance among Salmonella enterica isolates from broiler meat, pork, and other meat sources
The occurrence of third generation cephalosporin resistance was very high among isolates from imported broiler-meat-isolates (66%), compared to 0% in Dutch/EU broiler meat (Table 6.1.5). The situation was different for fluoroquinolones, with very high resistance levels in both Dutch/EU and imported broiler meat, and this has not changed over last 5 years.
In figure 6.1.3, trends in resistance levels of *Salmonella enterica* isolates from broiler meat are presented. Trends should be interpreted with caution, as the resistance levels presented are also influenced by the distribution of serotypes and the origin (Dutch/EU or imported meat) of the samples.

Figure 6.1.3 *Trends in resistance (%) of Salmonella enterica isolated from broiler meat in the Netherlands, 2001-2024*

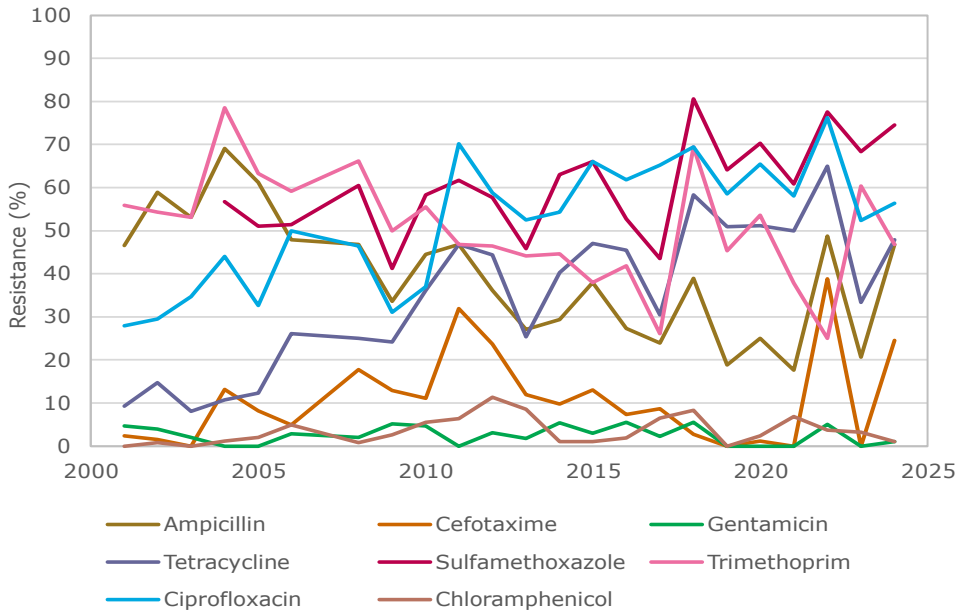


Table 6.1.5 *Resistance (%) of Salmonella enterica isolated from different types of raw meat in the Netherlands in 2024*

	Dutch/EU broiler meat[a]	Imported broiler meat	Pork	Other meat[b]
	N = 59	N = 35	N = 18	N = 12
ampicillin	32.2	71.4	44.4	8.3
cefotaxime	0.0	65.7	0.0	0.0
ceftazidime	0.0	65.7	0.0	0.0
gentamicin	1.7	0.0	0.0	0.0
tetracycline	32.2	74.3	38.9	8.3
sulfamethoxazole	74.6	74.3	50.0	0.0
trimethoprim	71.2	5.7	22.2	0.0
ciprofloxacin	55.9	57.1	0.0	8.3
nalidixic acid	55.9	57.1	0.0	0.0
chloramphenicol	0.0	2.9	11.1	0.0
azithromycin	0.0	0.0	0.0	0.0
meropenem	0.0	0.0	0.0	0.0
tigecycline	3.4	8.6	0.0	0.0
amikacin	0.0	2.9	0.0	0.0

[a] Fresh chicken meat sampled at retail and chicken neck skin from verification projects.
[b] Other meat includes beef (7), duck (2), lamb (1), sheep (1), and turkey (1).

Conclusions

- Although the resistance percentages for sulfamethoxazole and tetracycline among human *S*. Typhimurium isolates were high in 2024 (38% and 37%, respectively), and also higher than in the previous year, there has been a steady downward trend in resistance levels to these antibiotics over the time period 2015–2023.
- Among animal *S*. Typhimurium isolates, comparable high levels of resistance to sulfamethoxazole and tetracycline were found, although assessing trends over time is challenging due to the limited sample size.
- Among human *S*. Enteritidis isolates resistance against fluoroquinolones is high (31% in 2024), and it has been increasing since 2010. At the same time, 2024 fluoroquinolone resistance in a small sample of *S*. Enteritidis isolates from layers and boilers was much lower (2% and 5%), although significant fluctuations over time have been observed.
- Over the past 5 years, the proportion of ESBL-producing *Salmonella* isolates from humans has remained stable, with a prevalence of 1.4% (20/1470) in 2024.
- None of the tested human and non-human *S.* enterica isolates were found to produce carbapenemases.
- With regard to broiler meat, the occurrence of resistance against third generation cephalosporins appears to be limited to *S*. enterica isolated from imported broiler meat. This is however not the case for fluoroquinolone resistance levels, as these levels were very high in both Dutch/EU and imported broiler meat (both around 60%).

References

1. SWAB richtlijn antimicrobiële therapie voor acute infectieuze diarree, 2023. https://swab.nl/nl/exec/file/download/259

6.2 Campylobacter

Introduction

According to the European legislation[1] antibiotic resistance monitoring of zoonotic bacteria includes *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) as important food-borne pathogens. In this chapter, the occurrence and trends in antimicrobial resistance in *C. jejuni* and *C. coli* are described. Isolates were obtained from samples collected from food animals, meat and humans.

Methods

Isolation of campylobacters from faecal samples was performed according to the guidelines of the EURL Campylobacter by direct inoculation of faecal material on two different media: mCCDA and Butzler. After 48 hours incubation at 37 ^{0}C under aerophilic conditions presumptive campylobacter colonies are pure cultured and identified by MALDI-TOF mass spectrometry (MALDI Biotyper®, Bruker). In case of confirmed identification, one *C. coli* and one *C. jejuni* is analysed. Susceptibility testing is performed by broth microdilution according to ISO 20776-1:2019 in a standard antimicrobial panel designed for testing campylobacter which contains six antibiotics representing antibiotic classes relevant for treatment of human infections caused by Campylobacter (erythromycin, gentamicin and ciprofloxacin) or included for monitoring purposes (chloramphenicol, ertapenem and tetracycline)[2]. Within this monitoring program non-wild type susceptible isolates (exhibiting MIC-values above the ECOFF), obtained from farm animals and food are classified as resistant. These isolates most probably contain an acquired resistance mechanism, but may not be clinically resistant.

Data on antimicrobial resistance in human campylobacteriosis cases were extracted from the Infectious Diseases Surveillance Information System for Antimicrobial resistance (ISIS-AR) at the RIVM. In case of patients with multiple isolates of the same species within 60 days, only the first isolate was selected, to avoid repeated sampling causing bias in the calculation of resistance levels and time trends.

Results

6.2.1 Campylobacter in animals and food

Bacterial isolates

In 2024, 61 *C. jejuni* and 47 *C. coli* isolates obtained from caecal samples of broilers, 219 *C. jejuni* and 87 *C. coli* from caeca of veal calves as well as 286 *C. coli* from caecal samples of slaughter pigs were tested for antimicrobial susceptibility. In addition, 202 *C. jejuni* and 73 *C. coli* isolates obtained from chicken meat and neck skin were included in the monitoring.

Resistance levels

Table 6.2.1.1 shows the aggregated MIC distributions and resistance rates for all *C. jejuni* and *C. coli* strains isolated in 2024 from caecal samples of broilers, veal calves, and pigs. The resistance rates of *C. jejuni* and *C. coli* from caecal samples of broilers, veal calves, and pigs,

as well as neck skin and chicken meat, are shown in table 6.2.1.2. Trends in resistance of *C. jejuni* in broilers and poultry meat are shown in figures 6.2.1.1a and 6.2.1.1b and trends in resistance of *C. coli* in broilers and poultry meat are shown in figures 6.2.1.2a and 6.2.1.2b. National surveillance data for *Campylobacter* spp. isolated from humans are shown in figure 6.2.1.3 (from 2002) and table 6.2.1.3 (from 2009).

Broiler chickens, neck skin and chicken meat
In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from caecal samples of broilers as well as from samples of neck skin and chicken meat. Figure 6.2.1.1a and 6.2.1.1b demonstrate continuous high levels of resistance for ciprofloxacin and tetracycline in *C. jejuni* from broilers and chicken meat in the period 2010 - 2024. Table 6.2.1.2 shows high levels of tetracycline (>50%) in *C. jejuni* isolates in 2024 for isolates from chicken meat and neck skin as well as for isolates from caecal samples of broilers. High levels of resistance to ciprofloxacin were also observed in broilers (62.3%), chicken meat (66.9%) and neck skin (75.9%). Resistance to chloramphenicol, erythromycin and gentamicin was not detected in *C. jejuni* from poultry sources in 2024.

Figure 6.2.1.2a and 6.2.1.2b displays fluctuating high levels of resistance to tetracycline in *C. coli* isolates from broilers and chicken meat. During the period 2020 -2024, ciprofloxacin resistance in *C. coli* from broilers increased to extreme high levels of resistance above 90% from 2023 onwards. A high level of resistance to ciprofloxacin was also observed in *C. coli* isolates from chicken meat (87.7%) and chicken neck skin (87.5%). As in former years, gentamicin resistance in *C. coli* from broilers and poultry meat was completely absent. Unlike *C. jejuni*, macrolide resistance is observed annually in *C. coli*, with resistance rates less than 10% since 2019.

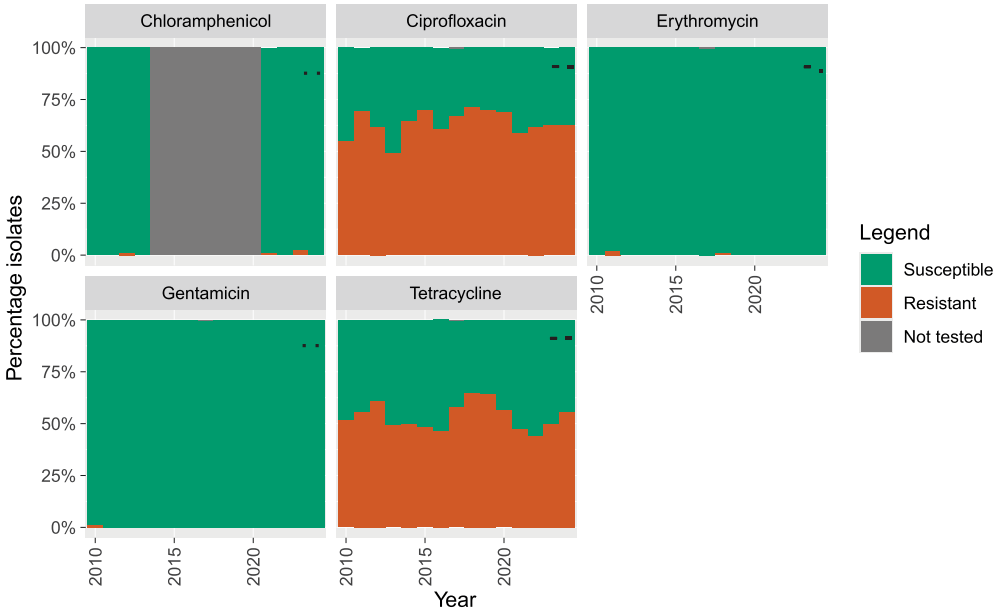
Higher resistance proportions were observed for almost all antimicrobials in *C. coli* isolates from broilers and chicken meat, compared to *C. jejuni* isolates from the same sources.

Table 6.2.1.1 *MIC distributions (in %) for Campylobacter jejuni (N = 280) and C. coli (N = 420) isolated from caecal samples of broilers, veal calves and pigs in 2024*

***C. jejuni,* broilers and veal calves**	**MIC (%) distribution mg/L**															**R%**	**95% CI**
(N = 280)	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol						14.3	67.5	14.3	3.9							0.0	0,0 - 1,3
Ciprofloxacin		38.6	8.9	1.4	0.4			20.7	20.7	8.2	1.1					51.1	45,1 - 57,1
Erythromycin					42.9	44.6	12.5									0.0	0,0 - 1,3
Ertapenem		62.9	26.8	5.7	2.1	1.4	1.1									-	-
Gentamicin			87.9	10.7	1.4											0.0	0,0 - 1,3
Tetracycline				16.4	2.5	2.1		0.7	0.7	2.9	14.6	60.0				81.1	76,0 - 85,5

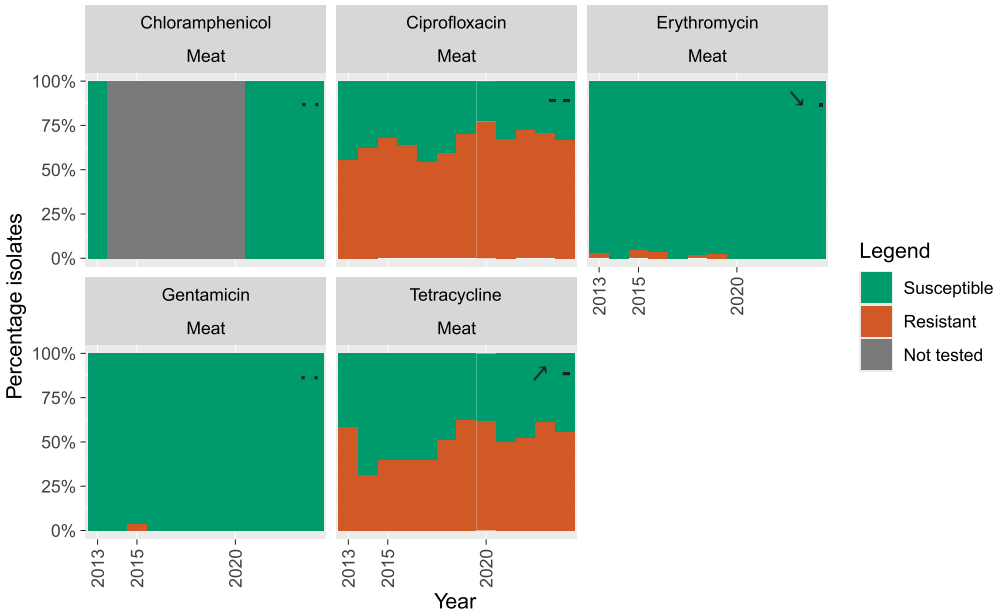
***C. coli,* broilers, veal calves and pigs**	**MIC (%) distribution mg/L**															**R%**	**95% CI**
(N = 420)	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol						2.1	37.4	46.9	13.6							0.0	0,0 - 0,1
Ciprofloxacin		45.0	15.5	2.9			2.1	11.4	14.8	8.1	0.2					36.7	32,1 - 41,5
Erythromycin					32.6	31.4	20.7	2.6	0.5		0.2			1.0	11.0	12.6	9,6 - 16,2
Ertapenem		29.3	28.6	14.0	13.3	7.9	4.5	2.4								-	
Gentamicin			33.6	64.8	1.0					0.7						0.7	0,2 - 2,1
Tetracycline				17.9	3.1	1.9	0.2	0.2	1.2	2.9	8.3	64.3				77.1	72,8 - 8,1

Figure 6.2.1.1a *Trends in resistance of Campylobacter jejuni from broilers*



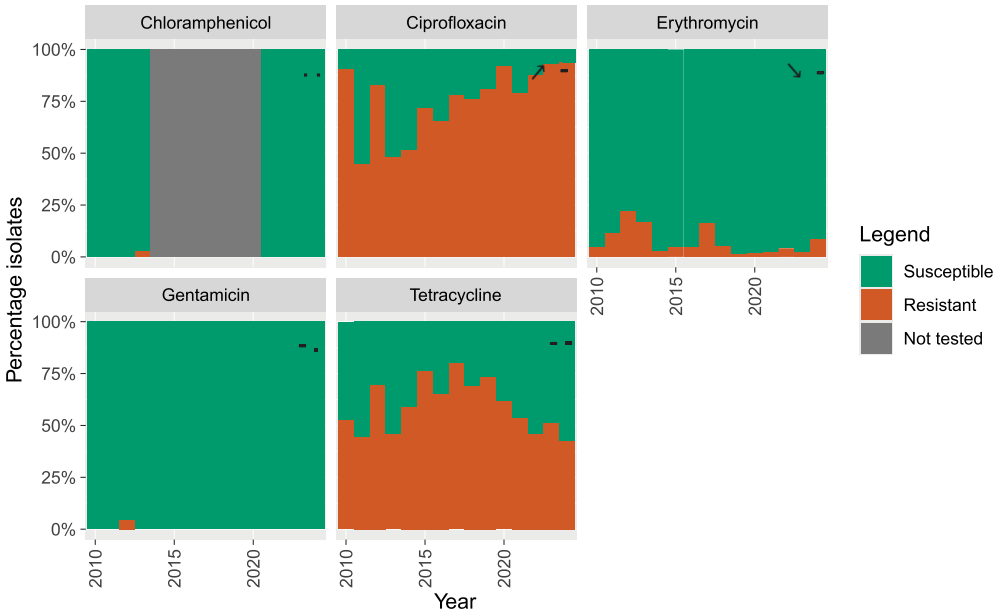
Percentage (%) of resistant C. jejuni isolates against different antibiotics, isolated from faecal samples of broilers in the period 2010-2024 in the Netherlands. The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Figure 6.2.1.1b *Trends in resistance of Campylobacter jejuni from chicken meat.*



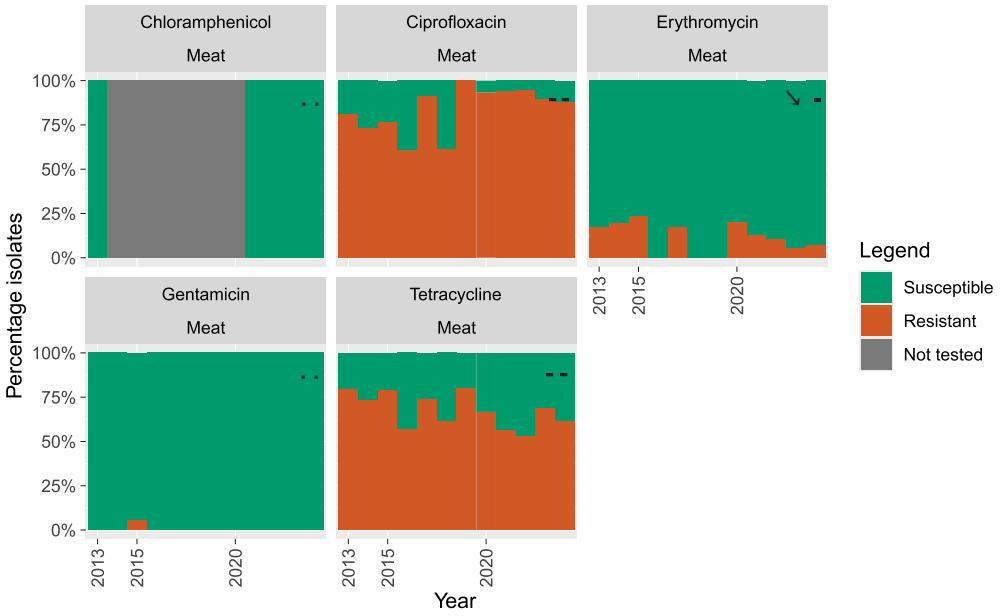
Percentage (%) of resistant C. jejuni isolates against different antibiotics, isolated from chicken meat samples in the period 2013-2024 in the Netherlands. The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2013-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Figure 6.2.1.2a *Trends in resistance of Campylobacter coli from broilers*



Percentage (%) of resistant C. coli isolates against different antibiotics, isolated from faecal samples from broilers in the period 2010-2024 in the Netherlands. The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Figure 6.2.1.2b *Trends in resistance of Campylobacter coli from chicken meat*



Percentage (%) of resistant C. coli isolates against different antibiotics, isolated from chicken meat samples in the period 2013-2024 in the Netherlands. The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2013-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Table 6.2.1.2 *Resistance percentages of C. jejuni and C. coli isolated from faecal samples, nek skin and meat in 2024*

	***C. jejuni* (R%)**				***C. coli* (R%)**				
	Broilers	**Chicken meat**	**Chicken neck skin**	**Veal calves**	**Broilers**	**Chicken meat**	**Chicken neck skin**	**Veal calves**	**Pigs**
	N=61	**N=148**	**N=54**	**N=219**	**N=47**	**N=57**	**N=16**	**N=87**	**N=286**
Chloramphenicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	62.3	66.9	75.9	47.9	93.6	87.7	87.5	74.7	15.7
Erythromycin	0.0	0.0	0.0	0.0	8.5	7.0	0.0	44.8	3.5
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0
Tetracycline	55.7	55.4	63.0	88.1	42.6	61.4	68.8	93.1	78.0

Veal calves and fattening pigs

From 2021 onwards , the mandatory monitoring of antimicrobial resistance in *Campylobacter* has been extended to *C. jejuni* and *C. coli* from veal calves (< 1 year) as well as *C. coli* obtained from slaughter pigs. Resistance proportion are depicted in table 6.2.1.2. As in former years, very high proportions of tetracycline resistance were measured for *C. jejuni* and *C. coli* from veal calves (88.1 - 91.1%) as well as for *C. coli* from pigs (78.0%). In veal calves resistance to ciprofloxacin was also high in both *C. jejuni* and *C. coli* (47.9 – 74.7%). Substantially lower levels of ciprofloxacin resistance were detected in *C. coli* from pigs (15.7%). In *C. jejuni* from veal calves resistance was completely absent for chloramphenicol, erythromycin and gentamicin. As in previous years, erythromycin resistance was highly prevalent in *C. coli* from veal calves (44.8%), while gentamicin resistance was observed at low levels (<5%) and resistance to chloramphenicol was completely absent. In *C. coli* from pigs resistance to erythromycin was rare (<5%) and resistance against chloramphenicol and gentamicin was not detected.

6.2.2 *Campylobacter in humans*

In 2024, 5389 campylobacteriosis cases were registered in ISIS-AR, which is almost similar to 2023 (n = 5199) and pre-COVID years (average 2015-2019, n = 5120). During the COVID-19 pandemic the number of isolates was lower (average 2020-2021, n = 4070). Resistance levels in isolates from human patients were determined for ciprofloxacin, tetracycline and erythromycin, and are shown in table 6.2.1.3 and figure 6.2.1.3. Figure 6.2.1.3 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance. In 2020 to 2022, however, resistance levels for all measured antibiotics dropped, most likely due to a substantial reduction in travel-related campylobacteriosis as a result of the COVID-19 lockdown, which is associated with higher resistance levels than domestically acquired campylobacteriosis. Because data on travel history it not available, this cannot be confirmed.

In 2024, the resistance level for ciprofloxacin in human *Campylobacter* spp. isolates was 62.3%, which is a public health concern. However, resistance is still lower compared with pre-pandemic years 2018 and 2019, with a 63.6% and 68.9% resistance for ciprofloxacin, respectively. Tetracycline resistance had a similar trend and was even slightly lower than in 2022 and 2023, with a 45.0% resistance compared with 48.8%

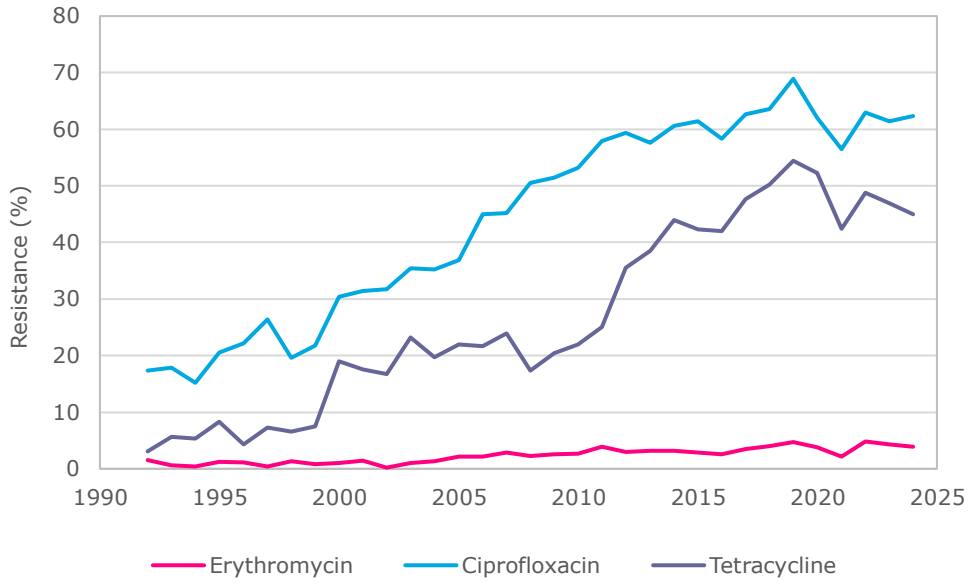
and 47.0%. Erythromycin resistance was 3.9% in 2024. This is slightly lower than in the two preceding years, but higher than in 2020 and 2021.

Table 6.2.1.3 shows the resistance levels for human *C. jejuni* and *C. coli* isolates since 2017, with higher resistance proportions for *C. coli* isolates than *C. jejuni* isolates. Overall, the resistance levels in human *C. jejuni* and *C. coli* isolates for all three antimicrobials show an increasing trend until 2019 and a reduction in resistance levels in 2020 and 2021. In 2022-2024, the resistance levels for *C. jejuni* and *C. coli* isolates stabilized and were back at pre-pandemic levels, except for fluoroquinolone and tetracycline resistance in *C. jejuni*, which were still lower than before the pandemic.

Table 6.2.1.3 *Resistance in C. jejuni and C. coli isolated from humans from 2017 – 2024*

	C. jejuni						***C. coli***					
	Fluoro-quinolone		**Tetracycline**		**Erythromycin**		**Fluoro-quinolone**		**Tetracycline**		**Erythromycin**	
	N	**R%**	**N**	**R%**	**N**	**R%**	**N**	**R%**	**N**	**R%**	**N**	**R%**
2017	2965	62.0	2642	50	3026	2.6	274	77.0	256	70.3	275	22.9
2018	3224	63.3	2965	53.9	3285	2.1	296	73.0	275	69.8	303	26.4
2019	3338	66.6	3116	54.0	3395	1.9	400	78.3	379	73.1	402	20.7
2020	2508	59.6	2352	50.3	2523	2.1	208	68.3	198	73.2	209	20.1
2021	2904	53.2	2768	40.2	2954	1.3	224	69.2	215	64.7	234	15.8
2022	3578	61.0	3383	45.5	3624	2.5	353	72.5	344	66.0	361	19.4
2023	3913	60.0	3652	44.9	3932	2.6	358	76.5	338	69.8	363	22.6
2024	4458	60.8	4260	42.3	4452	1.8	490	76.1	469	69.5	491	22.6

Figure 6.2.1.3 *Trends in resistance (%) of Campylobacter spp. isolated from humans between 1992 and 2024*



Conclusions

- In 2024, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof remained at a high level for quinolones and tetracycline.
- Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, and was observed at low levels in *C. jejuni* in veal calves and *C. coli* in broilers and poultry meat. As in former years, a notably higher level of erythromycin resistance was observed in *C. coli* from veal calves.
- The level of ciprofloxacin resistance in human *Campylobacter* spp. isolates remained high in 2024 and continues to be a public health concern. However, the strong upward trend observed over many years has now stabilized at a level that is slightly lower than before the COVID-19 pandemic.
- Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

References

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2. European Food Safety A, Aerts M, Battisti A, et al. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. *EFSA J*. Jun 2019;17(6):e05709.

6.3 Shiga-toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC)

Shiga-toxin producing *Escherichia coli* (STEC) is a bacterial zoonotic agent associated with human disease with varying clinical manifestations, including diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), a leading cause of acute renal failure among children. The natural reservoir of STEC is the gastrointestinal tract of ruminants, especially cattle and small ruminants[1]. Although, therapeutic treatment of STEC infections with antimicrobials is not regularly advised, monitoring AMR in STEC from symptomatic human cases is useful in assessing the risk of transmission of resistant bacteria, and resistance genes, from ruminants to humans.

Isolates from human clinical cases (N=336) consisting of multiple STEC/aEPEC/tEPEC[1*] O157 and non-O157 serotypes were tested for susceptibility. The set consisted of 73 STEC/EPEC O157 isolates and 263 STEC/EPEC non-O157 isolates. The most frequently occurring non-O157 isolates consisted of: O26 (n=31), O103 (n=28), O145 (n=28), O146 (n=25), O91 (n=13), O71 (n=11), O63 (n=10), and other O-types (n=117). All isolates were obtained from the RIVM national laboratory surveillance of STEC. Table 6.3.1 shows the MIC results for *E. coli* O157 isolates from humans; Table 6.3.2 shows resistance proportions of *E. coli* O157 and STEC/EPEC non-O157 isolates; Figure 6.3.1 presents the trends over time for STEC O157; Figure 6.3.2 presents the trends in resistance over time for STEC/EPEC non-O157.

In comparison to 2023, an increase in resistance proportions among STEC O157 can be seen for ampicillin, gentamicin, tetracycline, sulfamethoxazole, trimethoprim, and chloramphenicol (Figure 6.3.1). Following a sharp increase in 2021, the resistance proportions for tetracycline (20.6%) and sulfamethoxazole (22.2%) declined to 5.9% and 8.8% in 2023, respectively, before rising again in 2024 to 11.0% and 15.1%, similar to those in 2022. Resistance levels for gentamicin and chloramphenicol increased, in line with the trend observed in 2019. Resistance remained below 5% for gentamicin but exceeded 5% for chloramphenicol. There was no resistance detected for ciprofloxacin among STEC O157 in 2024. No ESBL-producing isolates were detected in 2024 among STEC O157 reflected by the absence of resistance for both cefotaxime and ceftazidime.

[1*] aEPEC = atypical enteropathogenic *E. coli*, which share the LEE-pathogenicity island with STEC but lack shiga-toxin genes a well as the EPEC adherence factor plasmid. tEPEC = typical enteropathogenic *E. coli*, which possesses the LEE-pathogenicity island as well as the EPEC adherence factor plasmid, but lack shiga-toxin genes.

Table 6.3.1 *MIC distribution (in %) and resistance percentages (R% incl 95% CI) for E. coli STEC O157 (N=74) isolated from humans in the Netherlands in 2024*

E. coli	**MIC (%) distribution mg/L**																		**R%**	**95% CI**
N = 73	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**		
Amikacin									97.3	2.7									0.0	0.0 - 0.0
Ampicillin							1.4	1.4	84.9	1.4			11.0						11.0	3.6 -18.2
Cefotaxime					100.0														0.0	0.0 - 0.0
Ceftazidime					95.9	4.1													0.0	0.0 - 0.0
Gentamicin						79.5	15.1	1.4			2.7	1.4							4.1	-0.5 - 8.7
Tetracycline								80.8	8.2				11.0						11.0	3.6 -18.2
Sulfamethoxazole										39.7	41.1	4.1					15.1		15.1	6.6 - 23.4
Trimethoprim					84.9	8.2						6.8							6.8	0.9 - 12.7
Ciprofloxacin	91.8	8.2																	0.0	0.0 - 0.0
Nalidixic acid									98.6	1.4									0.0	0.0 - 0.0
Chloramphenicol										87.7	2.7			9.6					9.6	2.6 - 16.4
Azithromycin								58.9	39.7			1.4		0.0					1.4	-1.3 - 4.0
Colistine							98.6	1.4											0.0	0.0 - 0.0
Meropenem		98.6	1.4																0.0	0.0 - 0.0
Tigecycline					98.6	1.4													0.0	0.0 - 0.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints.

Figure 6.3.1 *Trends in resistance (in %) of E. coli STEC O157 isolated from humans in the Netherlands from 1999 - 2024*

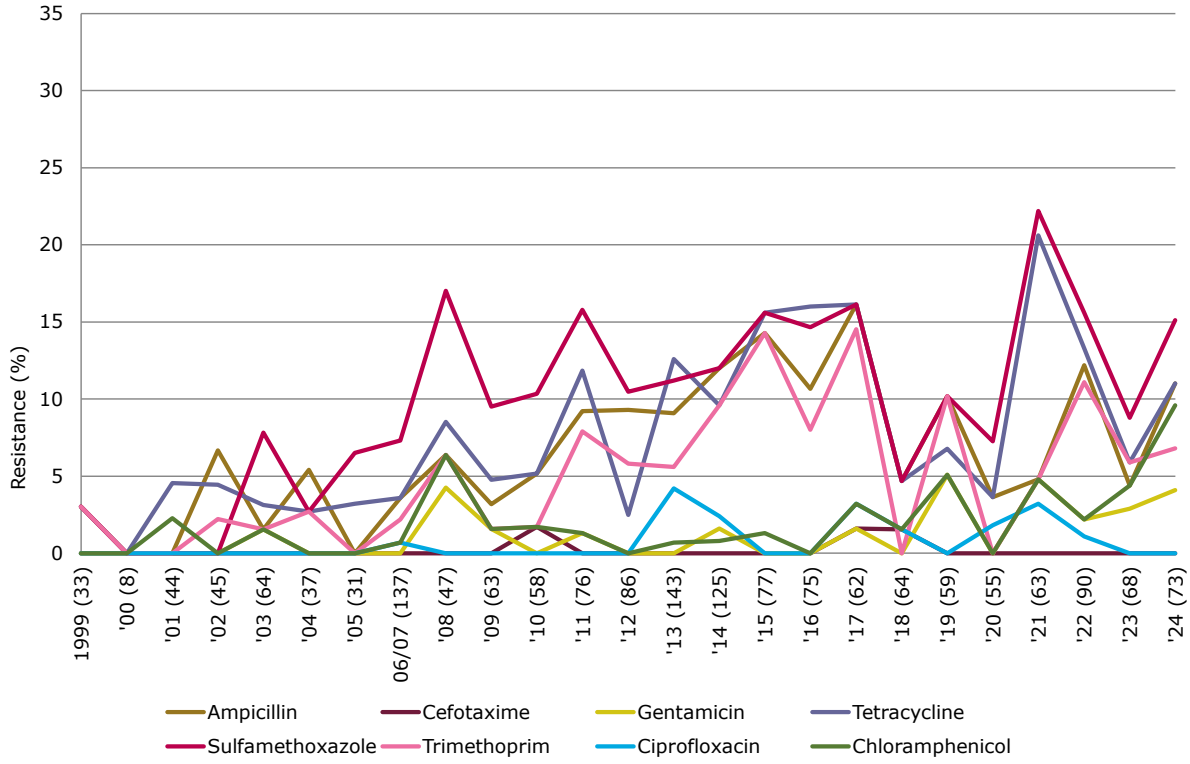


Figure 6.3.2 *Trends in resistance (in %) of E. coli STEC/EPEC non-O157 isolated from humans in the Netherlands from 2020-2024*

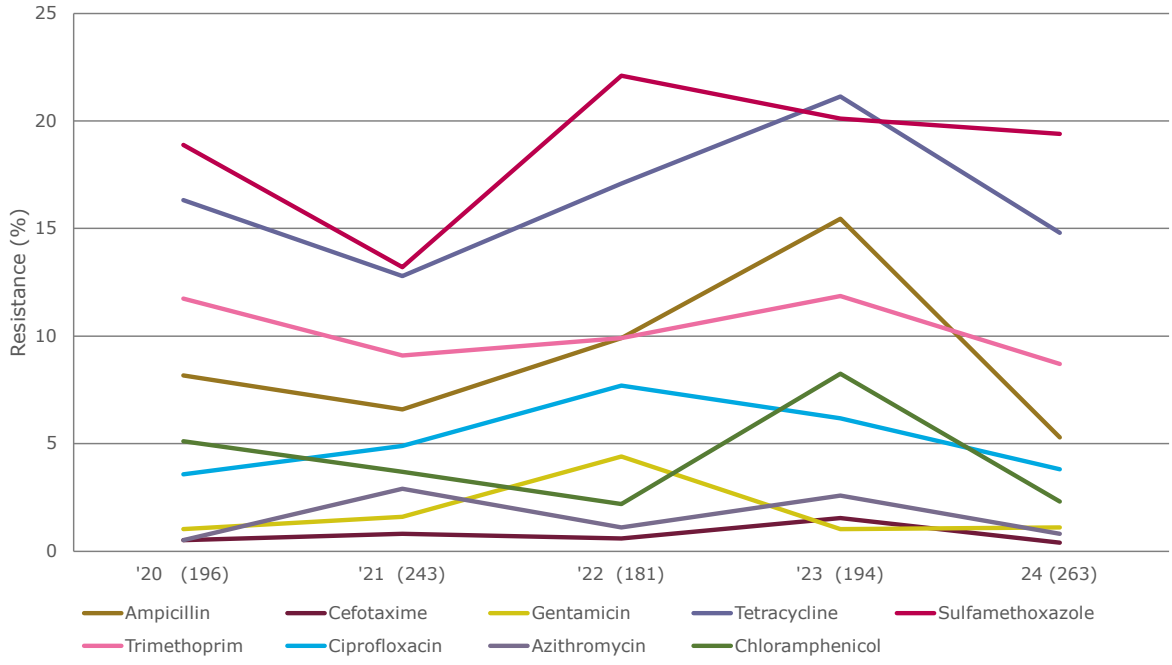


Table 6.3.2 shows differences in proportion of resistance between STEC O157 and STEC/EPEC non-O157 isolates. Statistical differences in proportion is observed for chloramphenicol (χ^2, p =0.004) with a higher resistance level for O157 isolates compared to non-O157 isolates. The proportion of multidrug resistance[2*] was not significantly different at 15.1% in STEC O157 and 19.8% in non-O157 isolates, with 11 out of 73 and 52 out of 263 isolates, respectively. Furthermore, from the multidrug resistant isolates the most frequently occurring non-O157 isolates, were of the serovar O26:H11 (n=31), O103:H2 (n=28), and O145:H28 (n=28). Resistance to 3rd gen cephalosporins (cefotaxime and ceftazidime) was detected in one non-O157 isolate; O103:H2 (stx1, eae and heam positive). Resistance marker detection in the genome sequence confirmed the presence of the ESBL gene $bla_{CTX\text{-}M\text{-}55}$, .

Table 6.3.2 *Resistance percentages (R%) of pathogenic E. coli from humans in the Netherlands in 2024*

E. coli	**O157**	**Other serotypes**
	N=73	**N=263**
Amikacin	0.0	0.0
Ampicillin	11.0	5.3
Cefotaxime	0.0	0.4
Ceftazidime	0.0	0.4
Gentamicin	4.1	1.1
Tetracycline	11.0	14.8
Sulfamethoxazole	15.1	19.4
Trimethoprim	6.8	8.7
Ciprofloxacin	0.0	3.8
Nalidixic acid	0.0	2.3
Chloramphenicol	9.6	2.3
Azithromycin	1.4	0.8
Colistin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0
Multidrug resistence	15.1	19.8

[2*] Multidrug resistance defined here as resistant against ≥2 classes of antimicrobials

Conclusions

- In human STEC O157 isolates, an increase in proportions of resistance against ampicillin, gentamicin, tetracyclin, sulfamethoxazole, trimethoprim, and chloramphenicol compared to 2023 was observed.
- The proportion of resistance of chloramphenicol was higher in human STEC/EPEC O157 *E. coli* than in human STEC non-O157.
- No ESBL-producing isolates were detected in STEC O157, but resistance to third generation cephalosporins was detected in one STEC/EPEC non-O157 *E. coli* isolate, containing *bla*$_{\text{CTX-M-55}}$.

References

1. L. Mughini - Gras, W. van Pelt, M. van der Voort, M. Heck, I. Friesema E. Franz, Attribution of human infections with Shiga toxin - producing *Escherichia coli* (STEC) to livestock sources and identification of source - specific risk factors, The Netherlands (2010–2014), *Zoonosis and Public Health*, Volume65, Issue1, February 2018 https://doi.org/10.1111/zph.12403

6.4 Yersinia

Introduction

Human food-borne yersiniosis is primarily caused by the *Yersinia enterocolitica* species. *Y. enterocolitica* is a zoonotic gram-negative bacterial pathogen that mostly infects humans through contaminated food. The majority of infections cause mild, self-limiting gastrointestinal disease with diarrhea that generally does not require antimicrobial treatment. Abdominal pain related to mesenteric lymphadenitis may occur, and may be mistaken for appendicitis. Bacteremia is rare, and mostly limited to young or immunocompromised children. Post-infectious sequelae such as reactive arthritis or erythema nodosum may occur, especially in older children and adults.

Pigs and rodents represent *Y. enterocolitica's* main natural reservoir, from where it spreads to humans via contaminated meat and/or environmental routes such as contact with soil or water. *Y. pseudotuberculosis* is a distinct *Yersinia* species complex that can be found in an array of mammals and birds, and is the second most commonly reported *Yersinia* species causing human infection. Clinical disease with acute pseudo-appendiceal pain is more common with *Y. pseudotuberculosis* than with *Y. enterocolitica*. Other zoonotic species such as *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii* may cause gastrointestinal disease in humans as well, but such infections are rare.

The evidence-base for appropriate antimicrobial treatment of human yersiniosis is limited.[1] However, ciprofloxacin and third-generation cephalosporins are recommended treatment options for infections with susceptible strains. Worldwide, beta-lactamase production among *Yersinia* species is common, resulting in wide-spread amoxicillin/ampicillin and first-generation cephalosporin resistance.[2]

Methods

This year for the first time, human yersiniosis data from the past 5 years were extracted from the ISIS-AR-surveillance database. ISIS-AR's selection criteria are described in Chapter 5.1.1. Antimicrobial resistance proportions of *Y. enterocolitica* were determined for amoxicillin/ampicillin, third-generation cephalosporins (ceftazidime and cefotaxime/ceftriaxone), cotrimoxazole, gentamicin, ciprofloxacin, and meropenem/imipenem. The limited amount of available AMR-related data for *Yersinia* did not allow for a reliable analysis of macrolide resistance.

Results

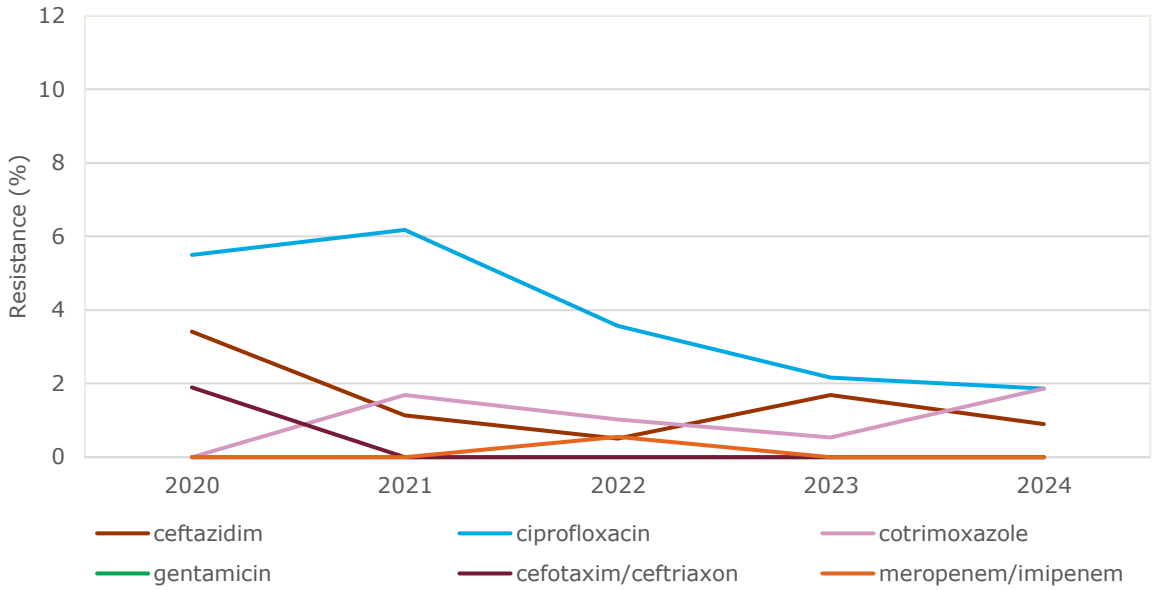
The majority of human yersiniosis cases was caused by *Y. enterocolitica* (82%), followed by *Y. frederiksennii* (4%) (Table 6.4.1). Over the past five years, the levels of resistance to amoxicillin/ampicillin in *Y. enterocolitica* have been very high (98%). In that same time period, resistance levels against ciprofloxacin for this species decreased from 6% in 2021 to 2% in 2024, while the proportions of resistance to third-generation cephalosporins, cotrimoxazole, and gentamicin have remained stable at levels below 2% in the same period (Figure 6.4.1).

Table 6.4.1 *Number of tested Yersinia spp. in the Netherlands in 2024, based on ISIS-AR data*

Type of species	Tested isolates, N
Yersinia enterocolitica	244
Yersinia frederiksennii	13
Yersinia pseudotuberculosis	7
Yersinia rohdei	7
Other *Yersinia* spp. and *Yersinia* n.s.	26

n.s. not specified.

Figure 6.4.1 *Trends in resistance (%) of human Y. enterocolitica isolates, 2020-2024*



Amoxicillin/ampicillin resistance levels are not shown, but have remained consistently high at approximately 98%.

Conclusions

- *Y. enterocolitica* was the Yersinia species most commonly identified in human yersiniosis cases (82%).
- Amoxicillin/ampicillin resistance was present in almost all *Y. enterocolitica* isolates.
- Over the past five years, ciprofloxacin resistance in *Y. enterocolitica* has decreased from 6% to 2%. During the same time period, resistance to third-generation cephalosporins and cotrimoxazole, has remained consistently below 2%.

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7 Antimicrobial resistance in commensal bacteria from farm animals and food

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract from farm animals and derived meat in accordance with EFSA guidelines [1] and European legislation [2]. The level of antimicrobial resistance (AMR) in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. In addition, the level of antibiotic resistance in commensal bacteria in food is monitored closer to the consumer to track the spread of resistant genes and bacteria through the food chain. *E. coli* is included as indicator organism for the Gram-negative bacterial population. After a discontinuation of seven years, enterococci were included in the AMR monitoring program again as representatives of the Gram-positive bacterial population. Reintroduction of Enterococcus to the program allows for better tracking of the prevalence and spread of AMR among Gram-positive bacteria. This report provides resistance data for enterococci in broiler chickens. EFSA [1] prescribes the sampling strategy and isolation methodology of bacteria from caeca of randomly selected food-producing animals at slaughter with the aim to detect the occurrence and trends in resistance at the bacterial population level in food-producing animals. In the Netherlands, this monitoring is conducted in slaughter pigs and broilers since 1998. From 2005 onwards, resistance in isolates from both dairy cattle, veal calves and meat samples have been included. In the years 2010 and 2011, samples of individual dairy cattle were collected at slaughter houses; in all other years pooled or individual faecal samples were collected at dairy farms. Until 2012, pooled veal calf samples were collected at farms. Monitoring programs in veal calves at farms stopped in 2012. From then onwards, the monitoring program for veal calves was carried out similar as for pigs and poultry by collecting samples from caeca of individual veal calves at slaughterhouses, and resistance levels were reported separately for white and rosé veal calves.

7.1 General AMR surveillance

7.1.1 Escherichia coli from farm animals and food

Introduction

In this chapter, information is presented on resistance in *E. coli,* as indicator organism for the occurrence and trends in resistance in Gram-negative bacteria in the gastro-intestinal tract of food-producing animals and meat in the Netherlands.

The EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU) was implemented in 2021. Indicator commensal *E. coli* isolates obtained from samples of caecal content taken at slaughter, and from samples of fresh meat taken at the border control posts have to be gathered and examined.

Methods

Bacterial isolation of *E. coli* is performed on MacConkey agar plates, either by direct inoculation of the sample using a cotton swab (faecal samples) or after overnight incubation of a 10% suspension in Buffered Petone Water (meat samples). Per sample one randomly picked colony is pure cultured and identified by MALDI-TOF mass spectrometry (MALDI Biotyper®, Bruker). In case of confirmed identification, one *E. coli* isolate is analysed per sample. This includes susceptibility testing by broth microdilution according to ISO 20776-1:2019 with mandatory panels of antimicrobials. Test results are interpreted using epidemiological cut-off values (ECOFFs) published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[3] In this chapter, non-wild type susceptible isolates (exhibiting MIC values above the ECOFF) are classified as resistant. These isolates probably harbour acquired resistance mechanism, but may not be clinically resistant for some antibiotics.

Results

Bacterial isolates

In 2024, randomly isolated *E. coli* obtained from caecal samples of broilers (n=301) , pigs (n=302), veal calves (n=297), faecal samples of dairy cattle (n=296) and dairy goats (n=500) were tested for antimicrobial susceptibility. In addition, 577 *E. coli* isolates obtained from fresh meat were included in the monitoring, mainly derived from chicken (retail n=213; import n=199) and lamb (retail n=104). Furthermore, a small number of *E. coli* isolates derived from turkey meat collected at retail (n=18) and import (n=42) were included.

Resistance levels

Table 7.1.1.1 shows resistance levels, presented as MIC-distributions, of 1196 *E. coli* isolates obtained from caecal samples from broilers, pigs, veal calves collected at slaughter and faecal samples of dairy cows collected at farms in 2024. Table 7.1.1.2 presents resistance percentages per animal species and includes for the first time resistance data of *E. coli* obtained from faecal samples of dairy goats collected at farms. Trends in resistance levels from 1998 to 2024 are shown in figure 7.1.1.1 and information on trends in multidrug resistance in the different animals sectors is shown in figure 7.1.1.2.
Table 7.1.1.3 presents resistance percentages of 756 *E. coli* isolates collected from fresh poultry meat (chicken and turkey) at retail and import and fresh lamb collected at retail in the Netherlands in 2024. Figure 7.1.1.3 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2024 isolated from fresh chicken meat at retail.

In 2024, trend-analysis was done for broilers, pigs, dairy and veal calves (including white and rosé) to estimate the trend in resistance levels in the period since 2010 until now, and the recent 5 years (2020-2024). Statistical analysis was based on the methods described by Hesp et al. (2019), estimating the incidence rate ratio (IRR) based on the proportion of resistant isolates for every livestock species and antibiotic using a generalized linear model with a Poisson distribution. Analysis was performed in R software. The results of these analysis are depicted in figures 7.1.1.1a-d and 7.1.1.3a-b.

Table 7.1.1.1 *MIC distribution (in %) and resistance percentages (R%) for all E. coli (N=1196) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2024*

E. coli	**MIC (%) distribution mg/L**																			
N = 1196	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**R%**	**95% CI**
ampicillin							0.8	19.7	52.3	7.9	0.1	0.5	18.6						19.2	17,0 - 21,6
cefotaxime					99.7	0.1		0.1		0.2									0.3	0,0 - 0,9
ceftazidime					94.2	5.5		0.1		0.1	0.1								0.3	0,0 - 0,7
gentamicin						68.2	29.1	1.5	0.1	0.1	0.1	0.9							1.2	0,6 - 2,0
tetracycline								64.2	14.3	0.6	0.4	0.7	19.8						20.9	18,6 - 23,3
sulfamethoxazole										35.4	32.9	11.5	0.1			0.2	20.1		20.2	18,0 - 22,6
trimethoprim					33.0	40.6	10.8	0.8	0.1		0.1	14.6							14.8	12,8 - 16,9
ciprofloxacin	77.2	15.5	0.3	0.9	3.4	1.3	0.4		0.2	0.7	0.2								7.0	5,6 - 8,6
nalidixic acid									91.9	2.8	0.2	0.3	1.0	3.8					5.3	4,1 - 6,7
chloramphenicol										85.8	6.6	0.8	0.9	5.9					7.6	6,2 - 9,3
azithromycin*								6.3	55.5	34.6	2.5	0.2	0.0	0.9					1.1	0,6 - 1,9
colistin							99.0	1.0											0.0	0,0 - 0,3
meropenem		99.7	0.3																0.0	0,0 - 0,3
tigecycline					96.5	3.5	0.0	0.0	0.0	0.0									0.0	0,0 - 0,3
amikacine									97.2	2.7	0.1								0.1	0,0 - 0,5

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, chloramphenicol and colistin the ECOFF and clinical breakpoint are identical.
* tentative ECOFF set by EURL established by EFSA data.

For most drugs or drug classes, resistance levels varied between the different animal sectors (Table 7.1.1.2). As in previous years, highest resistance levels were observed in broilers, slaughter pigs and white veal calves, lower levels in rosé veal calves, and the lowest levels of resistance were observed in isolates from dairy cattle and dairy goats. Overall, the highest resistance levels were detected for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used classes in veterinary medicine in the Netherlands. In addition, high levels of resistance were also observed for (fluoro)quinolones in broilers and for chloramphenicol in white veal calves. The use of chloramphenicol has been banned for many years from the veterinary sector, but resistance to chloramphenicol can be selected by the use of florfenicol. Low resistance was noticed for azithromycin, cefotaxime, ceftazidime and gentamicin. Resistance to colistin, meropenem and tigecycline was not detected.

Table 7.1.1.2 *Resistance percentages (R%) of E. coli isolated from faecal samples of broilers, pigs, veal calves (white and rosé), dairy cows and dairy goats in the Netherlands in 2024*

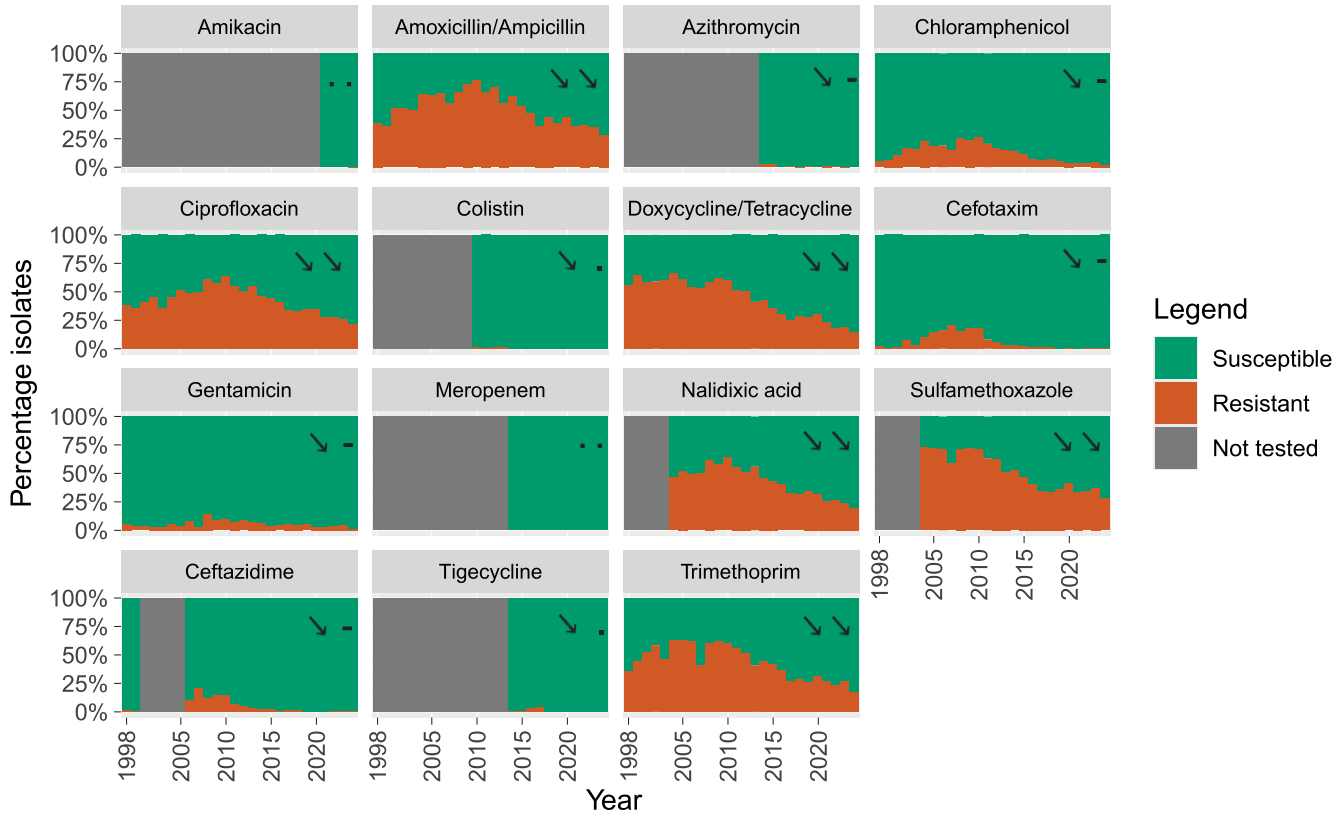
	Broilers	**Pigs**	**Veal calves**		**Dairy cows**	**Dairy goats**
Faecal samples	**N = 301**	**N = 302**	**White, N = 185**	**Rosé, N = 112**	**N = 296**	**N = 500**
ampicillin	28.2	19.9	34.1	15.2	1.7	2.6
cefotaxime	0.7	0.0	0.5	0.9	0.0	0.0
ceftazidime	0.7	0.0	0.5	0.0	0.0	0.0
gentamicin	1.3	0.3	4.9	0.0	0.0	0.0
tetracycline	14.3	26.2	53.5	18.8	2.7	5.2
sulfamethoxazole	27.9	24.2	34.1	14.3	2.0	3.6
trimethoprim	16.9	20.5	28.1	5.4	2.0	1.6
ciprofloxacin	21.9	2.3	4.3	0.0	1.0	0.2
nalidixic acid	19.6	0.3	1.1	0.0	0.3	0.2
chloramphenicol	2.0	9.9	22.7	9.8	0.7	0.6
azithromycin	0.0	1.7	3.8	0.9	0.0	0.0
colistin	0.0	0.0	0.0	0.0	0.0	0.0
meropenem	0.0	0.0	0.0	0.0	0.0	0.0
tigecycline	0.0	0.0	0.0	0.0	0.0	0.0
amikacin	0.3	0.0	0.0	0.0	0.0	0.0

Fluoroquinolone resistance
Fluoroquinolones (FQ) resistance in *E. coli* from broilers was high, but decreased from 28.0% in 2022 to 21.9% in 2024 for ciprofloxacin (Table 7.1.1.2). In *E. coli* from other animal sectors FQ resistance was low or completely absent: 4.3% in white veal calves, 2.3% in pigs, 1.0% in dairy cattle, 0.2% in dairy goats and undetected in rosé veal calves. Resistance to fluoroquinolones in *E. coli* isolated from meat was tested in 2024 for chicken and turkey meat collected at retail and import, as well as for *E. coli* from lamb collected at retail (Table 7.1.1.3). FQ resistance levels were relatively high in retail chicken and turkey meat (22.2 – 24.9%) and even higher in imported meat (35.7 – 38.2%). In *E. coli* from lamb FQ resistance was low (2.9%).

Resistance against extended-spectrum cephalosporins (cefotaxime and ceftazidime)
The prevalence of resistance against extended-spectrum cephalosporins (ESC-R) has declined over time in randomly selected indicator *E. coli* to levels close to the detection limit. As a result, ESC-R *E. coli* is only incidentally observed since 2019. In 2024, four ESC-R *E. coli* isolates were identified: two isolates from broiler chickens and two isolates from veal calves (one white and one rosé). No ESC-resistant indicator *E. coli* were observed in randomly selected *E. coli* isolates from caecal samples of slaughter pigs and dairy cattle (Table 7.1.1.2). Amongst indicator *E. coli* obtained from meat samples, no ESC-R *E. coli* were detected in fresh poultry meat from the retail in contrast to 11.1% ESC-R *E. coli* in imported chicken meat and 4.8% ESC-R *E. coli* in imported turkey meat (Table 7.1.1.3).

Broiler chickens
Although resistance proportions of commensal *E. coli* isolated obtained from caecal samples of broiler chickens were lower compared to 2023, still relatively high levels of resistance were observed for ampicillin, (fluoro)quinolones (ciprofloxacin and nalidixic acid), sulfamethoxazole, trimethoprim and tetracycline (Table 7.1.1.2). Resistance to cefotaxime, ceftazidime and gentamicin remained low (<5%), where resistance to amikacin, azithromycin, colistin, meropenem and tigecycline was not detected amongst indicator *E. coli*. Trend-analysis (based on linear regression) of resistance in *E. coli* over the period 2020-2024 showed decreasing trends in the level of resistance for ampicillin, (fluoro)quinolones, tetracycline, sulfamethoxazole and trimethoprim mainly due to a decrease in 2024 (Figure 7.1.1.1a). This decline in antibiotic resistance in broiler chickens is likely primarily due to a switch to slower-growing breeds that use fewer antibiotics. This reduces selection pressure and lowers resistance to indicator *E. coli*.

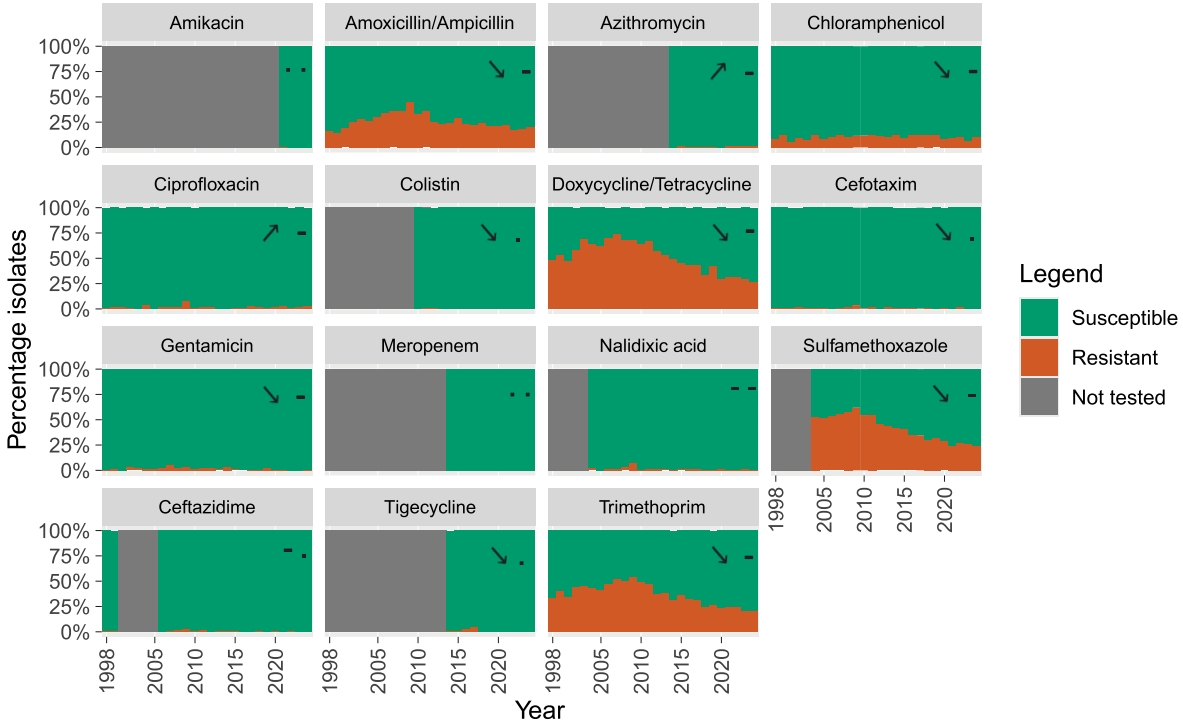
Figure 7.1.1.1a (broilers - faeces) *Percentage (%) of resistant indicator E. coli isolates against different antibiotics, isolated from faecal samples from broilers in the period 1998-2024 in the Netherlands.*



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Slaughter pigs
Resistance levels stayed below 30% in slaughter pigs for all antibiotic classes for the second year in a row (Table 7.1.1.2). The overall resistance proportion stabilised in slaughter pigs with some fluctuation in resistance between the different antibiotic classes (Figure 7.1.1.1b). For sulfamethoxazole, tetracycline and trimethoprim resistance levels stabilised between 20% and 30% whereas resistance to ampicillin remained below 20% for the third year in row. Chloramphenicol resistance increased to 9.9% compared to 2023 (6.3%). Low levels of resistance (<5%) were observed for azithromycin, and (fluoro)quinolones, whereas resistance to amikacin, cefotaxime, colistin, gentamicin, meropenem and tigecycline was not detected. Based on linear regression analysis, the level of resistance of indicator *E. coli* from slaughter pigs stabilised in the last five years.

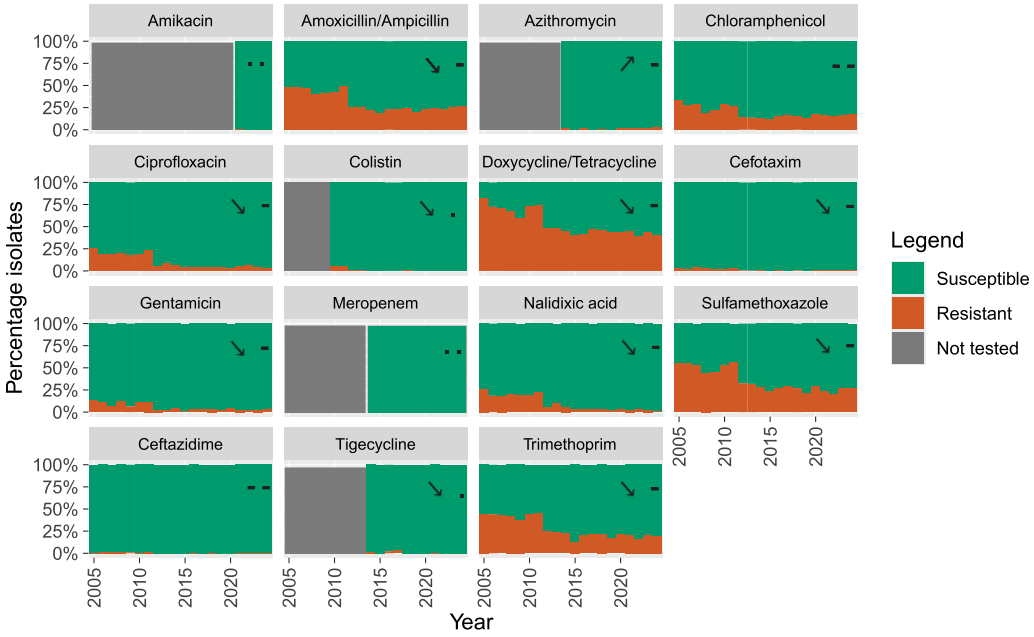
Figure 7.1.1.1b (pigs - faeces) *Percentage (%) of resistant indicator E. coli isolates against different antibiotics, isolated from faecal samples from pigs in the period 1998-2024 in the Netherlands*



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

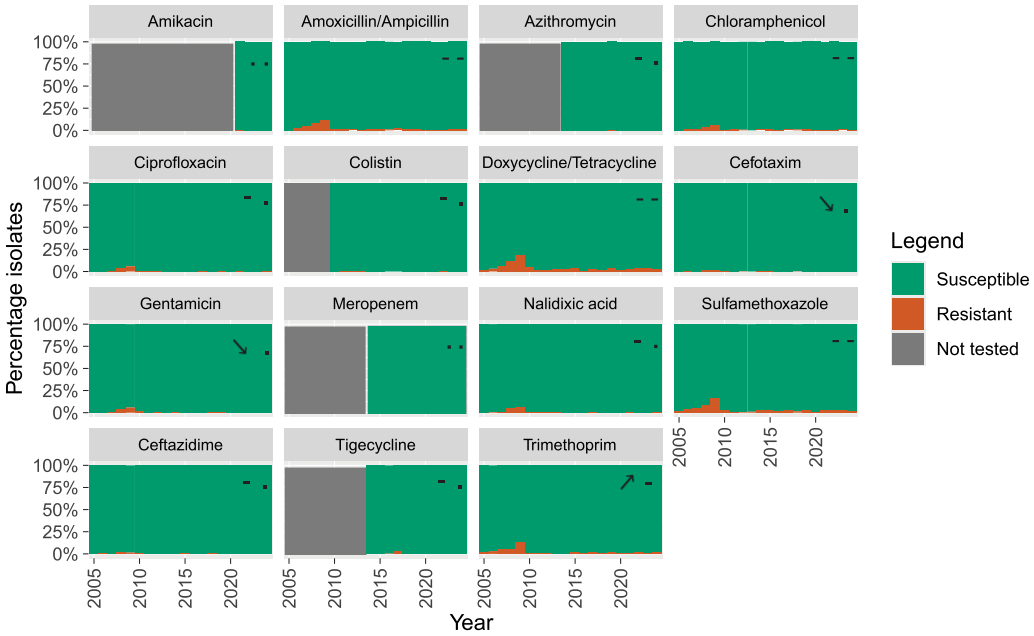
Veal calves
Resistance data on white and rosé veal calves are reported separately, due to the difference in production systems. As seen in previous years, higher resistance levels were measured in isolates from white, compared to those from rosé veal calves (Table 7.1.1.2). Figure 7.1.1.1c illustrates the trends in resistance in *E. coli* isolated from both types of veal calves combined. Resistance levels were relatively stable over time, with a clear decrease in 2012, which was the year in which the sampling strategy changed from sampling at farm at variable ages to sampling at slaughterhouse. This has influenced the results from 2012 onwards, because most antibiotic usage is in the younger calves and less in the period before slaughter. The ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40% in 2016, and to 70/30% in 2017 onwards, which better reflects the proportions of slaughtered white and rosé calves in The Netherlands. After 2017, resistance levels in veal calves stabilised (Figure 7.1.1.1c) with large differences between the two husbandry types (Table 7.1.1.2). In 2024, resistance was the highest in white veal calves compared to the other animals sectors for ampicillin, chloramphenicol, tetracycline, sulfamethoxazole and trimethoprim ranging from 22.7%-53.5%. In rosé calves, resistance levels for these antibiotics were lower ranging from 5.4%-18.8%. Resistance against azithromycin, gentamicin and fluoroquinolones was low are completely absent in the different types of veal calves. In addition, no resistance was observed for amikacin, colistin, meropenem and tigecycline (Table 7.1.1.2). Based on linear regression analysis, amongst indicator the levels of resistance of *E. coli* from veal calves stabilised in the last five years.

Figure 7.1.1.1c (veal calves - faeces) *Percentage (%) of resistant indicator E. coli isolates against different antibiotics, isolated from faecal samples from veal calves in the period 2005-2024 in the Netherlands*



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Figure 7.1.1.1d (dairy cows - faeces) *Percentage (%) of resistant indicator E. coli isolates against different antibiotics, isolated from faecal samples from dairy in the period 2005-2024 in the Netherlands*



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

Dairy cattle
Resistance in *E. coli* isolated from dairy cattle was traditionally low compared to pigs, broilers and veal calves (Table 7.1.1.2), reflecting the low use of antibiotics in dairy farming. As in previous years resistance to the 3rd generation cephalosporins was not detected.

Dairy goats
In 2024, indicator *E. coli*, obtained from faecal samples of dairy goats, was analysed as part of a policy-supporting project that also analysed other animal sectors not included in the European antimicrobial resistance monitoring program. In general, resistance of *E. coli* from dairy goats were very low with less than 5% resistance for almost all antibiotic classes ranging from 0 – 5.4%. No resistance was detected for amikacin, azithromycin, cefotaxime, colistin, meropenem and tigecycline. This low level of resistance is comparable to that in dairy cattle and reflects the low use of antibiotics in this livestock sector.

Multidrug resistance
Data to determine multidrug resistance (resistant to ≥3 different antibiotic classes) is based on resistance against the following antimicrobial classes: aminopenicillins (ampicillin), 3rd gen. cephalosporins (cefotaxime), carbapenems (meropenem), aminoglycosides (gentamicin and amikacin), tetracyclines (tetracycline), tigecycline, sulfonamides (sulfamethoxazole), folate pathway inhibitors (trimethoprim), (fluoro)quinolones (ciprofloxacin), phenicols (chloramphenicol), macrolides (azithromycin) and polymyxins (colistin). The data with the determined level of multidrug resistance over the years are shown in figure 7.1.1.2.

In general, the level of multidrug resistance decreased in for *E. coli* from broiler chickens as well as in slaughter pigs. In broilers a sharp decrease was observed in the period 2020-2024 with 38.1% in 2020, dropping to approximately 31% in the years 2021-2023 and ending at 22.6% in 2024. In pigs, a more long-term decrease in the level of multidrug resistance was observed in the last decade (2014 – 2024) from 33.9% in 2014 to 17.9% in 2024. In veal calves, the level of multidrug resistance is fluctuating in the last decade between 20.7%-28.1%. As in former years, multidrug resistance *E. coli* in dairy cattle was extremely low (1.7%) compared to the other animals species reflecting the low antibiotic use in this animal sector.

Figure 7.1.1.2 *Percentage (%) of indicator E. coli isolates, isolated from faecal samples from broilers and pigs (1998-2024), dairy and veal calves (2005-2024), in the Netherlands, being resistant to none (0), 1, 2 or 3 or more antibiotic (AB) classes.*

Isolates resistant to 0 antibiotic classes are classified as fully susceptible isolates, isolates resistant to 3 or more antibiotic classes are classified as multi-resistant isolates.

The decrease in the level of multidrug resistance resulted in an increase of the proportions of complete susceptibility (pan-susceptible) *E. coli* isolates. As a result, the percentage of pan-susceptible isolates ranged from 53.2% in broilers) to 54.0% in pigs and 55.9% in veal calves in 2024. Traditionally, the proportion of pan-susceptible isolates was very high in dairy cattle with 96.6%.

E. coli from meat

Table 7.1.1.3 presents resistance percentages of *E. coli* isolated from fresh chicken and turkey meat sampled from retail as well as from imported meat sampled at border control posts by the Dutch Food and Consumer Product Safety Authority (NVWA). In addition, fresh lamb was sampled at retail in 2024. Fresh retail meat comprises meat produced in The Netherlands, but also in other EU countries. Imported meat originates from outside EU. This includes the UK and countries outside Europe.

Table 7.1.1.3 *Resistance percentages (R%) of E. coli isolated from raw meat in the Netherlands in 2024*

	Chicken	Chicken	Turkey	Turkey	Lamb
	Fresh, retail	Fresh, import	Fresh, retail	Fresh, import	Fresh, retail
Products	**N = 213**	**N = 199**	**N = 18**	**N = 42**	**N = 104**
ampicillin	32.9	43.2	50.0	50.0	9.6
cefotaxime	0.0	11.1	0.0	4.8	1.0
ceftazidime	0.0	8.0	0.0	2.4	0.0
gentamicin	3.3	13.6	5.6	9.5	1.0
tetracycline	23.5	37.7	27.8	76.2	14.4
sulfamethoxazole	23.0	37.2	22.2	73.8	5.8
trimethoprim	17.8	26.6	22.2	42.9	4.8
ciprofloxacin	24.9	38.2	22.2	35.7	2.9
nalidixic acid	23.5	35.7	22.2	23.8	1.9
chloramphenicol	4.2	11.1	0.0	31.0	1.9
azithromycin	2.3	0.0	0.0	0.0	1.9
colistin	0.0	0.5	5.6	0.0	0.0
meropenem	0.0	0.0	0.0	0.0	0.0
[1]tigecycline	-	-	-	-	-
amikacin	1.9	1.0	0.0	2.4	1.9

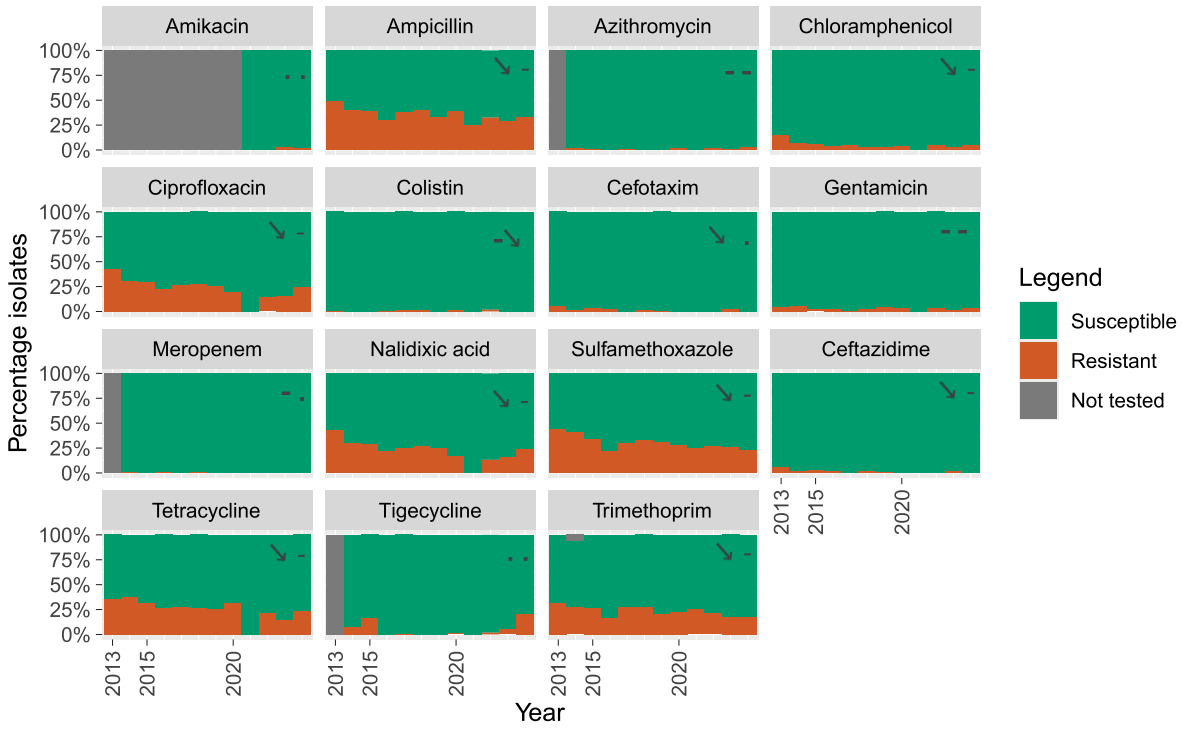
[1] Tigecycline results invalid due to light sensitivity of the compound

In general, levels of resistance in *E. coli* from chicken retail meat were comparable to *E. coli* from caecal samples of broilers with relative high levels of resistance for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. Resistance to third generation cephalosporins in *E. coli* isolates from imported poultry meat was high (11,1%, n=199 compared to retail poultry meat (0,0%, n=231).

Although the number of tested *E. coli* isolates from turkey meat are rather low, the results show higher resistances in *E. coli* derived from imported chicken and turkey meat, compared to fresh retail meat, particularly for third generation cephalosporins and fluoroquinolones. In *E. coli* from lamb meat (retail) levels of resistance were low (< 5%) for all antibiotic classes.

Figure 7.1.1.3a shows the trends in resistance of indicator *E. coli* from fresh chicken meat collected at retail. In *E. coli* isolates from chicken meat decreasing levels of resistance are observed with a tendency to stabilize in the more recent years. The decrease in resistance between 2020 and 2024 for various antibiotics, as observed in *E. coli* isolates from caecal samples, is not observed in *E. coli* isolates from broiler chicken meat. This difference in trends could possibly be explained by the fact that the chicken meat samples in retail are selected randomly and may also originate from other EU countries, while the caecal samples come exclusively from Dutch broiler flocks.

Figure 7.1.1.3a (chicken - meat retail) *Percentage (%) of resistant indicator E. coli isolates against different antibiotics, isolated from chicken retail meat in the period 2013-2024 in the Netherlands*



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2013-2024, the second symbol indicates the trend during the last 5 years (2020-2024), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing trend in resistant isolates, - no trend. • means statistical trend analysis could not be performed, due to a lack of (resistant) isolates.

7.1.2 Enterococci from farm animals

Introduction
According to the European legislation[2] antibiotic resistance monitoring of enterococci includes *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*) as indicator bacteria for resistance in Gram-positive bacteria. Enterococci are a non-mandatory part of the monitoring program. In 2024, enterococci from caecal samples of broilers were analysed.

Methods
Enterococci are isolated from caecal material by bacterial culturing on Slanetz and Bartley agar plates. Presumptive colonies are pure cultured and identification is performed by MALDI-TOF mass spectrometry (MALDI Biotyper®, Bruker). In case of confirmed identification, one *E. faecium* and one *E. faecalis* is analysed. Susceptibility testing is performed by broth microdilution according to ISO 20776-1:2019 in a standard antimicrobial panel designed for testing enterococci which contains thirteen different antibiotics representing most antibiotic classes relevant for treatment of human infections caused Gram-positive bacteria. Test results are interpreted using epidemiological cut-off values (ECOFFs) published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[3] In this chapter non-wild type susceptible isolates are classified as resistant. These isolates probably contain acquired resistance mechanism, but may not be clinically resistant for some antibiotics.

Results
Bacterial isolates
In 2024, 269 *E. faecium* and 98 *E. faecalis* isolates were isolated from 301 pooled samples of caecal material from broilers (each pool contained material from ten chickens from the same flock) and tested for antimicrobial susceptibility with broth microdilution.

Resistance levels
Table 7.1.2.1 presents resistance percentages of *E. faecium* and *E. faecalis* isolates obtained from broilers. In *E. faecium*, resistance levels were relatively high for Synercid (quinopristin/dalfopristin; 41.6%), tetracycline (30.5%), erythromycin (21.6%), and ampicillin (10.4%). In *E. faecalis*, relatively high resistance levels were also observed for tetracycline and erythromycin, with complete absence of resistance to ampicillin and Synercid. Resistance to other antibiotics of critical or high importance to human health (ciprofloxacin, daptomycin, gentamicin, linezolid, tigecycline, teicoplanin, and vancomycin) according to the List of Medically Important Antimicrobials of the WHO[4] was low (<1%) or not detected in both subspecies.

Table 7.1.2.1 *Resistance percentages (%) of Enterococcus faecalis and E. faecium isolated from broilers in the Netherlands in 2024*

Broiler chickens	***E. faecium***	***E. faecalis***
2024	**N=269**	**N=98**
ampicillin	10.4	0.0
chloramphenicol	0.4	0.0
ciprofloxacin	0.0	0.0
daptomycin	0.0	0.0
erythromycin	21.6	27.6
gentamicin	0.4	1.0
linezolid	0.4	0.0
synercid	41.6	0.0
teicoplanin	0.4	0.0
tetracycline	30.5	71.4
tigecycline	0.4	1.0
vancomycin	0.0	0.0

Conclusions

- Over the period 2020-2024, levels of resistance in *E. coli* from broilers decreased for ampicillin, (fluoro)quinolones , tetracycline, sulfamethoxazole and trimethoprim.
- In the same period, levels of resistance stabilised in pigs and veal calves for most antibiotics in the last five years, whereas resistance in dairy cattle remained traditionally low.
- In 2024, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were relatively high amongst indicator *E. coli* in broilers, pigs, and white veal calves.
- Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers (21.9%) in contrast with the prevalence (<5%) in pigs, veal calves and dairy cattle.
- Resistance to third generation cephalosporins was low (<1%) or not detected amongst (randomly isolated) indicator *E. coli* from caecal samples of all animal species.
- For most antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.
- In dairy goats, resistance levels of indicator *E. coli* were similarly low as in dairy cattle, reflecting the low antibiotic use in this sector.
- *E. coli* isolates from imported chicken and turkey meat showed higher levels of resistance compared to fresh retail meat, particularly for third generation cephalosporins and fluoroquinolones.
- In *E. coli* from lamb meat (retail) levels of resistance were low (< 5%) for all antibiotic classes.
- In enterococci (*E. faecium* and *E. faecalis*) obtained from caecal samples of broilers resistance was relatively high (>20%) for tetracycline and erythromycin, but absent or low (<1%) for ciprofloxacin, daptomycin, gentamicin, linezolid, tigecycline, teicoplanin, and vancomycin.

References

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7.2 Specific AMR surveillance

7.2.1 Extended-spectrum cephalosporins resistant Enterobacterales in farm animals and food

Third and fourth generation cephalosporins, collectively referred to here as extended-spectrum cephalosporins (ESC), are considered one of the highest priority critically important antimicrobials, and resistance can occur through several mechanisms. Two of these mechanisms, Extended-spectrum beta-lactamases (ESBLs) and AmpC genes, can occur on plasmids and be exchanged between various species of the Enterobacterales family, including *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*. These isolates are described in this chapter as ESBL/pAmpC producers. In *E. coli*, a third mechanism for resistance to ESC is the upregulation of the chromosomally located *AmpC*, a gene present in all *E. coli* that does not confer resistance in its wildtype form. These isolates are described here as AmpC promotor mutants. Due to its lower prevalence in clinical isolates and the lack of spreading between isolates, this type of resistance was previously perceived as less relevant for human health[2].

This section describes the results of the non-selective and selective screening for ESC-resistant *E. coli* and *Salmonella* in farm animals and food. Since the implementation of whole genome sequencing (WGS) in EU legislation(2020/1729/EU) was implemented in 2021, all ESC-resistant *E. coli* from farm animals and food are analysed by WGS. In addition to the description of the detected resistance mechanisms, a genetic comparison based on WGS data is made at the end of this section. All ESC-resistant Salmonella from human samples, livestock and meat are also confirmed by WGS.

7.2.1.1 Randomly isolated ESC-resistant *E. coli* from farm animals and food products

Random isolation of commensal *E. coli* from caecal samples of broilers, slaughter pigs, veal calves and dairy cows and food products is described in chapter 7.1.1. The prevalence of ESC-resistance in these *E. coli* provides data on the prevalence of the total population of *E. coli* that are present in the livestock sector and food in the Netherlands. The phenotype of these bacteria was determined by measuring the minimum inhibitory concentration (MIC) and comparing these to the epidemiological cut-off values described by EUCAST.

E. coli are considered suspected ESBL/pAmpC producers or AmpC promotor mutants when a reduced susceptibility of the isolate is measured against the ESC cefotaxime and/or ceftazidime. After confirmation of the phenotype, WGS is performed using the Illumina Nextera sequence technology. A standardised analysis pipeline was used to asses quality control and perform assembly of the WGS data[3]. Analysis of the resistance mechanisms was determined using Resfinder 4.5 including Pointfinder[4].

Results
The screening of randomly isolated *E. coli* for ESC-resistant isolates has been performed since 1998, see figure 7.2.1.1.1. Between 2000 and 2009, the prevalence increased considerably, but since the reduction of antimicrobial usage in farm animals, the prevalence has decreased again. While no ESC-resistant isolates were found in any livestock sector in 2019, approximately 2-5 isolates have been found annually since then. In 2024, two ESC-resistant isolates were found in samples from broilers, which were both confirmed to contain the SHV-12 gene. One isolate from a white veal calf contained the CTX-M-1 gene. Both of these gene type are also commonly found in these sectors in previous years, see table 7.2.1.1.1 and in the selective isolation of ESC-resistant *E. coli*, see chapter 7.2.1.2.

In food products, randomly selected *E. coli* that were ESC-resistant were only detected in imported poultry meat. ESC-resistant *E. coli* were detected in 19 out of 160 batches, 11,9%.

Figure 7.2.1.1.1 *Trends in ESC resistance (%) of E. coli randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows*

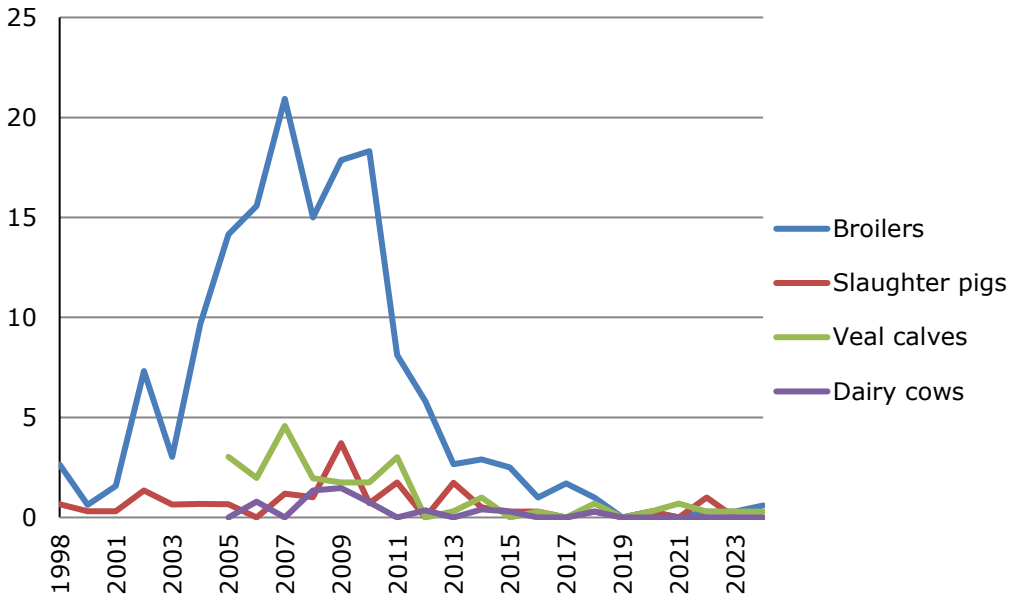


Table 7.2.1.1.1 *ESBL/pAmpC-genes and chromosomal AmpC mutations found in E. coli isolates with reduced susceptibility to ESC derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2024*

Year	ESC-resistant *E. coli* isolated from						Beta-lactamase genes											
	[c]Broilers	Veal calves	Slaughter pigs	[d]Dairy cows	Turkeys	Total ESBL suspected (n)	[a]CTX-M-1-group	CTX-M-2	CTX-M-9-group	TEM-52	TEM-20	[b]SHV-12	SHV-2	CMY-2	Chromosomal *ampC*	no gene found	Total *E. coli* (n)	% ESBL of total *E. coli*
2007	9	6	2	0	*n.t.*	**17**	3	1		3				1	2	7	539	**3.2**
2008	66	4	3	2	*n.t.*	**75**	38	5	1	9			2	12	3	5	1,026	**7.3**
2009	53	2	11	2	*n.t.*	**68**	34	7		2	1	8	1	12	3		894	**7.6**
2010	52	3	2	2	*n.t.*	**59**	21	6		5	1	9	4	5	3	5	1,002	**5.9**
2011	23	5	5	0	6	**39**	9			8		9	2	3	3	5	1,096	**3.6**
2012	26	2	0	1	0	**29**	8			4		8		5		4	1,328	**2.2**
2013	13	1	4	0	*n.t.*	**18**	7			4		3		3	1		1,371	**1.3**
2014	11	3	2	0	*n.t.*	**16**	8			1		4			1	2	1,519	**1.1**
2015	10	0	1	1	*n.t.*	**12**	3		2	1		1		2	3		1,283	**0.9**
2016	3	1	1	0	*n.t.*	**5**	2			1				1	1		1,492	**0.3**

[a] All were *bla*$_{\text{CTX-M-1}}$, only in 2011 one *bla*$_{\text{CTX-M-3}}$ gene was found in an isolate from a veal calf.
[b] One combination of *bla*$_{\text{SHV-12}}$ together with *bla*$_{\text{TEM-52}}$ occurred in 2012 in one broiler isolate.
[c] In broilers, three combinations were found: in 2008: *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{CTX-M-2}}$; in 2009: *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{SHV-12}}$ and *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{SHV-12}}$ and *bla*$_{\text{CMY-2}}$.
[d] In dairy cows, one combination of *bla*$_{\text{CMY-42}}$ with *bla*$_{\text{TEM-190}}$.
n.t. : not tested.

Table 7.2.1.1.1 (continued) *ESBL/pAmpC-genes and chromosomal AmpC mutations found in E. coli isolates with reduced susceptibility to ESC derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2024*

Year	ESC-resistant *E. coli* isolated from						Beta-lactamase genes											
	[c]Broilers	Veal calves	Slaughter pigs	[d]Dairy cows	Turkeys	Total ESBL suspected (n)	[a]CTX-M-1-group	CTX-M-2	CTX-M-9-group	TEM-52	TEM-20	[b]SHV-12	SHV-2	CMY-2	Chromosomal *ampC*	no gene found	Total *E. coli* (n)	% ESBL of total *E. coli*
2017	5	0	0	0	*n.t.*	**5**	2			1			2				1,194	**0.4**
2018	3	2	0	0	*n.t.*	**5**	2					3			2		1,198	**0.4**
2019	0	0	0	0	*n.t.*	**0**											1,209	**0.0**
2020	1	1	1	0	*n.t.*	**3**	1						1			1	1,103	**0.3**
2021	0	2	0	0	*n.t.*	**2**	1									1	1,206	**0.2**
2022	1	1	3	0	*n.t.*	**5**	1			1					2	1	1,276	**0.4**
2023	1	1	0	0	*n.t.*	**2**	2										1,185	**0.2**
2024	2	1	0	0	*n.t.*	**3**	1					2					1,196	**0.3**
Total	279	35	35	8	6	**363**	143	19	3	40	2	47	12	44	24	31	21,117	**1.7**

[a] All were *bla*$_{\text{CTX-M-1}}$, only in 2011 one *bla*$_{\text{CTX-M-3}}$ gene was found in an isolate from a veal calf.
[b] One combination of *bla*$_{\text{SHV-12}}$ together with *bla*$_{\text{TEM-52}}$ occurred in 2012 in one broiler isolate.
[c] In broilers, three combinations were found: in 2008: *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{CTX-M-2}}$; in 2009: *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{SHV-12}}$ and *bla*$_{\text{CTX-M-1}}$ with *bla*$_{\text{SHV-12}}$ and *bla*$_{\text{CMY-2}}$.
[d] In dairy cows, one combination of *bla*$_{\text{CMY-42}}$ with *bla*$_{\text{TEM-190}}$.
n.t. : not tested.

7.2.1.2 Selectively isolated ESC-resistant *E. coli* from farm animals and food products

The randomly selected ESC-resistant *E. coli* described in chapter 7.2.1.1 provide insight into the total prevalence of ESC-resistance in the farm animal population. The selectively isolated *E. coli* described here provide insight of the prevalence at the level of individual animals. Selection is performed according to protocols provided by the European Reference Laboratory for Antimicrobial Resistance. Isolation from faeces and caecal content occurs by incubating 1 gram of material in 9 ml of buffered peptone water overnight at 37 °C. While samples from pigs, veal calves and dairy cows represent individual animals per flock, for broiler chickens the caecal contents of 10 animals from a single flock are pooled since 2022 due to a change in EU legislation (2020/1729/EU). Selective isolation is performed on MacConkey agar plates supplemented with 1 mg/L of cefotaxime according to the EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal samples.[5]

The isolation from food products is performed by adding 25 grams of product to 225 ml of buffered peptone water and incubating overnight at 37 °C. Selective screening is performed on plates of MacConkey agar plates supplemented with 1 mg/L of cefotaxime according to the EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from meat samples.[6]

Presumptive resistant *E. coli* colonies are subcultured and species identification is performed using MALDI-TOF (Bruker Biotyper). The MIC of isolates is determined as described in chapter 7.1.1 using a panel of antibiotics specifically aimed at beta-lactamase producing Enterobacteriaceae, EUVSEC-2. The genotype of all ESC-resistant *E. coli* was confirmed using WGS, as described in chapter 7.2.1.1.

Results of selective isolation and molecular typing of ESC-resistant E. coli from farm animals

The results of the selective isolation in 2024 of ESC-resistant *E. coli* from farm animals are described table 7.2.1.2.1. Both the number of suspected ESC-resistant isolates is included, as well as the number of isolates for which the genotype could be confirmed via WGS. In figure 7.2.1.2.1 the prevalence of the confirmed ESC-resistant *E. coli* from farm animals are compared between the livestock sectors over time. These are supported by trend analyses over three time periods: 1) for the complete period since selective isolation started in 2014 up to 2024, 2) for the period of 2014 up to 2019, and 3) for the period of 2020 to 2024. These trend analyses are similar as described for resistance in indicator *E. coli* described in chapter 7.1.1. The genes responsible for the ESC-resistant phenotype based on WGS are summarized per livestock sector in table 7.2.1.2.2. It is currently unclear why certain resistance mechanisms become more or less prevalent over time.

The prevalence of ESC-resistant *E. coli* has most obviously decreased in **broilers** between 2014 and 2024, from 66.8% to 14.6%. The lowest prevalence was detected in 2020 with 10.2%. The trend analysis over shorter periods shows that the period of 2014 to 2019 also results in a significant decrease, while analysis over the past 5 years, from 2020-

2024, shows a small but significant increase again. It is expected that this increase is mostly caused by the change in sampling in 2022, since when the caecal contents of ten birds from a flock is now pooled, instead of testing individual animals per flock.

Over the past decade, the plasmid encoded AmpC gene CMY-2 has steadily decreased from a proportion of 28.6% in 2014, while in 2024 for the first time the gene was not detected in ESC-resistant *E. coli* from broilers. Over this period, the proportion of the ESBL-gene CTX-M-1 has also decreased from 43.2% to 22.7%, although this is still the second most common ESBL gene. The proportion of SHV-12 has fluctuated over the years between 9.9 and 41.2%, which was 27.3% in 2024. TEM-52B was first detected in broilers in 2022 but has now increased to 15.9%. CTX-M-55 was also rarely detected in broilers before 2021 but was present in 18.2% of ESC-resistant *E. coli* in 2024.

The **pig** sector is the production sector in the Netherlands where chromosomal mutations in the promoter region of the AmpC gene are most often found to be responsible for the ESC-resistant phenotype in *E. coli*. Although this resistance mechanism somewhat fluctuates, there was no significant trend. Only the trend analysis from 2014 to 2024 shows a small but significant reduction of ESBL and plasmid encoded AmpC from 12.3 to 8.9%, analyses of the shorter time periods 2014 to 2019 and 2020 to 2024 do not result in a significant reduction. CTX-M-1 and the AmpC promoter mutants are always the most frequent resistance mechanisms detected in pigs. SHV-12 is detected nearly every year between 1 and 4%, but in 2024, this mechanism was seen at a higher proportion in 12.2% of the ESC-resistant isolates.

The **veal** production sector consists of white and rosé veal calves which are monitored independently because of the differences in management. In both sectors, an increase in detection of ESC-resistant *E. coli* was noted in 2016 which was not explained. The trend analysis from 2014 to 2024, 2014 to 2019, and the 5-year trend show fluctuations in the prevalence in rosé veal calves from which no trend can be detected. Due to the lower slaughter volume, a smaller number of samples is collected in the rosé veal sector, making it more prone to show fluctuations over time. As in most years, the ESBL-genes CTX-M-1 and CTX-M-15 were most prevalent in rosé veal calves, respectively at a proportion of 32 and 36% of ESC-resistant *E. coli*.

The trend analysis from 2014 to 2024 shows a significant increase in ESC-resistant *E. coli* in white veal calves, which is confirmed in the analysis of the 2014 to 2019 time period. Despite some fluctuation, the 5-year trend analysis is not significant indicating that the prevalence is currently not increasing further. Similar to the rosé sector, CTX-M-1 and CTX-M-15 have long been the predominant ESBL genes, respectively at a proportion of 19.3% and 32.5% in 2024. However, over the past 5 years, the gene CTX-M-32 has become more prevalent, rising from 4.8% in 2019 to 26.5% in 2024 of the ESC-resistant isolates. The prevalence of ESC-resistant *E. coli* has recently started to increase in **dairy cattle**. No significant trend was detected in analysis of the time period 2014 to 2019, but the period from 2014 to 2024 shows an increase from 8.7% of the population in 2014, which increased to 22.9%

in 2024. Analysis of the period 2020 to 2024 confirms that the increase was most significant in this period. While in 2014 a mixture of resistance mechanisms was detected in the dairy cattle population, consisting of the ESBL gene CTX-M-1 (30.8%), the plasmid encoded AmpC gene CMY-2 (15.4%) and the chromosomal AmpC promotor mutant (26.9%), over the past decade a shift has been seen in which CTX-M-15 has become the most prevalent gene at a proportion of 65.2% in 2024.

In 2024, manure samples were collected for the first time at **dairy goat** farms, also described in chapter 3 for the indicator *E. coli*. Samples from dairy goats were collected on farms, instead of at the slaughter house, comparable the collection of samples from dairy cattle. A total of 102 dairy goat farms were included based on a voluntary basis from the farmer. In contrast to the other livestock sectors, 5 fresh manure samples were included per farm, which were individually tested for the presence of ESC-resistant *E. coli*. Despite the increased sample size per farm, the prevalence on dairy goats farms was relatively low at 2.4%. The isolated *E. coli* included 11 confirmed ESBL and plasmid-encoded AmpC producing isolates, from which CTX-M-1 and CTX-M-15 were the most prevalent genes. One isolate was detected in which a chromosomal AmpC promotor mutation was responsible for the resistant phenotype.

Figure 7.2.1.2.1 *Trends in prevalence of confirmed ESBL/pAmpC-producing E. coli and chromosomal AmpC-mutant E. coli in faecal samples of broilers, pigs, white and rosé veal calves and dairy cows from 2014-2024 determined by using selective isolation*

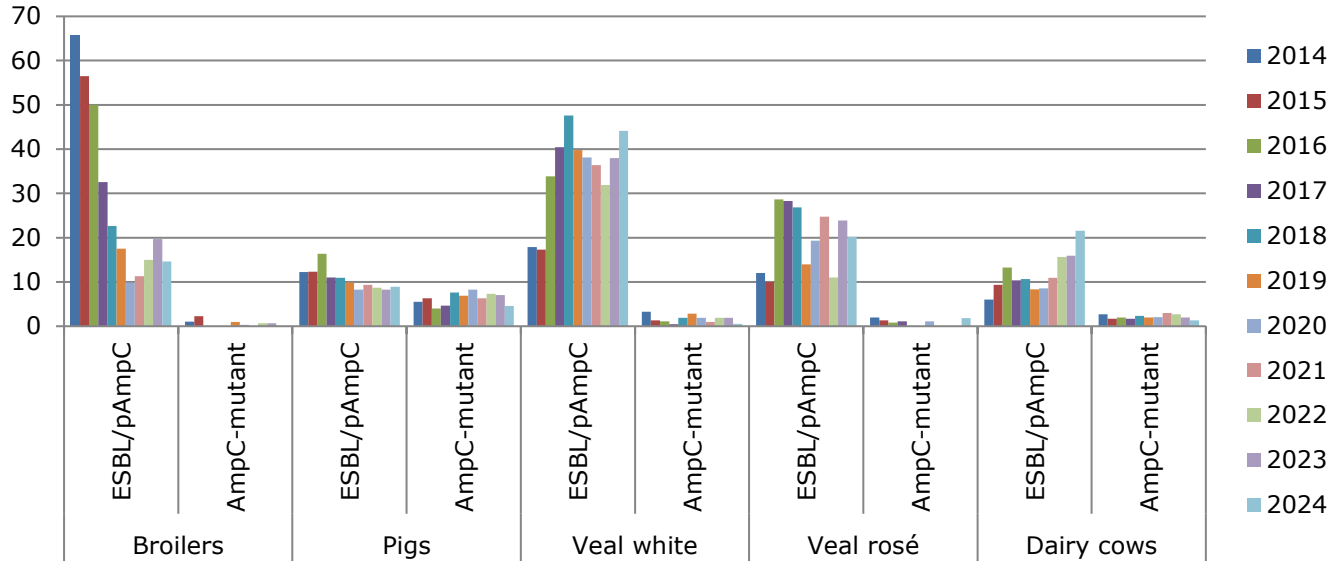


Table 7.2.1.2.1 *Proportion of E. coli isolates showing resistance to ESC derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves, dairy cows and dairy goats collected in 2024*

	N samples	N ESC-resistant E. coli	% ESC-resistant E. coli	N ESBL/ pAmpC carrying E. coli	% ESBL/ pAmpC carrying E. coli	N AmpC promotor mutants	% AmpC promotor mutants
Broilers	301	44	14.6	44	14.6	0	0.0
Pigs	302	41	13.6	27	8.9	14	4.6
Veal calves white	186	83	44.6	82	44.1	1	0.5
Veal calves rosé	114	25	21.9	23	20.2	2	1.8
Dairy cows	301	69	22.9	65	21.6	4	1.3
Dairy goats	510	12	2.4	11	2.2	1	0.2
Total	1714	274	16.0	252	14.7	22	1.3

Table 7.2.1.2.2 *Beta-lactamases identified in E. coli derived from selective culturing of faecal samples of broilers, slaughter pigs, veal calves, dairy cows and dairy goats in 2024*

		Broilers	Slaughter pigs	Veal calves		Dairy cows	Dairy goats	Total
				White	Rose			
CTX-M-1 group	CTX-M-1	10	16	16	8	5	3	58
	CTX-M-15	3	3	27	9	45	3	90
	CTX-M-32		1	22	2	3		28
	CTX-M-55	8		5	1	2		16
CTX-M-2 group	CTX-M-2				1	1		2
CTX-M-3 group	CTX-M-3			1				1
CTX-M-8/25 group	CTX-M-8					1		1
CTX-M-9 group	CTX-M-9	1	1					2
	CTX-M-14					2		2
	CTX-M-27					1		1
	CTX-M-65					1		1
TEM	TEM-52B	7						7
	TEM-52C	2	1	9	1		1	14
SHV	SHV-12	12	5	2		2	2	23
	SHV-2	1						1
CMY	CMY-2					2	1	3
DHA	DHA-1				1		1	2
Chromosomal ampC	-42 C>T		14	1	2	4	1	22
Total		**44**	**41**	**83**	**25**	**69**	**12**	**274**

Results of selective isolation and molecular typing of ESC-resistant E. coli in food products

Selective isolation of ESC-resistant *E. coli* was performed from food for human consumption, including samples from fresh meat, poultry, fish and fresh herbs and imported goods from outside the EU. Similar to the selective isolation of ESC-resistant *E. coli* from farm animals, described above, the selective isolation is more sensitive than the non-selective isolation method described in chapter 7.1.1.

In 2024, 1727 samples were analysed, of which 1185 were domestic produce and 601 were samples of food imported from outside the EU, table 7.2.1.2.3. A total of 208 isolates were suspected ESC-resistant *E. coli* of which 183 were confirmed ESBL or plasmid-encoded AmpC producing isolates, and 14 isolates contained mutations in the chromosomal AmpC promotor which are associated with ESC-resistance.

The prevalence of ESC-resistant *E. coli* are compared to previous years in figure 7.2.1.2.2 for the domestically produced conventional food sources. The genes responsible for the ESC-resistant phenotype based on WGS are summarized per food category in table 7.2.1.2.4.

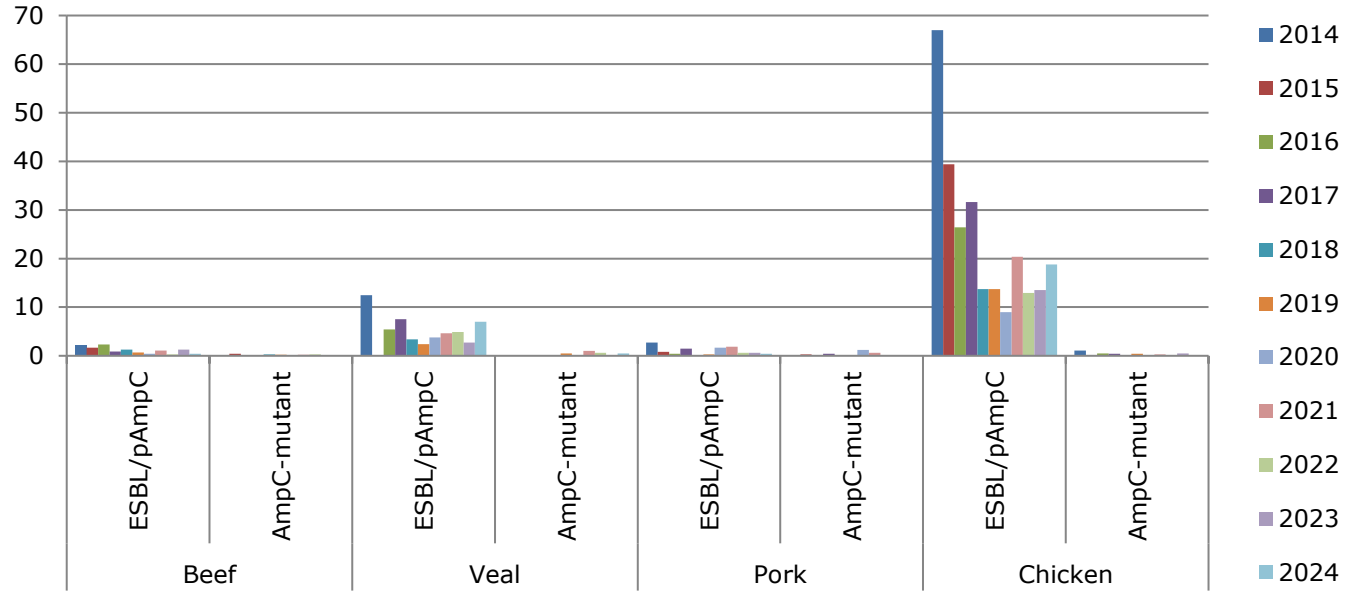
Table 7.2.1.2.3 *Prevalence of ESC-resistant E. coli isolates from raw meat, poultry, aquaculture and fresh herbs in the Netherlands in 2024*

Animal source	N samples	N ESC-resistant E. coli	% ESC-resistant E. coli	N ESBL/ pAmpC carrying E. coli	% ESBL/ pAmpC positive	N AmpC promotor mutants	% AmpC promotor mutants
Beef	284	1	0.4	1	0.4	0	0.0
Veal	186	14	7.5	13	7.0	1	0.5
Pork	274	1	0.4	1	0.4	0	0.0
Chicken	404	86	21.3	76	18.8	0	0.0
Turkey	21	3	14.3	3	14.3	0	0.0
Non-conventional meat	16	2	12.5	2	12.5	0	0.0
Imported chicken	134	69	51.5	60	44.8	11	8.2
Imported turkey	68	24	35.3	22	32.4	2	2.9
Imported aquaculture/ wild caught fish	300	3	1	3	1.0	0	0.0
Imported non-conventional fish	40	1	2.5	1	2.5	0	0.0
Fresh herbs	59	1	1.7	1	1.7	0	0.0
Total	**1727**	**205**	**11.9**	**183**	**10.6**	**14**	**0.8**

Table 7.2.1.2.4 *Beta-lactamases identified in E. coli from raw meat products, herbs and aquaculture in the Netherlands in 2024*

	Beta-lactamase gene	Chicken	Turkey	Pork	Beef	Veal	Unconventional meat	Imported chicken	Imported turkey	Imported aquaculture/wild caught fish	Imported unconventional fish	Total
CTX-M-1 group	CTX-M-1	7		1		7					1	16
	CTX-M-15		1		1		2	2	2	2		10
	CTX-M-32					2						2
	CTX-M-55	11						32	12			55
	CTX-M-55; CMY-2							1				1
CTX-M-2 group	CTX-M-2							7				7
CTX-M-8 group	CTX-M-8							12	2			14
CTX-M-9 group	CTX-M-14							1				1
	CTX-M-27		2									2
	CTX-M-65							3	4			7
TEM	TEM-52B	25										25
	TEM-52C	1				1						2
SHV	SHV-12	32				3			2			37
	SHV-12; CMY-2							1				1
	SHV-2							1				1
CMY	CMY-2							9	2			11
DHA	DHA-1									1		1
Chromosomal *ampC*	-42 C>T					1						1
Total		**76**	**3**	**1**	**1**	**14**	**2**	**69**	**24**	**3**	**1**	**194**

Figure 7.2.1.2.2 *Trends in prevalence of confirmed ESBL/AmpC-producing E. coli and chromosomal AmpC-mutant E. coli in fresh meat of broilers, pigs, veal calves and dairy cows from 2014-2024 determined by using selective isolation*



The prevalence of ESC-resistant *E. coli* on domestically produced **beef** and **pork** are usually relatively low compared to domestically produced veal and chicken. In 2024, only 1 CTX-M-15 producing *E. coli* was isolated from beef and 1 CTX-M-1 producing *E. coli* was isolated from pork.

In **veal**, ESC-resistant *E. coli* were isolated from 7.5% of meat samples, which seems higher than the 2.7% detected in 2023, but is appears to fall within the natural variation that is detected per year. Although CTX-M-1 producing *E. coli* were most detected, the other ESBL-genes that were detected here are also detected on veal meat in previous years, and in *E. coli* isolated from caecal samples from veal calves.

The detection of ESC-resistant *E. coli* from domestically produced **chicken** increased from 14% in 2023 to 18.8% in 2024, but this is also considered to be part of the natural variation between the years. The prevalence for **imported chicken** is significantly higher at 53%, and includes meat imported from Argentina, Brazil, Chile, Thailand and the United Kingdom. Despite this difference in prevalence, the risk to consumers for ingesting ESC-resistant *E. coli* may not be higher since this meat is not sold fresh to consumers. While SHV-12, TEM-52B and CTX-M-55 are isolated at the highest prevalence from domestic meat, of these only CTX-M-55 is also detected from imported broiler meat, with a large variety of other ESBL and AmpC genes.

For **turkey** meat, the prevalence of ESC-resistant *E. coli* is also significantly lower from domestic produce at 14.3%, than from **imported turkey** at 35.3%. Due to the volume of imported product, mostly turkey imported from Brazil was examined in 2024, as well as some product from Chile. Similar to the *E. coli* isolated from imported

chicken, ESC-resistant *E. coli* from turkey meat mostly encoded CTX-M-55, with a variety of other ESBL and AmpC genes.

Uncommon sources of domestically produced meat included samples of deer, duck, rabbit and horse meat. ESC-resistant *E. coli* were isolated from 2 samples, both of which were characterized to produce CTX-M-15.

Imported fish from aquaculture or line-caught fish included pangasius, salmon, tilapia, tuna and shrimp, imported from Argentina, Bangladesh, Chile, China, Ecuador, Gambia, Guyana, Honduras, India, Indonesia, the Maldives, Marocco, New Zealand Nigeria, Peru, the Philippines, South Africa, Sri Lanka, Thailand, the United Kingdom, the United States, Venezuela and Vietnam. Out of 306 batches, 1% was confirmed to contain ESC-resistant *E. coli*, including shrimps from Bangladesh and tilapia from Vietnam. These isolates produce CTX-M-15 and DHA-1, the latter of which is an ESBL gene that is only detected sporadically in samples from food or farm animals from the Netherlands. The category of **imported uncommon fish** includes samples of Nile perch, sea bream, mussels and tuna from Kenya, New Zealand, Sri Lanka, Tanzania, Uganda, the United Kingdom and Vietnam. Out of 40 batches, one *E. coli* isolate, from Nile perch from Uganda producing CTX-M-1 was detected.

Fresh herbs that were studied included included 59 batches of different herb species from within and outside the EU. Although no ESBL/AmpC-producing *E. coli* were isolated, one CPE was detected, further described in chapter 7.2.2.

Results of genomic comparisons of ESC-resistant E. coli from food and farm animals
Since 2021, all ESC-resistant *E. coli* isolated from food and farm animals are analysed using whole-genome sequencing (WGS)[3]. Core-genome multi-locus sequence typing (cgMLST) is used to determine related clusters with less than 10 allelic differences[7]. Only clusters containing more than 4 isolates, of which at least 1 isolate was collected in 2024, were further studied. Pairwise analysis of isolates in the clusters was performed to determine the number of single-nucleotide polymorphisms (SNPs), where any isolates with less than 40 SNPs are considered a single clone[8].

A total of 1554 *E. coli* genomes from 2021 to 2024 were compared, from which 258 genomes were shown to be part of 31 clusters. The largest cluster consists of 45 *E. coli*, isolated from white and rosé veal calves, as well as 1 from a dairy cow and one from a pig. All isolates in this cluster are less than 40 SNPs removed from their closest relative and appear to have a common ancestor. As indicated above, CTX-M-32 has become more prevalent over the past 5 years in veal calves. A smaller cluster of 5 isolates from white veal calves and one from a broiler isolate also consists of isolates related to each other, but these are not related to the larger cluster.

CTX-M-1 is present in 5 clusters from a variety of genetic backgrounds with an average size of 6 isolates. On average, 4 of these isolates per

cluster are considered clonal. Two clusters were mostly detected in samples from broilers and chicken meat, one cluster from pigs, one cluster from veal meat, white veal calves and dairy cattle, while one cluster was detected in caecal samples and meat from different animal species.

CTX-M-15 was detected in 11 clusters, containing on average 7 *E. coli* isolates, consisting on average of 5 clonal isolates. Approximately half of the isolates belong to MLST sequence type 58. Some of the *E . coli* were isolated from pork, broilers, chicken meat and goats, but the majority of these were isolated from white or rosé veal calves or dairy cattle.

CTX-M-55 was detected in 31 isolates from 4 clusters, of which the majority of isolates were MLST sequence type 3776, isolated from broilers or chicken meat, with a small number of isolates from dairy cattle, white veal, pigs, lamb or veal meat. On average, 4 *E. coli* isolates per cluster were shown to be clonally related.

The second largest cluster contains 19 isolates which are all clonally related and were isolated from white veal, dairy cattle, rosé veal, pigs and goats, all containing SHV-12. This gene was further detected in 5 other clusters, on average of 5 isolates of which on average 3 isolates were shown to be clonally related. Most *E. coli* from these clusters were isolated from broilers or chicken meat, with a small number isolated from white veal or pigs.

A single cluster of *E. coli* was detected containing TEM-52, isolated from white veal and veal meat. The cluster contains 8 isolates from which 5 were shown to be clonal.

Two clusters of *E. coli* with AmpC chromosomal promotor mutants were detected in samples from pigs, with a single sample from white veal. The larger cluster consists of 9 isolates which all have a clonal relationship, while the second cluster consists of 5 isolates of which 4 have a clonal relationship.

As the collection of ESBL-producing *E. coli* isolates grows for which WGS data is available and comparisons are made using isolates from multiple years, it is expected that the number of detected clusters will grow, but clusters were only reported if they also contain isolates from 2024. Nonetheless, the increased detection of highly related *E. coli* containing CTX-M-32 which are mostly isolated from white veal calves is reason for concern as it is unknown why this *E. coli* lineage has become dominant in a relatively short amount of time, while the prevalence of ESC-resistant *E. coli* remains relatively high in this sector. Of the 45 isolates in this cluster since 2021, 17 were detected in 2024. It is currently unknown why this clone appears to spread in this sector. In contrast, the second largest cluster, containing SHV-12, is present in samples from a greater variety of sectors.

ESC-resistant Salmonella
Each year, *Salmonella* isolates are typed by the public health institute RIVM, and phenotypically analysed for antimicrobial resistance at WBVR. These include bacteria from various sources, but mainly from human patients, see chapter 6.1 for a full description.

In 2024, a total of 1759 *Salmonella* isolates were analysed from which 25 ESBL-suspected isolates were detected, and 23 isolates were confirmed ESBL/AmpC producing isolates. Twenty-two isolates are from human origin, while 1 isolate came from a bovine sample. Confirmation was performed by RIVM using whole-genome sequencing, and the detected resistance mechanisms are indicated per serovar in table 7.2.1.2.5.

The results of the detected resistance mechanisms over time from 2007 until 2024 are presented in table 7.2.1.2.6, which indicates that the prevalence of ESBL/AmpC-producing *Salmonella* isolates is relatively stable over time.

Table 7.2.1.2.5 *Beta-lactamases identified in Salmonella isolates in 2024 (n=23)*

Serovar	CTX-M-1	CTX-M-15	CTX-M-32	CTX-M-55	CTX-M-14b	CTX-M-65	SHV-12	CMY-2	TEM-52b	Total
I 4:b:-		1								1
Anatum							1			1
Bredeney				1						1
Chester				1						1
Dublin[a]			1							1
Enteritidis									2	2
Infantis	1					2				3
Kentucky				1	3					4
London		1								1
Minnesota								2		2
Muenster				1						1
Saintpaul				3						3
Typhi		2								2
Total	**1**	**4**	**1**	**7**	**3**	**2**	**1**	**2**	**2**	**23**

[a] 1 isolate from bovine origin.

Table 7.2.1.2.6 *Beta-lactamases identified in Salmonella isolates collected in 2007-2024*

Year	[a]CTX-M-1-group	[b]CTX-M-2	CTX-M-3	CTX-M-8	[c]CTX-M-9-group	TEM-52	TEM-20	[d]SHV-12	[e]CMY-2	ACC-1	DHA-1	DHA-12	Total ESBL	Total *Salmonella* tested	% ESBL of total *Salmonella*
2007	9	13				17	2	4	2				47	1514	3.1
2008	25	12		1	1	13	1		6	2			61	2149	2.8
2009	12	4			2	3		1	9				31	2232	1.4
2010	8	3			1	2		3	4				21	1715	1.2
2011	5	3			1	1		2	13				25	1444	1.7
2012	14	5			2	2			10	1			34	1795	1.9
2013	1	3		5	4	5	1		36				55	1369	4.0
2014	6			2	3	1			21				33	1688	2.0
2015	13	2			6	1			12				34	1761	1.9
[f]2016	7				15	2			10		1		36	2117	1.7
[g]2017	3				23			1	3		1		31	1697	1.8
[g]2018	2		1	1	8				2				14	1718	0.8
2019	4				11			1	3				19	1880	1.0
2020					4				2				6	1310	0.5
2021	2				5			1	2				10	1264	0.8
[h]2022	4	2		1	6			2	32				47	1503	3.1
2023	10			1	4	1		1	2			1	20	2139	0.9
2024	13				5	2		1	2				23	1759	1.3
Total	138	47	1	11	101	50	4	17	171	3	2	1	547	31054	1.8

[a] contains *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-3}$ and a combination with *bla*$_{CMY-2}$ (n=2, 2014, 2015).
[b] In 2008 one combination of *bla*$_{CTX-M-2}$ with *bla*$_{TEM-52}$ was found in *S*. Paratyphi B var Java.
[c] contains *bla*$_{CTX-M-9}$, *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-65}$.
[d] In 2007 three *S.* Concord were found containing both *bla*$_{SHV-12}$ and *bla*$_{CTX-M-15}$.
[e] In 2015 a combination of *bla*$_{CMY-2}$ and *bla*$_{TEM-52}$ was found in *S*. Oranienburg and a combination of *bla*$_{CMY-2}$ with *bla*$_{CTX-M-1}$ in *S*. Molade.
[f] In 2016, one *S.* Minnesota isolate obtained from poultry meat at NVWA was not included in the molecular analysis.
[g] In 2017 and 2018 only human isolates were molecularly characterised.
[h] in 2022 a total of 33 *Salmonella* isolates obtained from imported fresh or processed meat were included which results in an increase of the % ESBL-positive isolates compared to former years.

Conclusions

- ESC-resistant E. coli were rarely detected (<1%) amongst randomly selected E. coli from samples collected at slaughter houses and retail meat.
- Amongst randomly selected E. coli from food products, ESC-resistant E. coli were only detected in imported poultry meat 12%.
- A small but significant increase in the prevalence of ESC-resistant E. coli in broilers is likely caused by a change in the sampling method.
- A significant increase is detected in the prevalence of ESC-resistant E. coli in dairy cattle from 8.7% in 2014 to 22.9% in 2024.
- No trends were detected over time in the prevalence of ESC-resistant E. coli in pigs or veal calves.
- The ESBL-gene CTX-M-32 is increasingly detected in veal calves. Whole-genome sequencing confirms that these isolates are part of a related cluster.
- In meat produced in the EU, poultry still contains the highest prevalence of ESC-resistant E. coli.
- ESC-resistant E. coli is detected at a higher prevalence from imported poultry meat than in poultry meat produced in the EU.

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7.2.2 Carbapenemase-producing Enterobacterales (CPE) in farm animals, companion animals and food

7.2.2.1 Farm animals

Introduction
Carbapenem antibiotics are banned for use in farm animals in Europe under EU legislation to prevent the spread of antibiotic resistance, as carbapenems are last-resort drugs for serious human infections. Carbapenems are classified by the European Medicine Agency as Category A ("Avoid").[1] To identify potential sources for spread of Carbapenemase-producing Enterobacterales (CPE) via the food chain to humans, it is important to continuously monitor farm animals and meat. Although CPE is still rarely found in farm animals, the European Food Safety Authority (EFSA) has recently reported CPE in farm animals and meat thereof in a growing number of EU countries.[2] In the Netherlands, monitoring of CPE in farm animals was started in 2012. No CPE were found until 2024.

Methods
Passive screening
Based on the outcomes of the antimicrobial susceptibility testing, all randomly isolated indicator *E. coli*, *Salmonella* as well as selectively cultured ESC-resistant *E. coli* isolates are screened for resistance to meropenem as indicator for the presence of carbapenem resistance genes.

Active screening based on selective culturing
As part of the mandatory AMR program[3], faecal samples of farm animals are screened for the presence of CPE according to the EURL-AR protocol 'Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples' based on non-selective enrichment, followed by culturing on chromogenic plates.[4] In more detail, an overnight enrichment step in BPW (1 gram sample in 9 ml BPW) is followed by inoculation on two chromogenic agar plates (ChromID® CARBA agar and ChromID® OXA agar plates, BioMérieux). After incubation, plates are inspected visually for growth of CPE suspected colonies (both *E. coli* and other Enterobacterales) and identified by MALDI-TOF (MALDI Biotyper® Sirius System, Bruker).

Active screening based on PCR
To enhance the sensitivity of the screening, all faecal samples are screened in parallel for specific carbapenem resistance genes with an in-house Real-Time multiplex PCR. This is important in an environment with a very low anticipated prevalence of carbapenem resistance as PCR is expected to have a higher sensitivity than culture methods. Samples are grown overnight in Buffered Peptone Water (BPW) with 0.25 mg/L ertapenem and 50 mg/L vancomycin. After incubation, five individual samples are pooled in lysis buffer, and DNA is isolated with a bead method. A multiplex Real-Time PCR (In house) is targeting NDM, KPC, VIM, IMP, OXA-48, IMI and FRI carbapenemase genes. In case of a positive PCR result, a step-wise analysis is performed to confirm the results: 1. Singleplex Real-Time PCR-tests is performed on purified DNA of the 5 individual samples of the pool; 2. If the PCR test is positive, the

original faecal sample and corresponding broth culture of suspected positive samples were inoculated for bacterial isolation on commercial selective plates (ChromID® CARBA agar and ChromID® OXA agar and MacConkey agar plates with 0.125 mg/L ertapenem all selective for Enterobacterales) as well as sheep blood agar plates (HIS) with 0.125 mg/L ertapenem (selective for *Shewanella* spp); 3. DNA is isolated from bacterial isolates grown on the selective agar plates, Real-Time PCR is repeated and genes are confirmed with Sanger sequencing. Isolates that encode putative CPE genes are tested for reduced susceptibility using broth microdilution and the EUVSEC-2 panel. The presence of CPE genes is confirmed using whole-genome sequencing.

Results
In 2024, no CPE were identified within the passive screening nor within the active screening based on selective bacterial isolation. However, two OXA-244 producing *E. coli* were detected in two caecal samples from farm animals using active screening based on PCR and bacterial culturing on home-made MacConkey agar plates with ertapenem.

The PCR screening of 1204 faecal samples (collected from 301 broilers, 302 slaughter pigs, 300 veal calves, and 301 dairy cattle) resulted in eight OXA-48-like positive faecal samples (0.7%) which is similar to the period 2021 -2023 ranging from 0.6 – 0.9% OXA-48-like positive samples. In addition, 510 faecal samples of dairy goats were all tested negative by PCR. PCR-positive samples were identified in dairy cattle (n=5); pigs (n=2) and broilers (n=1). In two samples, the presence of OXA-48-like carrying *Shewanella* was confirmed by bacterial culturing and PCR. These results confirm the findings of the previous years where OXA-48-like genes have also been found in *Shewanella* obtained in faecal samples from livestock. Given the role of *Shewanella* spp. as natural progenitor of this carbapenemase family[5], these genes were considered of environmental origin and not a public health risk. Four samples tested positive for OXA-48 group in the enrichment broth by PCR, but culturing of *Shewanella* and Enterobacterales was negative. For the first time since the start of the selective screening in 2012, CPE-suspected *E. coli* were isolated from two samples (one broiler and one pig sample) on MacConkey agar plates with ertapenem. Both *E. coli* isolates were confirmed to be reduced susceptible to all three tested carbapenems, meropenem, imipenem and ertapenem. Whole-genome sequencing confirmed both isolates as OXA-244 producing *E. coli* with a similar genetic background (ST58).

Our results demonstrate the added value of implementing a more sensitive screening method for detecting CPE with low-level resistance to carbapenems like OXA-244. This issue is already under the attention of the EURL-AR due to other findings. As a result the EURL-AR advises to use an alternative commercial medium capable of detecting OXA-244-producing *E. coli* in the mandatory active screening method based on selective culturing. Such actions will improve future monitoring of CPE in farm animals and food.

7.2.2.2 Companion animals

Introduction
Carbapenemase producing Enterobacterales (CPE) in companion animals in Europe have been observed, but the prevalence is still relatively low. CPE have been found in pet dogs from Germany[6,7], Spain[8], France[9], UK[10], Portugal[11] and Switzerland[12]. Monitoring to detect introduction of CPE in companion animals in the Netherlands was initiated in 2015. The screening for CPE comprised of an initial retrospective study and a prospective study. Until 2016, CPE had not been detected in the Netherlands (MARAN 2017). In 2017, the first case of a OXA-48 producing *E. coli*, isolated from a faecal dog sample, was reported (MARAN 2018). The faecal sample was submitted to the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University for parasitology diagnostics. In 2018, two individual dog samples were found positive for *E. coli*, harbouring OXA-48 and OXA-181 genes. Both samples originated from different parts of the Netherlands and were sent to the VMDC for parasitology diagnostics. From 2019 – 2021, the continued monitoring performed at the VMDC did not reveal CPE in samples of dogs and cats. But in 2022, an OXA-48-producing *E. coli* was identified on one occasion in a faecal sample of a dog. In 2023 no CPE was found in samples of dogs and cats.

Methods
From each sample, 0.5 gram faeces was suspended in 4.5 ml TSB broth, supplemented with 50 mg/L vancomycin for enrichment. The suspension was directly inoculated on ChromID Carba-Smart agar plates (BioMérieux). Both the Smart Agar and the enrichment broth were cultured overnight at 37 °C. After enrichment, the broth was inoculated again and cultured on ChromID Carba-Smart agar (BioMérieux). In addition, total DNA of the enrichment broth was isolated for molecular screening by PCR for the targets NDM[13], KPC[14], IMP[15], VIM[15], OXA-group-23, -24, -51, -58[16] and OXA-group-48[17].

Results
In 2024, 101 faecal samples from dogs and 105 faecal samples from cats were examined. Samples were obtained through the VMDC. Because the expected prevalence of CPE in companion animals remains low and reported CPE are frequently multi-resistant, the inclusion criterion for dog faecal samples was recent antimicrobial treatment of the animal. This strategy is not feasible for cats, since cats are less frequently treated with antimicrobials. Therefore, in cats a randomized stratified subset of faecal samples from cats submitted to VMDC were included.

One faecal sample from a dog turned out positive for a NDM-1 harbouring *E. coli* after selective culturing. The sample was submitted to the VMDC for parasitological screening that turned out negative. The dog showed intermittent diarrhoea and was treated with metronidazole before sampling. In the molecular screening another dog faecal sample tested positive for OXA-10. However, OXA-10 enzymes belong to class D β-lactamases and lack carbapenemase activity. Bacterial culturing of this sample was negative and was therefore considered negative for CPE. No CPE-suspected isolates were found in the 105 faecal cat samples. These

findings indicate the importance of a continuous screening program followed by molecular analysis of potential carbapenemase-producing bacteria for confirmation. Screening for carbapenemase-producing isolates in companion animals is continued in 2025.

7.2.2.3 Food

Introduction

As part of the mandatory AMR program[3], samples fresh meat from retail and import are screened for the presence of CPE according to the EURL-AR protocol 'Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat' based on non-selective enrichment, followed by culturing on chromogenic plates.[18]

The presence of carbapenem resistant bacteria in food products is part in the annual AMR monitoring program in the Netherlands. In recent years, carbapenem resistant bacteria have been incidentally isolated from imported food products other than meat. In total one *E. coli* and 14 *E. cloacae* isolates have been found in food product between 2017-2023. Shrimps was with 9 isolates the most dominant source, followed by four isolates from Tilapia, one from Salicornia and one from fresh coriander. The identified carbapenem genes was diverse including: bla_{IMI}, bla_{FLC}, bla_{NDM}, bla_{FRI}, $bla_{OXA\text{-}9\text{-}10}$. This shows that food is a possible risk for spread of carbapenem resistance through the environment.

Methods

Active screening based on selective culturing

Food samples of pork, beef, calf, fresh herbs, fish, and shellfish are screened for the presence of CPE according to the EURL-AR protocol 'Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat' based on non-selective enrichment, followed by culturing on chromogenic plates.[18] In more detail, an overnight enrichment step in BPW (1 gram sample in 9 ml BPW) is followed by inoculation on two chromogenic agar plates (ChromID® CARBA agar and ChromID® OXA agar plates , BioMérieux). After incubation, plates are inspected visually for growth of CPE suspected colonies (*E. coli, Klebsiella and Enterobacter* and identified by MALDI-TOF (MALDI Biotyper® Sirius System, Bruker). In case a suspected carbapenem resistant colony was isolated the carbapenemase gene was confirmed by WGS analysis.

Active screening based on PCR

In addition to the culture based screening, a selection of imported food samples were screened with an in-house Real-Time multiplex PCR. This DNA-based screening is species independent and possibly more sensitive. Per samples 25 gr of food product was added to BPW and after overnight incubation for 20 h at 37 °C, 300 µl of the enrichment was used for extracting DNA. Three different multiplex Real-Time PCR methods targeting (IMP and OXA-48), (KPC, NDM and VIM) and (FRI and IMI) carbapenemase genes.

Results
In 2024, 300 batches of frozen fish originating from fish farms in South-East Asia and Africa as well as of shrimps, mainly from farms in Africa and Asia, were screened for the presence of CPE by WFSR through selective culturing. In addition, 59 batches of imported herbs, 274 batches of pork meat, 281 batches of beef, 425 batches of chicken meat, 186 batches of calf meat, and 172 batches of imported pig and beef meat were screened for the presence of CPE. From one shrimp sample and one fresh herb a CPE was isolated. These isolates were further analyzed for the presence of carbapenems genes by Whole genome sequencing. In one *Enterobacter cloacae* complex isolate a $bla_{\text{IMI-1}}$ gene was identified, and in one *Escherichia coli* isolate $bla_{\text{OXA-48}}$.

In the active screening based on PCR a total of 353 samples were analysed including 260 samples from frozen fish, shrimps and shellfish samples and 55 fresh herbs samples. DNA analysis with the three multiples qPCR. As in previous years a relative high number of samples of (123 out of 300) DNA-samples were positive with the OXA-48 primer set. A possible explanation for the high prevalence of OXA-48 is the link of these samples to water environments in which *Shewanella* spp. are commonly present often carrying chromosomal blaOXA-48-like genes.[5] In two imported samples from frozen shrimps a positive signal for the IMI gen was detected.

Our findings confirm earlier reports on the presence of CPE in imported seafood in the Netherlands [19, 20] reflecting the high consumption of antimicrobials in South-East Asia, specifically in aquaculture as an environment with a high selective pressure for resistant bacteria.

The active monitoring of (imported) food will be continued in 2025.

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7.2.3 Colistin resistance in farm animals and food

Introduction
The use of colistin in farm animals in Europe is increasingly restricted due to concerns about rising resistance. This restriction is due to the discovery of the plasmid-mediated resistance gene *mcr-1* in 2016[1] and the recognition of colistin as a crucial antibiotic for the treatment of multidrug-resistant infections in humans. In the same year, *mcr-1* was reported in farm animals and meat in the Netherlands.[2] In 2020, a mandatory diagnostic test before prescribing colistin was introduced, and overall sales of colistin for animal use in the EU fell significantly. According to the latest report of the Netherlands Veterinary Medicines Authority (SDa) colistin use in farm animals in the Netherlands has decreased for the fourth consecutive year (2021-2024)[3]. (Almost) no colistin is used in broilers, turkeys, dairy cattle, veal calves, other cattle, and meat rabbits. Within the pig sector, colistin is mainly used in weaned piglets.

To monitor the prevalence of *mcr*-positive bacteria in farm animals as a potential source linked to the food chain, screening of faecal samples for *mcr*-positive Enterobacterales began in 2016 and was implemented into the national AMR monitoring program in animal and food to date.

Methods
For this purpose, purified DNA of pooled BPW + 2mg/L colistine cultures (five samples per pool) of faecal samples of farm animals are tested for the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* using an in house designed multiplex Real-Time PCR based on the updated EURL-AR protocol[4]. From 2022 onwards, the PCR screening of the samples was extended with *mcr-6*, *mcr-7*, *mcr-8*, *mcr-9* and *mcr-10* using an extra in inhouse designed multiplex RT-PCR. In case of a PCR positive pool, individual samples are tested, followed by direct culturing of the original BPW broth on MacConkey agar with 2 mg/L colistin.

Results
In total, 1714 faecal samples (collected from 301 broilers, 302 slaughter pigs, 300 veal calves, 301 dairy cattle and 510 dairy goats) were analysed in 2024. As a result of the screening, *mcr*-1 positive *E. coli* were identified in two samples from broilers (0.6%) and one pig (0.3%). In addition, three broiler samples were tested positive for *mcr-1* with PCR, which could not be confirmed by bacterial culturing. In addition, *mcr*-9 was detected in 3 samples of white veal calves (1.0%) and one pig sample (0.3%). Bacterial culturing was negative for all *mcr-9*-positive samples which is most probably due to the fact that, in contrast to other *mcr*-genes, the presence of *mcr-9* does not always result in elevated colistin MICs which hinders detection of these bacteria by selective culturing. No colistin resistant indicator *E. coli* were identified from dairy cattle and dairy goats. Furthermore, no colistin was not detected in the randomly isolated indicator *E. coli* from faecal samples of farm animals (Table 7.1.1.2). These results confirm the long-term low prevalence of *mcr*-positive bacteria in farm animals reflecting the low use of this antibiotic.

Table 7.1.1.3 shows that colistin resistance was only observed in one *E. coli* isolate obtained from imported chicken meat (0.5%) and one *E. coli* isolate obtained from fresh turkey meat (5.6%). Both isolates harboured *mcr-1*.

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7.2.4 MRSA surveillance in livestock and humans living/working on farms

Introduction
During the last two decades, MLST clonal complex (CC) 398 has emerged in livestock and persons in contact with livestock in multiple countries, including the Netherlands. This subtype of MRSA is referred to as livestock-associated MRSA (LA-MRSA). The most important risk factor for carriage of LA-MRSA is professional contact with livestock, especially pigs, poultry and veal calves. During the last decade, the detected number of MRSA infected persons without direct contact with livestock, seems to be increasing. In 2018, a national project on surveillance of MRSA in humans, livestock, and meat products was started. The objective of this project was to assess possible changes in the rate or nature of MRSA transmission between animals and humans.

Methods
The project is a collaboration between NVWA, RIVM, WBVR and WFSR. MRSA isolates obtained from various sources result in prevalence data in different animal reservoirs over the years. In addition, the genomes of the MRSA isolates are analysed using various Illumina platforms. Animal related MRSA isolates are collected from three surveillance programs[1]:

1. Surveillance livestock project in livestock and persons living or working on a farm.
   This surveillance is described in van Duijkeren et al., 2025[1] and executed under Zoonoses Directive 2003/99/EC. Within this surveillance program every year one livestock sector is investigated for the presence of MRSA. In 2024 pig farms and persons working and/or living on these farms were sampled. Dust was collected using three wipes in one barn per farm that housed pigs older than 16 weeks of age. The farm was considered positive if at least one of the dust samples contained MRSA bacteria. Farmers and persons living and/or working on the farm sent in a nose swab by regular mail. Culture methods were used as described in van Duijkeren *et al.,* 2025.[1]
2. National MRSA surveillance in livestock.
   In this surveillance caecal /faecal samples collected at slaughter and/or at a farm are collected and investigated for MRSA as described in van Duijkeren et al., 2025.[1]
3. National MRSA surveillance in meat.
   Every year, Dutch retail meat samples and imported meat are collected and investigated for MRSA.

The genomes of the animal-related isolates are compared with the genomes of isolates collected in the Dutch national MRSA surveillance in humans. See for the description of the Dutch national MRSA surveillance in humans chapter 5.2.5.
The molecular comparison of the isolates obtained in 2024-2025, will take place next year when the sampling at pig farms is completed.

In 2024, susceptibility testing of MRSA was performed on all isolates originating from caeca of pigs (n=50) and veal calves (n=82) as well as one isolate per dust sample (n=90) from pig farms. MRSA isolates were tested for antimicrobial susceptibility with broth microdilution according to ISO standards in a harmonised antibiotic panel advised by EFSA using

commercially available Sensititre plates (Thermofisher Scientific, panel EUST). The MIC-values were interpreted with ECOFFs as advised by EUCAST (https://mic.eucast.org/)

Results

MRSA prevalence on farms in caecal samples and on meat

MRSA prevalence on farms

In 2024, 113 pig farms were sampled. The sampling of this animal sector was continued in 2025. The results described here are therefore preliminary results. Fourteen farms were organic farms, with a MRSA prevalence of 21% (95%CI 5-51%). This is significantly lower than among the 99 conventional farms sampled (77%, 95%CI 67-85%). The overall prevalence was 70.5% (Table 7.2.4.1). This prevalence is not significantly different than that found in 2020 when 149 farms were sampled and 113 (76%) was MRSA positive. However, the prevalence was significantly higher than for any other type of livestock farms sampled in previous years (Table 7.2.4.1). In 2024, 57 persons living and/or working on 37 pig farms participated in the study. From those 57 persons, twenty persons (derived from seventeen pig farms) carried MRSA (35.1% (24.0-48.1%)). This MRSA prevalence was higher than the MRSA prevalence among persons living and/or working on other types of livestock farms (0.9-12.7%) sampled in previous years (Table 7.2.4.1).

Table 7.2.4.1 *MRSA prevalence on farms and in persons living/working on the farm 2018-2024*

Year	Animal	MRSA positive farms (n)	Total no of farms sampled (n)	Preva-lence (%)	95% CI	Mrsa positive humans (n)	Total no of positive humans (n)	Preva-lence (%)	95% CI
2024	Pigs	79	113	70.0	60.9-77.6	20	57	35.1	24.0-48.1
2023	Sheep	7	156	4.5	2.2-9.0	5	80	6.3	2.7-13.8
2022	Veal calves	44	173	25.4	19.5-32.4	7	55	12.7	6.3-24.0
2020/ 2021	Dairy cows	11	181	6.2	3.4-10.6	1	107	0.9	0.2-5.1
2020	Pigs	113	149	75.8	68.4-82.0	ns	ns	-	-
2018/ 2019	Broilers	0	195	0.0	0.0-2.0	4	133	3.0	3.4-10.3

ns=not sampled.

MRSA prevalence in caecal samples

In 2024, caecal/faecal samples from the national surveillance taken at slaughter for the monitoring of antimicrobial resistance in zoonotic and commensal bacteria (EU decision (EU) 2020/1729) were investigated. Caecal samples are only collected from animals raised and slaughtered in the Netherlands.

MRSA was found in caecal samples from 50/302 (16.6%) pigs, 75/186 (40.3%) white veal calves and 7/114 (6.1%) rosé veal calves. Caecal samples from broilers and faecal samples from dairy cattle were not investigated in 2024 (Table 7.2.4.2).

The data should be interpreted with care, as caecal or faecal samples are not the preferred sample for MRSA. The data, however, are available from the routine surveillance and as they are collected in the same way

each year, trends in time can be analysed. The prevalence found in pigs and veal calves in 2024 was comparable to the prevalence found in previous years (2021 to 2023).

Table 7.2.4.2 *MRSA found in caecal samples collected in 2021-2024*

	Year			
Animal species	**2021 % (n/N)**	**2022 % (n/N)**	**2023 % (n/N)**	**2024 % (n/N)**
Pigs	15.1 (15/100)	16.0 (48/300)	13.7 (41/300)	16.6 (50/302)
White veal calves	44 .3 (31/70)	28.2 (58/207)	-	40.3 (75/186)
Rosé veal calves	0.0 (0/32)	2.0 (2/100)	-	6.1 (7/114)
Dairy cows	1.0 (1/102)	0.3 (1/300)	-	-
Broilers	1.0 (1/102)	-	0.3 (1/290)	-

MRSA prevalence on meat

In 2024, a total of 1,503 retail meat samples were investigated. Table 7.2.4.3 shows the amount tested and the number of MRSA positive samples per meat product over the years 2018-2024. In 2024, there were no significant differences in MRSA prevalence on meat compared to 2023. Pork, poultry meat, and veal are still the most contaminated products compared to sheep/goat meat, beef and other meat.

Table 7.2.4.3 *MRSA found on meat (products) between 2018-2024*

Year	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**2024**
Sample	**%(n/N)**	**%(n/N)**	**%(n/N)**	**%(n/N)**	**%(n/N)**	**%(n/N)**	**%(n/N)**
Sheep/goat meat	no data	no data	2.2	12.4	no data	4.6	4.3
			(1/46)	(37/299)		(7/151)	(7/162)
95% CI			0.0-11.5	8.9-16.7		1.9-9.3	1.8-8.7
Pork	5.9	8.4	3.5	no data	7.2	6.9	8.4
	(8/135)	(25/296)	(3/57)		(13/180)	(20/291)	(25/297)
95% CI	2.6-11.34	5.5-12.2	1.1-14.6		3.9-12.0	4.3-10.4	5.5-12.2
Beef	2.1	3.8	no data	no data	6.1	3.9	5.5
	(3/140)	(2/11)			(9/14)	(17/432)	(17/309)
95% CI	0.4-6.1	1.9-6.8			2.8-11.3	2.3-6.2	3.2-8.7
Poultry-meat	22.0	19.9	16.5	8.6	9.3	10.4	7.9
	(29/132)	(50/251)	(45/248)	(28/324)	(18/194)	(20/192)	(36/455)
95% CI	15.2-30.0	15.2-25.4	13.6-23.5	5.8-12.3	5.6-14.3	6.5-15.6	5.6-10.8
Veal	no data	no data	3.8	6.9	9.1	13.6	10.4
			(2/52)	(18/261)	(15/164)	(25/184)	(21/201)
95% CI			0.5-13.2	4.1-10.7	5.2-14.6	9.0-19.4	6.6-15.5
Other meat	no data	no data	0.0	20.0	14.3	4.5	0
			(0/4)	(4/20)	(3/21)	(1/22)	(0/106)
95% CI			0.0-60.2	5.7-43.7	3.1-36.3	0.1-22.8	0.0-3.4

* Most "other" samples in 2024 derived from venison from UK and New-Zealand.

Resistance levels of MRSA from pigs and veal calves
The proportion of resistance of the MRSA isolates from pigs and veal calves caeca and dust samples from pig farms to various antimicrobial drugs are depicted for each type of sample in table 7.2.4.4. In 2024, as expected for MRSA, nearly all isolates tested resistant against (benzyl)penicillin and cefoxitin. High levels of resistance were also observed for tetracycline. This is in line with the known levels (~100%) of tetracycline resistance in LA-MRSA.

The proportion of isolates that was resistant to clindamycin, erythromycin, gentamicin, kanamycin and trimethoprim from veal calves was much higher compared to the resistant proportion of pig MRSA isolates. For other antibiotics (chloramphenicol, quinupristin/dalfopristin and tiamulin) the resistant proportion of pig isolates was slightly higher than in veal calve isolates. Resistance levels in MRSA isolated from pig husbandry differ a little depending on isolation source. Overall, resistance levels for most antimicrobials (especially chloramphenicol, quinupristin/dalfopristin, tiamulin and trimethoprim) were higher in isolates collected from dust samples at the farm than from isolates derived from caeca at slaughter.

Compared to 2022, resistance levels in MRSA isolates from caecal samples of veal calves has slightly declined (NethMap/MARAN 2023). Resistance levels in MRSA isolates from pig caeca remained equal compared to 2023 except for chloramphenicol, this resistance level declined from 51% in 2023 to 14% in 2024 (NethMap/MARAN 2024). Finally, no or very low resistance was detected against the following reserved and highly critically important antibiotics for humans: fusidic acid, linezolid, mupirocin, rifampicin, or vancomycin.

Table 7.2.4.4 *Resistance percentages (R%) of MRSA isolated from dust samples from pig farms and from caeca from pigs and veal calves at slaughter in 2024*

Antibiotic	Pig farms dust (N=90)	Pigs caeca (N=50)	Veal calves caeca (N=82)
cefoxitin	98.9	100.0	98.8
chloramphenicol	21.1	14.0	3.7
ciprofloxacin	5.6	2.0	3.7
clindamycin	43.3	36.0	67.1
erythromycin	27.8	26.0	67.1
fusidic acid	1.1	0.0	1.2
gentamicin	15.6	28.0	80.5
kanamycin	8.9	18.0	81.7
linezolid	0.0	0.0	0.0
mupirocin	0.0	0.0	1.2
penicillin	100.0	100.0	100.0
quinupristin/dalfopristin	23.3	14.0	6.1
rifampicin	0.0	0.0	1.2
streptomycine	3.3	2.0	3.7
sulfamethoxazole	1.1	2.0	2.4
tetracycline	94.4	100.0	100.0
tiamulin	25.6	14.0	8.5
trimethoprim	63.3	52.0	95.1
vancomycin	0.0	0.0	0.0

Comparison of animal-related MRSA GG0398 isolates compared to MRSA isolates from the human Dutch national surveillance
A molecular comparison will be performed in 2026 when the sampling of the pig farms has been completed. Results of former comparisons are found in MARAN2024 and van Duijkeren et al., 2025.[1]

Discussion
Farms investigated in 2024 show a difference in MRSA prevalence between conventional farms and organic farms. This is in concordance to the results of a study on organic pig farms in 2011,[2] which described lower antibiotic usage on organic farms as explanation for a lower MRSA prevalence compared to conventional farming. The persisting high prevalence on conventional pig farms poses a potential threat to persons living and/or working on these farms, especially for persons in direct contact with the animals.
On meat, the highest prevalence is found on pork, veal calf and poultry meat. Generally, it is believed that contaminated meat is not an important transmission route for MRSA for the population at large, especially if the meat is heated before consumption. In some studies performed abroad, however, food handling has been implicated as a transmission route.[3,4]

Conclusions

- The preliminary results of the prevalence of LA-MRSA on pig farms show that still a high percentage of farms are MRSA positive. As a result there is also no decrease in MRSA prevalence visible in caecal samples from pigs at slaughter
- Preliminary results show that MRSA is also present on 21% of organic farms
- No changes are found in MRSA prevalence on meat: Pork, veal and poultry meat are most contaminated
- MRSA prevalence on white veal calve farms is higher compared to rosé veal calve farms.
- MRSA isolates from veal calves and pigs show very low to no resistance to antimicrobials that are important for human medicine: fusidic acid, linezolid, mupirocin, rifampicin, and vancomycin.

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8 Supplements

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8.2 Overview of antimicrobial resistance in human surveillance programs included in NethMap One Health 2025

Table S1 *Overview of antimicrobial resistance surveillance programs included in NethMap One Health 2025*

Surveillance program	Origin of isolates	Availability	Sources 2025	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance programs aimed at resistance surveillance in specific pathogens					
CPE	GP, hospital, LTCF	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, next generation sequencing, Nanopore long-read sequencing
CPPA	GP, hospital, LTCF	2020-	Nationwide	Central testing	Gradient testing, Carba-PCR, next generation sequencing, Nanopore long-read sequencing
CRAB	GP, hospital, LTCF	2022-	Nationwide	Central testing	Gradient testing, Carba-PCR, next generation sequencing, Nanopore long-read sequencing
MRSA	GP, hospital, LTCF	2008-	Nationwide	Central testing	MLVA typing, whole genome sequencing
Neisseria meningitidis	Hospital	1994-	Nationwide	Central testing	Gradient testing
Neisseria gonorrhoeae	SHC	2006-	15 out of 24 SHC	Decentral testing	Gradient testing

ISIS-AR = Infectious disease surveillance information system on antibiotic resistance; CPE = carbapenemase-producing Enterobacterales; CPPA = carbapenemase-producing Pseudomonas aeruginosa; CRAB = carbapenem-resistant Acinetobacter baumannii-calcoaceticus complex; MRSA = methicillin-resistant Staphylococcus aureus; GP = general practice; LTCF = long-term care facility; SHC = sexual health centres; UMCG = University Medical Centre Groningen; NIC-ErasmusMC = National Influenza Centre Erasmus Medical Centre location; WHO-CC = World Health Organization collaborating centre; MLVA = multiple-locus variable number of tandem repeat analysis

Table S1 (continued) *Overview of antimicrobial resistance surveillance programs included in NethMap One Health 2025*

Surveillance program	Origin of isolates	Availability	Sources 2025	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance programs aimed at resistance surveillance in specific pathogens					
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Whole genome sequencing, additional phenotypic testing
Influenza antiviral drugs	Community, GP, hospital	2005-	NIVEL GP sentinels, hospital/regional laboratories, Infectieradar	central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Whole genome Nanopore sequencing, Neuraminidase enzyme inhibition assay
HIV	Hospital	2003-	Nationwide	Decentral testing	Sequencing with viral mutation characterization for reverse transcriptase, protease, or integrase resistance-associated mutations
Resistance among anaerobic pathogens	Hospital	2010-	UMCG	Central testing	Gradient testing
Clostridioides difficile	Hospital, LTCF	2005-	5 hospitals	(de)central testing	Agar dilution
Azole resistance in *Aspergillus fumigatus*	Hospital	2011-	5 university hospitals + 5 teaching hospitals	Central testing	EUCAST microbroth dilution methodology

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