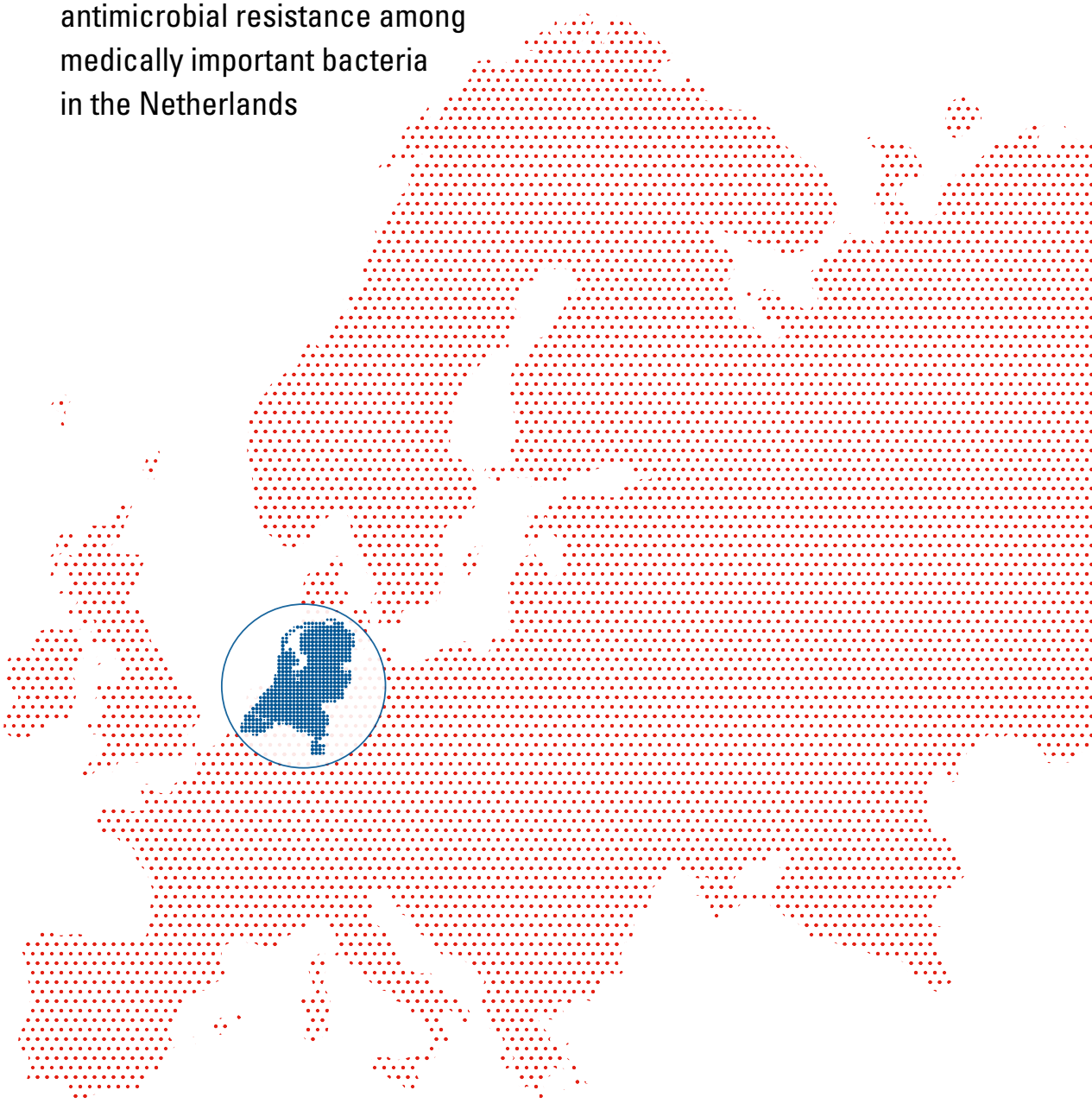
NethMap 2024

Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands



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

SWAB Stichting Werkgroep Antibioticabeleid

NethMap 2024

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2023

November 2024

Synopsis

NethMap/MARAN report

Every year in the Netherlands, it is determined for each type of bacterium which percentage is resistant to antibiotics. A resistant bacterium has become insensitive to antibiotics. In the Netherlands, the percentage of resistant bacteria in 2023 was around the same as in 2022. For some types of bacteria, the resistance percentage has dropped slightly over the past five years. Compared to other European countries, antimicrobial resistance in the Netherlands is still low.

However, highly resistant bacteria are increasingly being found. These are bacteria that are resistant to several antibiotics at the same time and to antibiotics that are used as a last resort in rare cases. These highly resistant bacteria are common in people who have been hospitalised abroad. Infections caused by these bacteria can be difficult to treat. Alertness to resistant bacteria therefore remains necessary.

Measures to prevent infections, such as thorough hand washing and other hygiene measures, are needed to prevent resistant bacteria from spreading. In addition, incorrect and unnecessary antibiotic use should be prevented as much as possible, because this makes bacteria resistant to antibiotics.

In 2023, general practitioners prescribed antibiotics approximately as often as in 2019, the year before the COVID-19 pandemic. This happened less often in the Netherlands in the period 2020–2022. In hospitals, slightly more antibiotics were used in 2023 than in the years before the COVID-19 pandemic. Furthermore, in 2023, hospitals and nursing homes reported more outbreaks of resistant bacteria than in 2022, but nevertheless fewer than in the years before the pandemic.

The measures that have been taken in the Netherlands to combat antimicrobial resistance go beyond human healthcare (one health). This is because resistant bacteria are also carried by animals and are present in food and the environment. Antibiotic use in Dutch livestock farming has fallen significantly since 2009, but this decline is now levelling off. The same applies to antimicrobial resistance in animals. Antibiotics that are crucial for the treatment of infections in humans are only used for livestock and other kinds of animals in extremely rare cases. As a result, resistance to antibiotics that are important for humans is rare.

These are the findings of the annual NethMap/MARAN report for 2024. In this report, a number of organisations present data about antibiotic use and antimicrobial resistance in the Netherlands for both humans and animals.

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

Publiekssamenvatting

NethMap/MARAN-rapport

Elk jaar wordt in Nederland per bacteriesoort geteld welk percentage resistent is tegen antibiotica. Een resistente bacterie is ongevoelig voor antibiotica. In Nederland was in 2023 het deel van de resistente bacteriën ongeveer even groot als in 2022. Bij sommige bacteriesoorten kwam resistentie in de afgelopen 5 jaar iets minder vaak voor. Vergeleken met andere Europese landen is de antibioticaresistentie in Nederland nog laag.

Toch worden zeer resistente bacteriën steeds vaker gevonden. Dat zijn bacteriën die resistent zijn tegen verschillende antibiotica tegelijk en tegen antibiotica die in zeldzame gevallen als laatste redmiddel worden gebruikt. Deze zeer resistente bacteriën komen regelmatig voor bij mensen die in het buitenland in het ziekenhuis hebben gelegen. Infecties door deze bacteriën kunnen moeilijk worden behandeld. Alertheid op resistente bacteriën blijft daarom nodig.

Maatregelen om infecties te voorkomen, zoals handen wassen en andere hygiënemaatregelen, zijn nodig om te voorkomen dat antibioticaresistente bacteriën zich verspreiden. Ook moet onjuist en onnodig gebruik van antibiotica zo veel mogelijk worden voorkomen, omdat bacteriën hierdoor antibioticaresistent worden.

In 2023 hebben huisartsen ongeveer even vaak antibiotica voorgeschreven als in 2019, het jaar voor de coronapandemie. In de periode 2020-2022 gebeurde dat in Nederland minder vaak. In ziekenhuizen is in 2023 juist iets meer antibiotica gebruikt dan in de jaren voor de coronapandemie. Ook zijn in ziekenhuizen en verpleeghuizen in 2023 meer uitbraken gemeld van infecties door resistente bacteriën dan in 2022, maar nog altijd minder dan in de jaren voor de pandemie.

De maatregelen die in Nederland zijn genomen om antibioticaresistentie te bestrijden, reiken verder dan de gezondheidszorg bij mensen (*one health*). Resistente bacteriën komen namelijk ook voor bij dieren, in voeding en in het milieu. Het antibioticumgebruik in de Nederlandse veehouderij is sinds 2009 flink gedaald, maar die daling vlakt nu af. Voor de antibioticaresistentie bij dieren geldt hetzelfde. De antibiotica die cruciaal zijn om infecties bij mensen te behandelen, worden alleen bij hoge uitzondering gebruikt voor landbouwhuisdieren. Hierdoor komt resistentie tegen antibiotica die voor mensen belangrijk zijn, weinig voor.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2024. Hierin presenteren verschillende organisaties samen de gegevens over het antibioticumgebruik en -resistentie in Nederland, voor mensen en dieren.

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

Colophon

This report is published under the acronym NethMap by the SWAB, the Dutch Foundation of the Working Party on Antibiotic Policy, in collaboration with the Centre for Infectious disease control (CIb) of the RIVM, the National Institute for Public Health and the Environment of the Netherlands. SWAB is fully supported by a structural grant from CIb, on behalf of the Ministry of Health, Welfare and Sports of the Netherlands. The information presented in NethMap is based on data from ongoing surveillance systems on the use of antimicrobial agents in human medicine and on the prevalence of resistance to relevant antimicrobial agents among medically important bacteria isolated from healthy individuals and patients in the community and from hospitalized patients.

NethMap 2024 and earlier versions are also available from the website of the SWAB: www.swab.nl and www.rivm.nl.

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Acknowledgements

We thank the Foundation for Pharmaceutical Statistics SFK, The Hague, for providing data on community usage of antimicrobial agents and all hospital pharmacists of the centres mentioned below for providing data on hospital usage.

We thank all participants of ISIS-AR, Dr E Bathoorn, Dr JA Severin, the Netherlands Reference Laboratory for Bacterial Meningitis in Amsterdam, GRAS, Prof Dr M van der Valk, Dr A van Sighem, Stichting HIV Monitoring, anaerobic pathogen surveillance, *C. difficile* surveillance, azole resistance surveillance, and the NIVEL for their important contributions; and the staff of the Publishing Department RIVM for preparing this report for printing.

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Centres contributing to the surveillance of resistance to antimicrobial agents (ISIS-AR)
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Franciscus Gasthuis en Vlietland; Rotterdam, Ikazia Ziekenhuis; Rotterdam, Maasstad Ziekenhuis; Rotterdam, Star-SHL; 's-Gravenhage, Haaglanden MC; 's-Gravenhage, HagaZiekenhuis; 's-Hertogenbosch, Jeroen Bosch Ziekenhuis; Sittard-Geleen, Zuyderland MC; Terneuzen, Microvida locatie ZorgSaam; Tilburg, Microvia locatie Elisabeth-Tweesteden Ziekenhuis; Utrecht, Diakonessenhuis; Utrecht, Saltro; Utrecht, UMC Utrecht; Veldhoven, Eurofins-PAMM; Velp, Ziekenhuis Rijnstate; Venlo, VieCuri; Zwolle, Isala

Contents

1 Introduction

This is NethMap 2024, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2023 and previous years. NethMap is a cooperative effort of the Dutch Working Group on Antibiotic Policy (SWAB; Stichting Werkgroep Antibiotica Beleid) and the Centre for Infectious Disease Control Netherlands (CIb) at the National Institute for Public Health and the Environment (RIVM). NethMap is issued back-to-back together with MARAN, reporting on trends in antimicrobial resistance and antimicrobial use in animal husbandry.

In 1996, SWAB was founded 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 major aim of the SWAB is to contribute to the containment of the development of antimicrobial resistance and provide guidelines for optimal use of antibiotics, taking into account results obtained from resistance surveillance and antibiotic use surveillance. Based on the national AMR surveillance system (ISIS-AR) performed by the CIb-RIVM, trends in antimicrobial resistance are monitored using routine antibiotic susceptibility testing data from microbiology laboratories in the Netherlands. Furthermore, the CIb subsidizes surveillance programs that focus on the monitoring of specific pathogens, or even specific resistance mechanisms. Finally, the CIb coordinates the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR) which aims to mitigate large-scale outbreaks of AMR in hospitals and long-term care facilities and to prevent spread to other healthcare facilities through early warning and reporting. Together these constitute the basis of the surveillance of resistance reported in NethMap and are used by CIb to monitor and inform the general public, professionals and policy makers about potential national health threats with regard to antimicrobial resistance.

NethMap parallels the monitoring system of antimicrobial resistance and antibiotic usage in animals in The Netherlands, entitled MARAN – Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands. Jointly, NethMap and MARAN provide a comprehensive overview of antibiotic usage and resistance trends in the Netherlands in humans and in animal husbandry and therefore offer insight into the ecological pressure associated with emerging resistance.

Each year, we try to further improve the report and highlight the most important trends. The trends and developments in highly resistant microorganisms (HRMOs) receive attention in separate chapters. Furthermore, the Antimicrobial Stewardship Monitor program is gaining footage in an increasing number of hospitals that participate, making the results and conclusions more robust. In the future, we will further integrate the human and veterinarian data. For now, data on resistance amongst food-borne bacterial pathogens in humans are described in MARAN, but with a reference in NethMap in chapter 4.7.

We believe NethMap/MARAN continues to contribute to our knowledge and awareness regarding the use of antibiotics and the resistance problems that are present and may arise in the future. We especially thank all those who are contributing to the surveillance efforts, and express our hope that they are willing to continue their important clinical and scientific support to NethMap/MARAN and thereby contribute to the general benefit and health of the people.

The editors:
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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 (NethMap 2024) and the veterinary sector (MARAN 2024).

2.1 Most important trends in antimicrobial use

Outpatient use

- Total outpatient antibiotic use increased by 5.8%, from 8.32 DID in 2022 to 8.80 DID in 2023.
- Total outpatient systemic antibiotic use has returned to pre-COVID levels, with a similar distribution across antibiotic classes. Slight increases in antibiotics generally considered second-line, such as amoxicillin/clavulanic acid and macrolides are concerning.
- The increase of amoxicillin use as compared to 2019 (0.08 DID, +7.0%) might be explained by a high number of pneumonia diagnoses in 2023, for which amoxicillin is the first treatment choice.
- The use of tetracycline declined by 0.11 DID, although still the most commonly used antibiotic in the outpatient setting.

Inpatient use

- In 2023, compared to 2022, inpatient systemic antibiotic use increased by 4.5%, from 92.1 to 96.3 DDD/100 patient-days. When expressed as DDD/100 admissions, the use of systemic antibiotics increased by 6.3%, reaching 359.5 in 2023. When measured in DDD/1,000 inhabitant-days (DID), systemic antibiotic use increased by 2.5% to 0.766.
- Most antibiotic classes exhibited an increase, with notable deviations observed for β-lactamase-resistant penicillins (flucloxacillin) and nitrofurantoin. Nevertheless, flucloxacillin remains the most used antibiotic in terms of DDD in the hospital setting. The largest relative increases were seen for tetracyclines and lincosamides.

- From 2019 (pre-COVID) to 2023, there was a 22% increase in DDD/100 patient-days, with most antibiotic classes showing increases except for aminoglycosides, which decreased. The decline in aminoglycosides was primarily due to reduced use of gentamicin (-24%).
- The largest relative increases were seen in tetracyclines (+65%) and third-generation cephalosporins (+45%).
- The most pronounced absolute increases were in third-generation cephalosporins (+3.5 DDD/100 patient-days) and β-lactamase-resistant penicillins (+2.9 DDD/100 patient-days).

Long-term care facilities (LTCFs)

- In 2023, the total use of antibiotics and their distribution across classes in LTCFs was similar to pre-pandemic levels.
- The most common used antibiotics are as before the combination of penicillins (amoxicillin with clavulanic acid), nitrofurantoin and fluoroquinolones. The high use of broad-spectrum fluoroquinolones remains concerning.

2.2 Most important trends in antimicrobial resistance and implications for treatment

In the Netherlands, the Infectious disease Surveillance Information System on Antibiotic Resistance (ISIS-AR) is used to monitor antimicrobial resistance for a wide range of pathogens in different settings. In addition, a number of surveillance programs exist that focus on specific pathogens, or even specific resistance mechanisms. These programs often include susceptibility testing and confirmation of important resistance mechanisms and molecular typing in national reference laboratories. In table 2.2.1 an overview is provided of surveillance programs that are included in NethMap 2024.

Table 2.2.1 Overview of antimicrobial resistance surveillance programs included in NethMap 2024

Surveillance program	Origin of isolates	Availability	Sources 2023	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in a wide range of pathogens					
ISIS-AR	GP, Hospital, LTCF	2008-	48 laboratories	Decentral testing	Various methods used in routine 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-	16 out of 24 SHC	Decentral testing	Gradient testing
***Mycobacterium tuberculosis* complex**	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

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

Over the last years, resistance rates in the Netherlands are mostly stable. Noteworthy, 2020 and 2021 have been exceptional years due to the COVID-19 pandemic. In 2023, the resistance rates did not increase for most pathogens and antibiotics.
In the summary below, the most important trends of 2023 and implications for therapy are provided. As implications differ by category of patient and indication of use, the summary is organized as such. There are significant differences in susceptibility of pathogens by patient category. In particular for ICU patients, resistance levels are generally higher and routine culturing with susceptibility testing remains mandatory to tailor therapy to the individual patient. If broad spectrum therapy is initially chosen, susceptibility test results should be used to narrow down antimicrobial therapy to prevent further emergence of resistance and cultures have to be repeated when indicated.
Of importance, resistance rates reported in NethMap are based on data on the first isolate per patient per year. Resistance of bacteria in the individual patient, especially in those patients staying longer in the hospital, is often higher than reported here. On the other hand, resistance may be overestimated in patients visiting general practitioners (GP) or living in long-term care facilities (LTCF), since cultures are usually only performed after failure of initial therapy. In 2019, EUCAST has redefined the category 'I' from a definition of 'intermediate or uncertain therapeutic effect' to the definition 'susceptible, increased exposure'. In 2021, the Dutch Society of Medical Microbiology (NVMM) has encouraged all laboratories in the Netherlands to use this new definition. At present, most Dutch laboratories have redefined the category 'I' according to recommendations by EUCAST. Nevertheless, because in the analysis for this report the percentage of resistant isolates ('R') was calculated, and calculations were based on reinterpretation of the raw test values according to EUCAST breakpoints 13.1 (2023), the new definition did not influence the presented resistance percentages or trends.
It should be borne in mind that the majority of conclusions below are based on agents used as intravenous therapy, except for agents that are available as oral drugs only or have a specific indication such as urinary tract infections (UTI).

General practices

Urinary tract infections

- 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 levels decreased slightly to 20%.
- 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 empirical choice antibiotic for the empirical treatment of complicated UTI in primary care, was still high but decreased to 25% in *E. coli* and was stable at 16% in *Klebsiella pneumoniae*. Resistance levels for co-trimoxazole, third empirical choice antibiotic for this indication, was 18% in *E. coli* and 8% 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

- Antibiotic resistance levels in *Staphylococcus aureus* were relatively low except for clindamycin (including inducible resistance, 13%), erythromycin (14%) and fusidic acid (23%). Resistance to clindamycin (including inducible resistance) and macrolides in *S. aureus* remained stable over the last five years.
- MRSA was found in 4% of isolates of primary care patients which was stable over the previous 5 years.
- The rise in resistance to doxycycline/tetracycline (28%) in β-haemolytic *Streptococcus* spp. group A over the last five years is worrisome. Resistance to clindamycin (including inducible resistance, 7%) remained stable after an initial rise.

Respiratory infections

- Resistance to doxycycline/tetracycline (17%) and erythromycin (15%) in *S. pneumoniae* was high in GP patients, most probably due to the more frequent use of these antibiotics in primary care.

Outpatient departments

Enterobacterales and P. aeruginosa

- For the three most important oral antibiotics used in the outpatient department (OPD) setting, (i.e. co-amoxiclav, co-trimoxazole and ciprofloxacin) a similar decreasing or stable trend was found as observed in primary care. In *E. coli*, resistance to co-amoxiclav was still high but decreased to 30% and was stable at 18% in *K. pneumoniae*. Resistance to co-trimoxazole was stable at 22% in *E. coli* and 12% in *K. pneumoniae*. Resistance levels for ciprofloxacin were 15% in *E. coli*, 13% in *K. pneumoniae* and 11% in *P. aeruginosa*.
- Although administration of these three antibiotics may limit the probability of successful treatment when used as empirical treatment, combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole in all Enterobacterales was low (≤5%).

S. aureus

- Antibiotic resistance levels in *S. aureus* were generally low with the exception of resistance to clindamycin (including inducible resistance, 17%), erythromycin (18%) and fusidic acid (9%), Resistance to clindamycin seems to have slightly increased over the last five years.
- Resistance to fusidic acid was much lower in *S. aureus* isolates of OPD patients (9%) than in *S. aureus* isolates of GP patients (23%).
- MRSA was found in 2% of isolates of OPD patients which was stable over the previous 5 years.

Hospital departments

Inpatient hospital departments (excl. ICU)

Enterobacterales and P. aeruginosa

- For both Enterobacterales and *P. aeruginosa*, resistance levels for all tested antibiotics were comparable to resistance levels in isolates of OPD patients.
- For all Enterobacterales, resistance to second and third generation cephalosporins seemed to have plateaued over the past five years. In 2023, resistance to cefuroxime was 11% in *E. coli* and 13% in *K. pneumoniae*. Resistance to cefotaxime/ceftriaxone was 6% in *E. coli* and 8% in *K. pneumoniae*. Patients that are infected with *K. pneumoniae* or *E. coli* have a 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, reducing likelihood of resistance to 3% or less, might be a suitable option.
- Although meropenem resistance in *K. pneumoniae* was less than 1%, there was a significant increase over the last five years from 0.1% to 0.4% in this specific healthcare setting.
- For the three most important oral antibiotics, co-amoxiclav, co-trimoxazole and ciprofloxacin, similar resistance levels and a similar trend was found as observed in isolates from primary care and OPD patients.
- For *P. aeruginosa*, resistance to ceftazidime, piperacillin/tazobactam and ciprofloxacin were 3%, 6% and 9%, respectively. Empirical treatment with ceftazidime when infections are potentially caused by *P. aeruginosa* remains therefore adequate.
- Resistance in *Acinetobacter* spp. for all tested antibiotics remained low at 5% or less.

S. aureus, β-haemolytic Streptococcus spp. groups A, B and C/G
- In *S. aureus* resistance was high for clindamycin (including inducible resistance, 17%) and increased significantly over the last five years. This indicates that culture and susceptibility testing are advised before starting treatment with this drug.
- 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 8%, respectively. Resistance to doxycycline/tetracycline increased over the last five years and was 24% in 2023. This might be due to the increased use of tetracyclines in hospitals over the last years.
- In β-haemolytic *Streptococcus* spp. group B and C/G, resistance levels for clindamycin (18% group B, 15% group C/G), and erythromycin (22% group B, 16% group C/G) were higher than for group A.

S. pneumoniae and Haemophilus influenzae
- In *S. pneumoniae*, resistance to β-lactam antibiotics was less than 1%. Resistance to doxycycline/tetracycline and macrolides was 10% hospital patients, which was lower than in GP patients, most probably due to the more frequent use of tetracyclines in primary care than in hospitals.
- In *H. influenzae*, resistance to amoxicillin and co-amoxiclav was 29% and 11%, respectively. Resistance to ciprofloxacin (4%) and doxycycline/tetracycline (2%) was lower.

Intensive Care Units

Enterobacterales, P. aeruginosa and Acinetobacter spp.
- For *E. coli*, *K. pneumoniae* and *P. aeruginosa*, resistance levels for β-lactam antibiotics were higher in intensive care unit (ICU) patients than in isolates from non-ICU patients.
- In *K. pneumoniae*, resistance to cefuroxime and ceftriaxone was 18% and 11%, respectively and remained stable over the last five years. In *E. coli* resistance to cefuroxime and ceftriaxone was 16% and 10%, respectively.
- A worrisome increase in resistance to meropenem in *K. pneumoniae* was found. Resistance in ICUs only increased from 0.3% to 1.5% over the last five years.
- In *P. aeruginosa* isolates from ICU patients, resistance to ceftazidime and piperacillin/tazobactam was 9% and 13%, respectively, 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.
- Resistance in *Acinetobacter* spp. in ICU patients was higher than for non-ICU patients but still remained low for all recommended antibiotics at 6% or less.

S. aureus

- MRSA percentage in ICU patients increased to 4% over the last five years. The MRSA level was higher than in non-ICU patients.

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 (27%) in *E. coli* from admitted patients remains a problem.
- Resistance to cephalosporines in *E. coli* and *K. pneumoniae* isolates from admitted patients is stable but higher than in other departments (17% and 11% to cefuroxime and ceftriaxone in *E. coli* and 18% and 13% to cefuroxime and ceftriaxone in *K. pneumoniae*, respectively).
- Resistance to ciprofloxacin in *P. aeruginosa* in admitted patients has increased from less than 10% to 16% over the last five years.

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%).

Highly resistant micro-organisms and specific pathogens

Extended spectrum β-lactamases

- From 2019 to 2023, the proportion of extended spectrum β-lactamase (ESBL) *E. coli* was stable in GP (4%), outpatient departments (6%), inpatient departments (6%) and ICUs (9%).
- From 2019 to 2023, the proportion of ESBL *K. pneumoniae* was stable in GP (5%), OPD (8%) and inpatient departments (9%).
- In the ICUs, ESBL *K. pneumoniae* showed a peak in proportions during the COVID-19 years and is decreasing in 2022-2023 (11%).

Carbapenem-resistant and carbapenemase-producing Enterobacterales

- The prevalence of CRE/CPE confirmed isolates among *E. coli* was 0.05% in 2023 which was comparable to previous years. Carbapenem resistance in *K. pneumoniae* is significantly higher compared to *E. coli*, and has slightly increased from 0.2% in 2020 to 0.4% in 2023 for all healthcare settings combined.
- In 2023, the number of carbapenemase-producing Enterobacterales isolates submitted to the RIVM (n=579) was considerably higher than in previous years (n=484 in 2022, n=245 in 2021, n=226 in 2020 and n=398 in 2019).

- The most frequently identified carbapenemase encoding genes in Enterobacterales were *bla*$_{OXA-48}$, *bla*$_{OXA-48}$-like, *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$.
- In 48% of patients, there is a relation with hospitalization abroad for more than 24 hours during the preceding two months (10% and 61% among persons with a diagnostic and screening isolate, respectively), which was lower than in 2022 but comparable to the years before 2022. Turkey, Ukraine, Egypt, and Morocco are the countries that are most often reported.

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

- In 2023, 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 (8%). On average, 20% of the isolates produced carbapenemase.
- The proportion of carbapenem-resistant *P. aeruginosa* in ICU patients returned to pre-COVID-19 levels in 2022 and remained stable in 2023, after two years during the COVID-19 pandemic in which this proportion was remarkably lower.
- The predominant (41%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was *bla*$_{NDM-1}$. In contrast, in 2021 the dominant carbapenemase encoding allele in CPPA was *bla*$_{VIM-2}$.

Carbapenem-resistant *Acinetobacter baumannii-calcoaceticus* complex

- In 2023, in ISIS-AR, 2% of *A. baumannii-calcoaceticus* complex in diagnostic isolates were resistant to carbapenems (1% in GP and outpatient departments, 5% in inpatient departments and ICUs).
- The *A. baumannii-calcoaceticus* complex population in the Netherlands was very diverse with a high variety of intrinsic and acquired carbapenemase encoding genes.
- The predominant (38%) carbapenemase-encoding gene combination was *bla*$_{OXA-23}$ and *bla*$_{OXA-66}$, and 12% (7/58) 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 2019-2023 was still at a low level of 3%. A higher proportion of 4% was seen for cultures requested by GPs and for ICUs.
- Percentages for MRSA obtained from diagnostic samples were quite stable over time, except in ICU patients in which the prevalence increased from 2% to 4% between 2019 and 2023.
- LA-MRSA MC0398 is no longer the predominant MRSA clade. With MC0008, MC0005 and MC0398 constituting 14%, 13% and 13% of the MRSA isolates in 2023, the most frequently identified MLVA-complexes are more equally distributed.
- In 2023, 40% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 18% of the screening isolates were PVL-positive.
- Although overall less outbreaks were reported to SO-ZI/AMR, a high number of MRSA outbreaks in hospitals was reported in 2023. Almost half of them were related to paediatric or neonatal wards.

Vancomycin-resistant *E. faecium*

- The number of reported hospital outbreaks with vancomycin resistant *Enterococcus faecium* (VRE$_{fm}$) in 2023 was comparable to 2022 and 2021, but still lower compared to 2019, which was probably related to the COVID-19 pandemic.
- The proportion of VRE in infection-related isolates with *E. faecium* in various healthcare settings is still low and varies marginally below 1%.

- The absolute number of positive screening VRE_{fm} isolates continued to increase to above the pre-COVID-19-period. The absolute number of diagnostic isolates does not show an increase.

SO-ZI/AMR

- In 2023, 44 outbreaks were reported to SO-ZI/AMR. This number was higher than in 2020-2022 (34, 27 and 36 in 2020, 2021 and 2022 respectively), but still lower than in 2017-2019, when around 60 outbreaks were reported each year.

Helicobacter pylori

- Although probably biased towards higher resistance levels, resistance was high for levofloxacin (24%), clarithromycin (57%) and metronidazole (52%).
- Resistance to amoxicillin/ampicillin traditionally has been low but has increased substantially to 10% over the last five years. Resistance to doxycycline/tetracycline remained low (1%).
- For the treatment of *Helicobacter pylori* infections, first choice combination treatment consists of amoxicillin and clarithromycin, of which combined resistance was 6% in 2023. If treatment fails, a combination of tetracyclin plus metronidazole or amoxicillin plus metronidazole is recommended. Both have combined resistance levels of less than 10%.

Neisseria meningitidis

- The number of invasive meningococcal disease isolates is still 35% lower in 2023 compared to 2019 (pre-COVID-19) but has more than tripled compared to the lowest recorded number in 2021.
- Penicillin resistance in *Neisseria meningitidis* isolates is rare in the Netherlands.

Neisseria gonorrhoeae

- No resistance to ceftriaxone, the current first-line treatment for gonorrhoea, has been reported. However, the MIC distribution has shifted towards higher MICs since 2019.
- Resistance to ciprofloxacin yearly increases and more than doubled since 2016, to 63.1% in 2023, despite the fact that ciprofloxacin is not prescribed for gonorrhoea, according to guidelines.
- Azithromycin resistance levels increased from 2.2% in 2014 to 31% in 2023.

Mycobacterium tuberculosis

- In 2023, there was an increase of 11.5% of tuberculosis cases in the Netherlands compared to 2022.
- Resistance to the first-line antibiotics to treat tuberculosis remained almost stable over the last years (any form of resistance in 9.1% of the isolates tested), although there may be somewhat larger fluctuations in isoniazid resistance.
- The number of multi-drug resistant (MDR) isolates was stable in recent years (average of 10 cases per year), in 2023 there was a slight increase in MDR cases to 16 (3.1% of culture confirmed cases).

Anaerobes

- The breakpoints of antibiotics for anaerobic bacteria have been extensively modified by EUCAST and are currently more adapted to the different anaerobic genera, each having its own characteristics.
- Metronidazole resistance among clinical *Bacteroides* and *Prevotella* isolates remained low (0.6% and 0.9%, respectively).
- The modified breakpoints for meropenem lead to an increased resistance rate among *Bacteroides* isolates.

Clostridioides difficile

- No *Clostridioides difficile* infection (CDI) outbreaks were notified to the national CDI Expert Center in 2023.
- Since COVID-19 an increase in severe CDI cases and community-onset CDI has been seen.
- Vancomycin minimum inhibitory concentration has increased, but no vancomycin resistance was detected in *C. difficile* isolates.
- The newly reported metronidazole-resistant RT955 was not found in the Netherlands.

Aspergillus fumigatus

- Triazole resistance frequency in 2023 was 11.6% in five UMCs and 3.6% in five teaching hospitals, which represents a resistance level similar to previous years.
- Overall, 81.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism, with TR-genotype variants especially frequent in TR_{46} isolates.

2.3 Antibiotic use and antimicrobial resistance in animals

The antibiotic reduction policies in the Netherlands has resulted in 76.4% reduction of sales of Antimicrobial Veterinary Medicinal Products (AVMPs) for veterinary use since 2009, leveling off during recent years. Over the last ten years antimicrobial resistance (AMR) has decreased simultaneously in *E. coli* isolates (indicator for AMR) from most livestock species, levels of resistance stabilised in more recent years. In spite of the reduction in antibiotic use of both classes in poultry continuous high levels of resistance are observed for ciprofloxacin and tetracyclines in *Campylobacter* isolates from humans and poultry. In 2023, the prevalence of ESBLs stabilized in most animal sectors, but unexpectedly increased in dairy cattle and broilers. Colistin resistance remains present at low levels. In 2023, no CPE was detected in samples from livestock or meat.

Antimicrobial use

- In 2023, a total of 117 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is an increase of 4.5% compared to 2022. However, a decrease in sales by 76.4% over the years 2009-2023 is attained (with 2009 considered a reference year by the Dutch Government).
- A small increase in sales of AVMPs in the Netherlands in 2023 is reflected by an overall increase in used mass of active substances as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized veterinary medicinal products.
- 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, because use in monitored AND unmonitored veterinary patients is reflected by overall (total) sales of AVMP's. Use and sales of polymyxins decreased in 2023. The overall decrease since 2011 is 83.4% in sales. Of the fluoroquinolones, 49% of sold mass is applied in sectors not yet monitored like companion animals; an overall decrease of 92.1% since 2011 is observed.

Antimicrobial resistance related to animals

Salmonella from livestock, meat and humans

- In 2023, 1413 clinical *Salmonella* isolates from humans and 726 isolates from non-human sources were tested for antimicrobial susceptibility. Overall, the highest resistance levels were observed for ciprofloxacin (21%), ampicillin (18%), sulfamethoxazole (19%), tetracycline (17%), trimethoprim (9%) and chloramphenicol (6%).

- The highest levels of resistance were observed for monophasic *S.* Typhimurium, *S.* Infantis, *S.* Chester, *S.* Paratyphi B var. Java, and *S.* Typhimurium.
- Notable is the ongoing increase in the resistance against ciprofloxacin among the *S.* Enteritidis (27% in 2023) which is the epidemiologically most relevant serovar among human infections with a primary reservoir in laying hens.
- In contrast to *S.* Enteritidis, the long-term trends for all resistances among *S.* Typhimurium is stable or declining. Epidemiologically, *S.* Typhimurium is the second most relevant serovar with a primary reservoir in pigs.
- In total, 20/1413 (1.4%) human clinical *Salmonella* isolates were found to be ESBL-producing, which is comparable to previous years (1.8% in 2022 and 1.4% in 2021).
- Levels of resistance among isolates from broiler meat were higher than among human clinical isolates: ciprofloxacin 52%, tetracycline 33% and sulfamethoxazole 68%). All three of these show an increasing trend over time while the other resistances tend to decline over time, indicating an increasing source of exposure to resistant *Salmonella*.
- One carbapenemase-producing *Salmonella* (OXA-48) was found in a human *Salmonella* isolate.

Campylobacter jejuni and *C. coli* from livestock, meat and humans

- In 2023, resistance levels in *Campylobacter jejuni* (*C. jejuni*) isolates from caecal samples of broilers and meat thereof remained at a high level for ciprofloxacin (resp. 63% and 71%) and tetracycline (resp. 50% and 61%). These figures all show an increasing trend over time.
- Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, but was present at low levels among *C. jejuni* from veal calves (2.5%) and among *C. coli* from broilers (2.3%) and poultry meat (4.2%). A notably higher level of erythromycin resistance was observed in *C. coli* from veal calves (38%).
- In 2023, again high levels of resistance against ciprofloxacin (61%) and tetracycline (47%) were observed in humans, but were not yet back at pre-COVID-19 pandemic levels. During the COVID-19 pandemic, resistance most likely dropped due to a decrease in travel related campylobacteriosis cases, which is associated with higher resistance proportions. Overall, the resistance levels for ciprofloxacin and tetracycline have been increasing over the last decades.
- Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low (4%).

Pathogenic *E. coli* (STEC/EPEC/aEPEC) from human patients

- In Shiga-toxin producing and Enteropathogenic *E. coli* serotype O157 (STEC/EPEC O157), a decrease in proportions of resistance against ampicillin (4% in 2023), tetracycline (6%), trimethoprim (6%) and sulfamethoxazole (9%) compared to 2022 was observed. The proportion of resistance of ciprofloxacin was higher in human STEC/EPEC non-O157 *E. coli* (6%) than in human STEC O157 (0%).
- In 2023, no ESBL-producing isolates were detected in STEC O157, but resistance to third generation cephalosporins was detected in three out of 262 (1%) STEC/EPEC non-O157 *E. coli* isolates through presence of ESBL genes in two isolates ($bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$). In a third isolate, a mutation in the AmpC promotor region was detected.

Indicator *E. coli* from livestock and meat products

- Amongst indicator *E. coli* obtained from caecal samples of broilers, pigs and veal calves, the levels of resistance stabilised for most antibiotics in the last five years. Resistance in *E. coli* from faecal samples of dairy cattle remained traditionally low.
- Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers (25.6%) in contrast to the low prevalence observed in pigs (1.7%) and veal calves (4.3%) and the complete absence in dairy cattle.
- Resistance to third generation cephalosporins was low or absent (0 - 0.3%) amongst randomly isolated indicator *E. coli* in all animal species.
- In *E. coli* isolates from chicken meat, decreasing levels of resistance were observed with a tendency to flatten in the more recent years.
- Except for the lower fluoroquinolones resistance in retail pork, resistance patterns in *E. coli* obtained from pork were comparable to broiler meat with the highest resistances monitored for ampicillin, trimethoprim, sulfamethoxazole and tetracycline.
- In isolates from bovine meat, levels of resistance are traditionally low with fluctuating percentages below 5% for most antimicrobials tested. In addition, *E. coli* obtained from imported beef showed similar low levels of resistance.

Specific resistances in Enterobacterales (ESBL/pAmpC/CPE/*mcr*) from various sources

- The prevalence of extended-spectrum β-lactamase (ESBL) resistance in randomly selected *E. coli* has been steadily low for several years in all livestock species.
- In contrast to randomly selected *E. coli*, selective culturing revealed an increase of ESBL resistance in dairy cattle and broilers in recent years. In dairy cattle, the prevalence of selectively isolated ESBL-resistant *E. coli* significantly increased over the past five years from 10.3% in 2019 to 18.0% in 2023.
- A significant increase in the prevalence of selectively isolated ESBL-resistant *E. coli* was also observed in broilers over the past four years from 10.2% in 2020 to 20.3% in 2023. However, the increase in broilers is partially attributed to a change in the sampling method from 2022 onwards in accordance with the updated European legislation.
- As in former years, Whole Genome Sequencing of ESBL-resistant *E. coli* shows that genetic clustering is mostly seen within production sectors, although some spill-over appears to occur.
- In 2023, no carbapenemase-producing Enterobacterales (CPE) were detected in livestock.
- As in former years, the prevalence of *mcr* genes encoding for colistin resistance, in *E. coli* was low in livestock and meat.

MRSA from livestock and meat

- In 2023, seven out of 156 of the investigated sheep farms (4.5%) was tested positive for MRSA.

2.4 Antimicrobial stewardship

Since 2014, all hospitals have established antimicrobial stewardship teams (A-teams) responsible for implementing an antimicrobial stewardship program, as recommended by the Dutch Health Care Inspectorate (IGJ) in response to the SWAB's statement to contain antimicrobial resistance.

The most important developments concerning stewardship in 2022 and 2023 are:

- Of the hospitals participating in the yearly A-team survey (70% of all hospitals), 100% had a formal A-team, but only 61% indicated that structural financial support for A-team activities is present. This year one of the newly added activities to the A-team survey was 'antibiotic allergy evaluation and delabeling'; 44% (n=22) of the hospitals indicate including this in their A-team activities.
- Twenty and 18 (~25%) acute care hospitals (2022, total n=72 and 2023, total n=69) extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor for 2022 and 2023, respectively.
- On average, in 59% and 58% of surgical interventions, cefazolin was used as backbone of surgical antimicrobial prophylaxis in 2022 and 2023, respectively.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 82% (range 76-96%) of surgical antimicrobial prophylaxis courses were discontinued on the day of surgery or the day after in both 2022 and 2023.
- Of all patients receiving empirical therapy upon admission, 16% (mean, range 8-25%) was switched to oral treatment within 24 hours in both 2022 and 2023, while 40% (mean, range 22-65%) and 42% (mean, range 22-69%) were switched to oral treatment between 48-96 hours in 2022 and 2023, respectively.

Data validation will enhance and further develop the antimicrobial stewardship monitor. Benchmarking against hospitals of the same type is essential for the improvement of antibiotic stewardship activities within hospitals and therefore relevant for the clinic.

2.5 Implications for public health and health policy

Although one might expect that by 2023 the impact of COVID-19 and the related measures would have gradually diminished, the figures have not y*et al*l returned to the levels seen in 2019 before COVID-19. In 2023, 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 slightly increased in specific healthcare settings. Moreover, the number of carbapenemase-producing Enterobacterales isolates submitted to the RIVM (n=579) was considerably higher than in previous years. For most of the patients there is a relation with recent hospitalization abroad. Fortunately, the number of HRMO outbreaks in healthcare institutes reported to the SO- ZI/AMR was still lower than in 2017-2019.
Overall in 2023, the prevalence of resistance of most pathogens was stable. Resistance percentages among Gram-negative micro-organisms in general practice, outpatient departments and inpatient departments were stable or declining in the previous five years, while resistance percentages on the intensive care

units were generally higher and sometimes increasing. Notably, resistance among Gram-positive micro-organisms are on the rise in the Netherlands, such as clindamycin resistance among *S. aureus*, which warrants special attention for antibiotic stewardship programs and surveillance of these pathogens as well.

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[1], 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[2] 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.
Although the EU incidence of bloodstream infections with MRSA indicated that the EU target had been reached, and the EU incidence of third-generation cephalosporin-resistant *E. coli* bloodstream infections is comparable with 2019 levels after a drop in 2020/2021, the incidence of carbapenem-resistant *K. pneumoniae* increased by over 50% between 2019 and 2023.
Based on the European Antimicrobial Resistance Surveillance Network (EARS-Net) wide variations in the occurrence of antimicrobial resistance across the EU/EEA exist.[3] Several AMR developments should be highlighted. First, although carbapenem resistance remained rare in *E. coli*, almost one third of EU/EEA countries reported carbapenem resistance percentages above 10% in *K. pneumoniae*. In addition, the EU incidence of carbapenem-resistant *K. pneumoniae* bloodstream infections increased by over 50% between 2019 and 2023, which indicates the need to rapidly strengthen prevention and control actions, in the EU and in Member States. There are widely varying estimated incidences and AMR percentages among countries, suggesting that there are further opportunities for reduction. The options for action are aimed at timely and appropriate diagnosis, high standards of infection prevention and control and antimicrobial stewardship. In contrast to the Netherlands, also combined resistance to different antimicrobial groups was high for *K. pneumoniae*, with over 40% of the clinical isolates reported to EARS-Net for 2023 being resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was a frequent occurrence with almost 25% of *K. pneumoniae* being resistant to at least three of the surveyed antimicrobial groups. In *E. coli*, combined resistance to at least three antibiotic groups was lower with a percentage of just above 12% in 2023.
Moreover, a risk assessment[4] 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, with sporadic cases also in the Netherlands in the previous years. 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. Previously, hvKp strains

[1] Naghavi, Mohsen, et al. "Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050." The Lancet 404.10459 (2024): 1199-1226.
[2] Council Recommendation on stepping up EU actions to combat antimicrobial resistance in a One Health approach (europa.eu)
[3] https://www.ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data
[4] https://www.ecdc.europa.eu/en/publications-data/risk-assessment-emergence-hypervirulent-klebsiella-pneumoniae-eu-eea

were primarily found in Asia, were mainly community-acquired, and were only rarely resistant to antibiotics. However, recent reports point to increasing geographic distribution, healthcare association and multidrug resistance. 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.
Finally, the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) described data of susceptibility of *Neisseria gonorrhoeae* in 23 EU/EEA countries in 2022. Although dual ceftriaxone and azithromycin resistance is exceedingly rare in the EU/EEA, the rapidly decreasing azithromycin susceptibility combined with the continued detection of occasional ceftriaxone resistance is still of concern and threatens the effectiveness of treatment and control of gonorrhoea. Also in the Netherlands, azithromycin resistance levels have increased from 2.2% in 2014 to 31% in 2023, and the MIC distribution for ceftriaxone, the current first-line treatment for gonorrhoea in the Netherlands, has shifted towards higher MICs since 2019.

Conclusions and discussion

The data presented in NethMap/MARAN 2024 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals. Although one might expect that by 2023 the impact of COVID-19 and the related measures would have gradually diminished, still, the interpretation of the data is complicated by the wide variety of changes that took place during the pandemic years.
For now, it is important to notice that the total use of antimicrobials described by general practitioners in humans is back to pre-COVID levels and that antimicrobial use in hospitals has increased above pre-COVID levels again. Antimicrobial resistance is stable in most of the clinically important species. The total use of antimicrobials in animals has decreased with over 77% compared to 2009 and antimicrobial resistance has decreased simultaneously among most livestock species. Worldwide, resistance and multidrug resistance in Enterobacterales (most notably *K. pneumoniae*) is of major concern, and needs ongoing close attention. In addition, vigilance is warranted for high resistance percentages among other groups of micro-organisms as well.
In addition to the search and destroy approach to control the spread of HRMO, antimicrobial stewardship programs and A-teams have been implemented universally in Dutch hospitals to further optimize antibiotic prescription practices. 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 National Action Plan on Antimicrobial Resistance 2024-2030[5] 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.

[5] https://www.rijksoverheid.nl/documenten/rapporten/2024/04/30/nederlands-actieplan-voor-het-terugdringen-van-antimicrobiele-resistentie-2024-2030

3
Use of antimicrobials

3.1 Outpatient antibiotic use

Methods

Data on outpatient antibiotic use in the Netherlands in 2023 were obtained from the Foundation for Pharmaceutical Statistics (SFK), The Hague. Antibiotic use was quantified using Defined Daily Doses (DDD) for each ATC-5 code. The SFK collects dispensing data from approximately 90% of Dutch outpatient pharmacies, which serve 93% of the Dutch population. These data are extrapolated to represent 100% coverage, encompassing prescriptions from general practitioners, outpatient hospital services, clinics, and dentists. Data are presented as DDD per 1,000 inhabitants per day (DID). In 2019, the World Health Organization (WHO) revised the DDD definitions for two drug classes: penicillins with extended spectrum and penicillins with β-lactamase inhibitors.[1] From 2019 onwards, the SWAB processed data using the updated DDD definitions. To facilitate comparison of data collected before and after 2019, the 2018 data are presented using both the old and new DDD definitions.

Results

Total outpatient antibiotic use increased by 5.8%, from 8.32 DID in 2022 to 8.80 DID in 2023 (Table 3.1.1). Compared to 2019 (pre-COVID), total outpatient use has increased by 1.4%. Tetracyclines (doxycycline), penicillins with extended spectrum (amoxicillin), nitrofuran derivatives (nitrofurantoin) and macrolides were the most often used antibiotics in outpatient care. Compared to 2022, the use of tetracyclines and β-lactamase resistant penicillins increased the most with a rise in macrolide use as well. Compared to 2019, β-lactamase resistant penicillin and lincosamides (clindamycin) use increased by 21% and 17%, respectively (Figures 3.1.1 and 3.1.2; The gray blocks in the figures indicate the COVID-19 years.). Conversely, the use of trimethoprim (-7%), tetracyclines (-6%), and nitrofurantoin (-6%) declined compared to 2019.

Discussion

Total outpatient systemic antibiotic use has returned to pre-COVID levels, with a similar distribution across antibiotic classes. Slight increases in antibiotics generally considered second-line, such as amoxicillin/clavulanic acid and macrolides are concerning. Amoxicillin is the first choice for treating pneumonia, which has been seen more in 2023 compared to previous years[2] and might explain the 0.08 DID (+7.0%) increase in use as compared to 2019. Meanwhile, the use of tetracycline declined by 0.11 DID, although still the most commonly used antibiotic in the outpatient setting. The use of nitrofurantoin and trimethoprim has shown a consistent downward trend since 2014, which may suggest more prudent prescribing practices for uncomplicated urinary tract infections, as recommended by the NHG guidelines.

Table 3.1.1 Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DID), 2014-2023 (source: SFK)

ATC Group	Therapeutic group	2014	2015	2016	2017	2018	DDD including changes as of 2019 (source: WHO) 2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	2.23	2.25	2.10	1.98	1.94	1.94	1.83	1.54	1.42	1.54	1.72
J01CA	Penicillins with extended spectrum	1.94	2.13	2.08	1.94	2.02	1.35	1.26	0.98	0.98	1.22	1.34
J01CE	Beta-lactamase sensitive penicillins	0.30	0.23	0.24	0.22	0.07	0.07	0.16	0.12	0.13	0.20	0.18
J01CF	Beta-lactamase resistant penicillins	0.44	0.43	0.46	0.46	0.49	0.49	0.48	0.47	0.48	0.53	0.58
J01CR	Penicillins + beta-lactamase-inhibitors	1.55	1.56	1.52	1.42	1.42	0.95	0.93	0.81	0.81	0.92	0.97
J01D	Cephalosporins & carbapenems	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.16	0.14	0.14	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11
J01EE	Sulphonamides + trimethoprim	0.28	0.28	0.28	0.29	0.30	0.30	0.33	0.33	0.33	0.35	0.37
J01FA	Macrolides	1.18	1.20	1.17	1.17	1.22	1.22	1.22	1.13	1.07	1.14	1.20
J01FF	Lincosamides	0.18	0.19	0.20	0.21	0.23	0.23	0.23	0.23	0.24	0.27	0.27
J01GB	Aminoglycosides	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.79	0.77	0.75	0.73	0.73	0.73	0.67	0.64	0.64	0.67	0.69
J01XE	Nitrofuran derivatives	1.40	1.40	1.39	1.36	1.35	1.35	1.30	1.24	1.24	1.23	1.22
J01XX01	Fosfomycin	0.03	0.04	0.05	0.05	0.07	0.06	0.06	0.07	0.07	0.07	0.07
	Others	0.04	0.04	0.02	0.05	0.04	0.04	0.03	0.03	0.03	0.03	0.00
J01	**Antibiotics for systemic use (total)**	**10.58**	**10.72**	**10.44**	**10.06**	**10.06**	**8.90**	**8.68**	**7.77**	**7.61**	**8.32**	**8.80**

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2014-2023 (source: SFK)

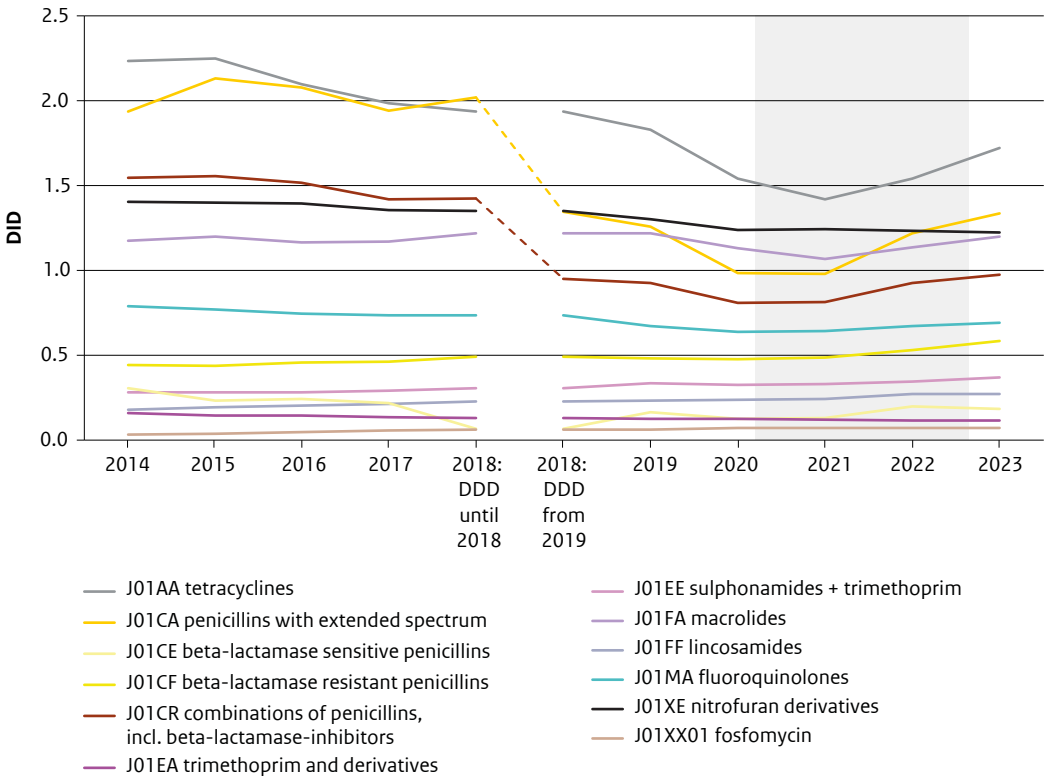
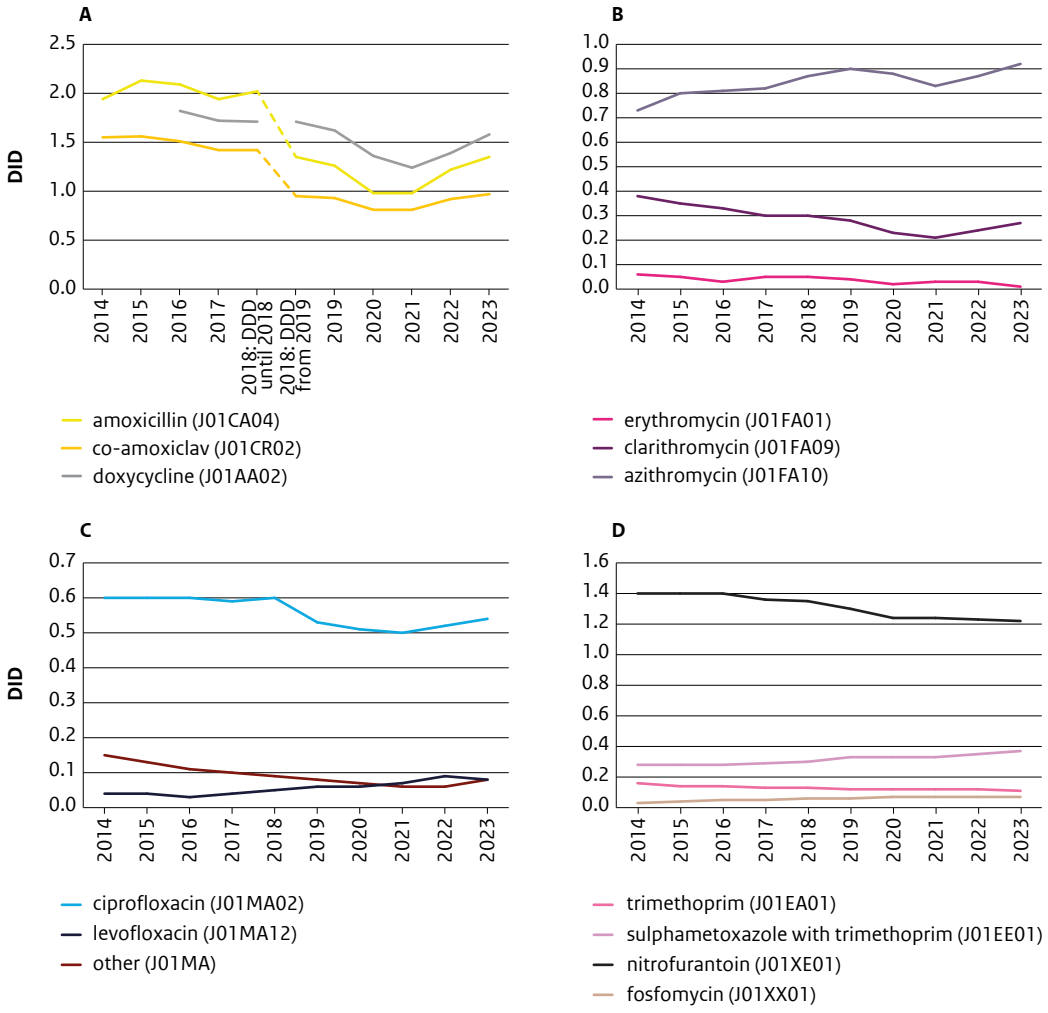


Figure 3.1.2 Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2014-2023 (source: SFK)



Antibiotic prescribing quality indicators in primary care

Introduction

To evaluate the 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). This is the third time NethMap reports these QI outcomes.

Methods

The RIVM yearly 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). Data are available from 2018 to 2023. For the analyses of 2023, GP practices with complete data for 2023 were included (n=686). For the time trend analysis, GP practices with complete data for the period 1 January 2018 – 31 December 2023 were included (n=406). We excluded GP practices with a ≥20% non-explainable difference in the number of registered patients between two consecutive years from 2018 to 2023. The median score for each QI was calculated with the interquartile range (IQR; 25 – 75 percentiles).

The fourteen analysed QIs for primary care are:
General QIs (total prescriptions and percentages of broad-spectrum antibiotics):

- Total number of systemic antibiotic prescriptions/1000 registered patients/year (excluding prescriptions for prophylaxis and/or chronic use)
- Percentage of amoxicillin/clavulanic acid prescriptions from total systemic antibiotic prescriptions
- Percentage of macrolide prescriptions from total systemic antibiotic prescriptions
- Percentage of quinolone prescriptions from total systemic antibiotic prescriptions
- Percentage of amoxicillin/clavulanic acid + macrolide + quinolone prescriptions from total systemic antibiotic prescriptions

Antibiotic prescribing percentages (episodes of specified diagnoses treated with antibiotics/episodes of specified diagnoses) for:

- Otitis media
- Upper respiratory tract infection (URTI)
- Lower RTI (LRTI)
- Impetigo

First choice antibiotic prescribing percentages according to the current Dutch guidelines[3] (episodes with first choice antibiotic prescribed/episodes with any antibiotic prescribed) for:

- Otitis media (amoxicillin)
- Tonsillitis (pheneticillin or phenoxymethylpenicillin)
- Pneumonia (amoxicillin or doxycycline)
- Cystitis in women (nitrofurantoin or fosfomycin)
- Impetigo (flucloxacillin)

Results

In 2023, the median number of antibiotic prescriptions per 1000 patients was 259 (IQR: 226 – 302; range: 115 – 766) (Table 3.1.2). Most QI outcomes show high variability between individual practices with respect to numbers of prescribed antibiotics (QI1) and prescribing quality (other QIs).

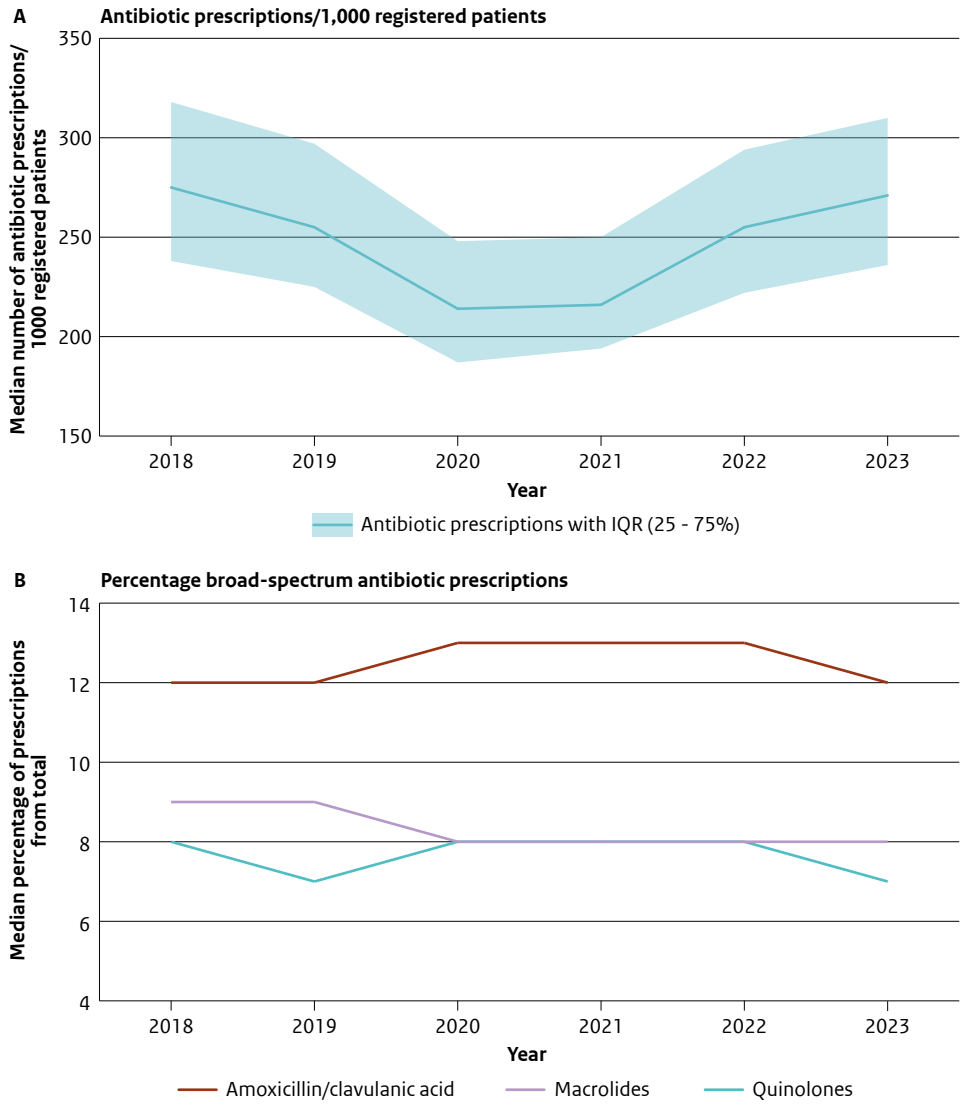
Table 3.1.2 Outcomes of the antibiotic prescribing QIs for GP practices (N=686) in 2023, STIZON

Quality Indicator	Median	IQR (25 - 75%)
General		
1. Antibiotic prescriptions/1000 registered patients	259	226 - 302
2. % Amoxicillin/clavulanic acid	12	11 - 14
3. % Macrolides	7	6 - 10
4. % Quinolones	7	6 - 8
5. % Amoxicillin/clavulanic acid + Macrolides + Quinolones	28	25 - 31
Antibiotic prescribing percentages		
6. Antibiotic prescribing % otitis media	42	35 - 51
7. Antibiotic prescribing % URTI	21	16 - 27
8. Antibiotic prescribing % LRTI	22	18 - 28
9. Antibiotic prescribing % impetigo	28	22 - 36
First choice antibiotic prescribing percentages		
10. % 1st choice antibiotic prescribing otitis media	87	80 - 92
11. % 1st choice antibiotic prescribing tonsillitis	55	38 - 66
12. % 1st choice antibiotic prescribing pneumonia	81	73 - 87
13. % 1st choice antibiotic prescribing cystitis (women)	86	83 - 89
14. % 1st choice antibiotic prescribing impetigo	72	55 - 83

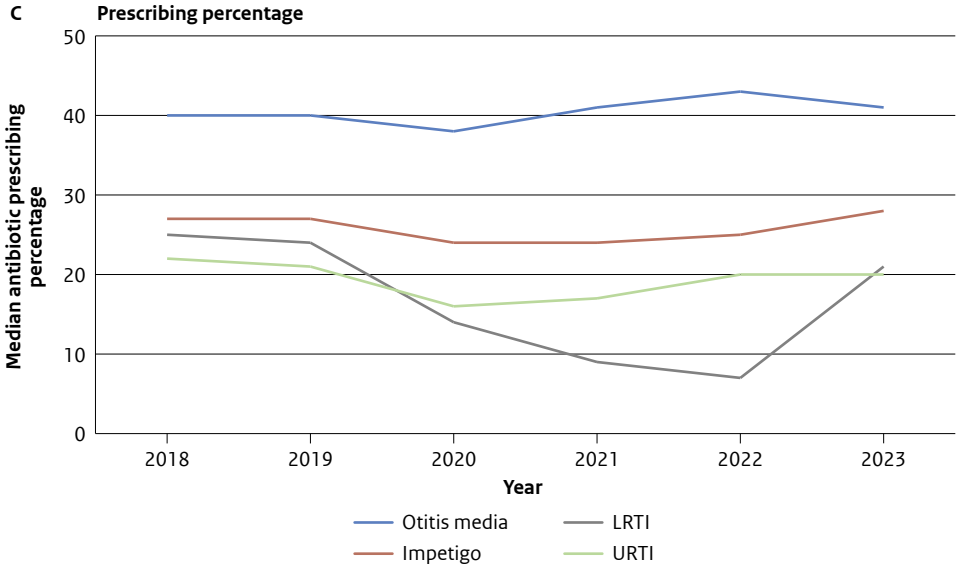
LRTI: Lower respiratory tract infection; URTI: upper respiratory tract infection

Trend analyses from 2018 to 2023 are shown in Figure 3.1.3. A sharp decline in the median number of antibiotic prescriptions per 1000 registered patients per year was seen during the COVID-19 pandemic (Figure 3.1.3A). In 2023, the median number of antibiotic prescriptions increased again to the level observed in 2018. The median percentages of amoxicillin/clavulanic acid, macrolide, and quinolone prescriptions were quite stable during the 2018 – 2023 period (Figure 3.1.3B). The median antibiotic prescribing percentages for otitis media and impetigo were quite stable, while for URTI and LRTI sharp decreases were seen during the COVID-19 pandemic. Prescribing percentage for URTI increased again in 2021 and for LRTI in 2023 (Figure 3.1.3C). The QIs of first choice antibiotic prescribing per clinical indication were stable, or increased over time (Figure 3.1.3D), particularly for impetigo and tonsillitis. First choice prescribing for pneumonia decreased during the COVID-19 pandemic but increased in 2023 to 82%.

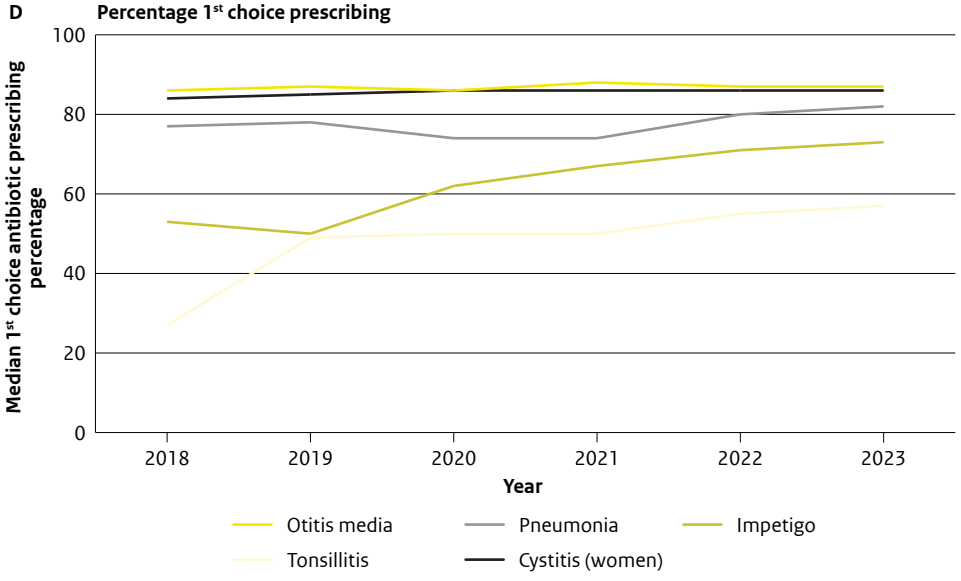
Figure 3.1.3 Trends in outcomes of the antibiotic prescribing QIs in primary care (N=406 GP practices), 2018 – 2023, STIZON. Median number of systemic antibiotic prescriptions/1000 registered patients/ year (A), percentages of amoxicillin/clavulanic acid, macrolide and quinolone prescriptions among total number of systemic antibiotic prescriptions (B), antibiotic prescribing percentages for episodes of otitis media, URTI, LRTI and impetigo (C), and first choice antibiotic prescribing for episodes of otitis media, tonsillitis, pneumonia, cystitis in women and impetigo (D). Please note that the Y-axis has a different range for Figures B, C, and D.



C


Prescribing percentage
Median antibiotic prescribing percentage
50
40
30
20
10
0
2018
2019
2020
2021
2022
2023
Year
Otitis media
LRTI
Impetigo
URTI

D


Percentage 1st choice prescribing
Median 1st choice antibiotic prescribing percentage
100
80
60
40
20
0
2018
2019
2020
2021
2022
2023
Year
Otitis media
Pneumonia
Impetigo
Tonsillitis
Cystitis (women)

Discussion

These QI outcomes provide insight into the antibiotic prescribing quality by GPs in the Netherlands. Data are available to the individual participating GP practices to benchmark their own prescribing behaviour. The feedback is also used in the pharmacotherapeutic consultations (FTO) organised by the regional antimicrobial resistance care networks.

The decrease of the antibiotic prescribing percentage for LRTI in 2020 – 2022 is being investigated and probably relates to an increase in number of episodes (denominator) due to the R83 ICPC code, which is, since the COVID-19 pandemic, also used for coding SARS-CoV-2 and long-COVID.

For next year, numbers of practices for which QI outcomes can be reported are expected to further increase with data from a second database (VIPLive), making the benchmark results even more robust and enabling stratification of results by, for example, region, practices that participated in an FTO or not, and practices with relatively more/less children.

Conclusions

- In 2023, a median of 259 antibiotics were prescribed per 1000 registered patients by GP practices for acute infectious diseases.
- There is wide variation in prescribing habits between GP practices.
- From 2018 to 2023, a sharp decline in the number of antibiotic prescriptions per 1000 registered patients was seen in 2020 and 2021 due to the COVID-19 pandemic, which increased again in 2022 and 2023 to the pre-COVID level. Also, the prescribing percentage for URTI showed a similar trend, while for LRTI the decrease continued in 2022 probably due to a (long-)COVID-19-related increasing denominator.
- These QI outcomes provide insight in the quantity and quality of antibiotic prescribing by GPs in the Netherlands.
- QI outcomes can be an effective tool to benchmark individual prescribing behaviour as part of antimicrobial stewardship activities.

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.

3.2 Inpatient antibiotic use

Methods

Data on antibiotic use in Dutch hospitals for 2023 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 and route of administration, at both the unit and product level.[3] Several changes in DDD definitions were implemented by the WHO in 2019.[1] For these antibiotic groups, DDDs calculated using the old and new WHO definitions are shown for 2018 to enable long-term comparison; the dashed line represents the transition to the new/updated DDD definition.

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. Hospital data and corresponding hospital statistics were used to estimate total inpatient consumption. Hospital-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 2023, complete data from 60 hospital pharmacies were obtained.

Comparison to 2022

Inpatient systemic antibiotic use increased by 4.5%, from 92.1 to 96.3 DDD/100 patient-days (Table 3.2.1). When expressed as DDD/100 admissions, the use of systemic antibiotics increased by 6.3%, reaching 359.5 in 2023 (Table 3.2.1). When measured in DDD/1,000 inhabitant-days (DID), systemic antibiotic use increased by 2.5% to 0.766 (Table 3.2.2).

Most antibiotic classes exhibited an increase, with notable deviations observed for β-lactamase-resistant penicillins (flucloxacillin) and nitrofurantoin. Nevertheless, flucloxacillin remains the most used antibiotic in terms of DDD in the hospital setting. The largest relative increases were seen for tetracyclines and lincosamides.

Comparison to 2019 (pre-COVID)

From 2019 to 2023, there was a 22% increase in DDD/100 patient-days, with most antibiotic classes showing increases except for aminoglycosides, which decreased (Figures 3.2.1 and 3.2.2). The decline in aminoglycosides was primarily due to reduced use of gentamicin (-24%, Figure 3.2.2 V). The largest relative increases were seen in tetracyclines (+65%) and third-generation cephalosporins (+45%). The most pronounced absolute increases were in third-generation cephalosporins (+3.5 DDD/100 patient-days) and β-lactamase-resistant penicillins (+2.9 DDD/100 patient-days).

There was considerable variation in systemic antibiotic use across Dutch hospitals (Figures 3.2.3, 3.2.4, and 3.2.5). However, antibiotic use was comparable between hospital types, with median values for university hospitals at 91, large teaching hospitals at 93, and general hospitals at 89 DDD/100 patient-days (Figure 3.2.4).

Cephalosporins

The most commonly used cephalosporin in university and large teaching hospitals was ceftriaxone (third generation), whereas cefuroxime (second generation) was most often used in general hospitals (Figure 3.2.6).

Antimycotics

Antimycotics were primarily used in university hospitals, at 12.1 DDD/100 patient-days. Of these, 60% were triazole derivatives such as fluconazole and voriconazole (Table 3.2.3).

Discussion

The trend observed in recent years continues: the increase in DDD per 100 patient-days (+4.5%) outpaces the increase in DID (+2.5%). This likely reflects shorter hospital stays coupled with more intensive treatment regimens for individual patients receiving antibiotics during their inpatient stays.

In 2023, there was a notable increase in respiratory infections, particularly *Mycoplasma* pneumonia, for which doxycycline is the antibiotic of choice. This might explain the increased use of tetracyclines.

Flucloxacillin remains the most commonly used antibiotic in terms of DDD. For severe *Staphylococcus aureus* infections such as periprosthetic infections, endocarditis, osteomyelitis, arthritis, and sepsis, it is administered in high-dose regimens of up to 12 grams per day.

Compared to pre-COVID levels, only the use of aminoglycosides has decreased, most probably attributed to changes in empirical treatment guidelines for sepsis and growing awareness of aminoglycoside toxicity.

The large variation in antibiotic use between Dutch hospitals remains difficult to explain. Local practice is likely influenced by guidance from culture results versus choice of empiric antibiotic regimens. Additionally, the average length of stay differs between hospital types (university hospitals 6.4 days, large teaching hospitals 4.7 days, general hospitals 4.2 days). Ensuring uniformity in data delivery and cleaning remains of relevance.

Table 3.2.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2014-2023 (source: SWAB)

ATC group	Therapeutic group	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡	2023‡
J01AA	Tetracyclines	1.90	1.89	1.96	1.97	2.05	2.05	2.10	2.00	1.87	2.83	3.47
J01CA	Penicillins with extended spectrum	8.42	9.24	10.9	10.2	11.1	5.26	4.92	5.01	4.86	6.02	6.34
J01CE	Beta-lactamase sensitive penicillins	2.40	2.39	2.55	2.50	2.26	2.26	2.49	2.60	2.40	2.95	3.13
J01CF	Beta-lactamase resistant penicillins	8.67	7.74	8.73	9.59	10.8	10.8	10.6	12.0	11.3	14.5	13.5
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	14.5	14.3	14.6	14.7	14.5	12.0	10.1	10.6	8.84	10.0	10.5
J01DB	First-generation cephalosporins	4.35	4.59	4.63	5.29	6.43	6.43	6.68	6.55	7.13	7.58	7.80
J01DC	Second-generation cephalosporins	4.98	5.33	5.75	5.87	7.99	7.99	7.99	8.48	6.77	7.63	8.92
J01DD	Third-generation cephalosporins	5.67	5.49	5.95	6.39	6.88	6.88	7.73	9.93	11.41	10.94	11.2
J01DH	Carbapenems	1.65	1.74	1.83	1.98	1.93	1.32	1.41	1.53	1.63	1.69	1.74
J01EA	Trimethoprim and derivatives	0.26	0.26	0.25	0.27	0.23	0.23	0.20	0.23	0.17	0.19	0.15
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.89	1.76	2.13	2.38	2.15	2.15	2.41	3.00	2.59	2.96	3.40
J01FA	Macrolides	2.88	2.74	2.97	2.82	2.66	2.66	2.75	3.18	2.50	2.72	2.93
J01FF	Lincosamides	2.30	2.35	2.45	2.43	2.54	2.54	2.36	2.34	2.08	2.71	3.32
J01GB	Aminoglycosides	3.57	3.66	3.70	3.62	3.76	3.76	3.34	2.97	2.83	2.84	2.95
J01MA	Fluoroquinolones	9.02	8.39	9.15	8.65	8.45	7.67	6.99	7.39	6.57	7.69	8.01
J01XA	Glycopeptides	1.59	1.60	1.62	1.72	1.73	1.73	1.99	2.39	2.46	2.52	2.55
J01XB	Polymyxins	0.19	0.23	0.23	0.24	0.14	0.11	0.15	0.14	0.16	0.19	0.15
J01XD	Imidazole derivatives	2.60	2.58	2.80	3.00	3.20	3.20	3.21	3.28	3.44	3.72	3.97
J01XE	Nitrofuran derivatives	1.55	1.42	1.67	1.73	1.63	1.63	1.40	1.77	1.70	2.00	1.84
J01XX	Other antibacterials *	0.09	0.12	0.13	0.28	0.24	0.24	0.28	0.31	0.31	0.29	0.36
	Others**	0.07	0.07	0.07	0.08	0.10	0.10	0.13	0.10	0.12	0.15	0.15
J01	**Antibiotics for systemic use (total)**	**78.6**	**77.9**	**84.1**	**85.7**	**90.7**	**81.0**	**79.3**	**85.8**	**81.1**	**92.1**	**96.3**
	expressed in DDD/100 admissions:											
J01	**Antibiotics for systemic use (total)**	**326.0**	**330.1**	**326.1**	**340.2**	**339.7**	**303.2**	**318.5**	**333.1**	**303.7**	**338.1**	**359.5**

* *fosfomycin, methenamine, linezolid, daptomycin*

** *J01BA, J01DE, J01DF, J01DI, J01EC and J01XC*

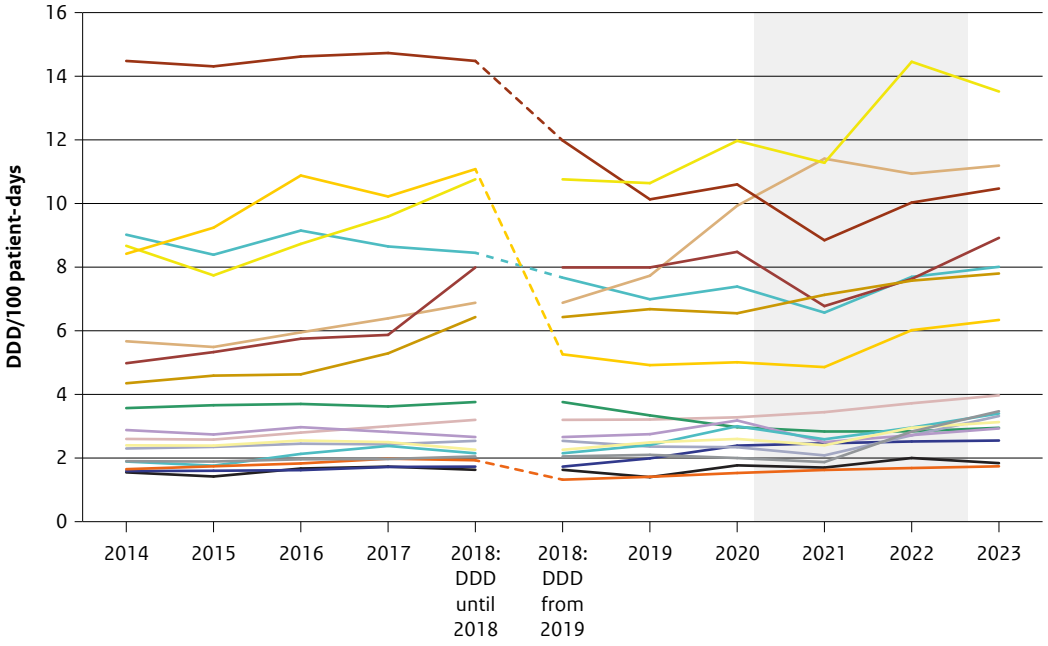
‡ *DDD including changes as of 2019 (source: WHO)*

Table 3.2.2 Ten years data on the use of antibiotics for systemic use (J01) in in-patient hospital care (DDD/1,000 inhabitant-days), 2014-2023 (source: SWAB)

ATC group	Therapeutic group	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡	2023‡
J01AA	Tetracyclines	0.023	0.025	0.022	0.021	0.023	0.023	0.021	0.019	0.016	0.022	0.026
J01CA	Penicillins with extended spectrum	0.101	0.118	0.125	0.117	0.110	0.052	0.063	0.050	0.044	0.048	0.051
J01CE	Beta-lactamase sensitive penicillins	0.028	0.028	0.029	0.029	0.033	0.033	0.024	0.022	0.021	0.024	0.024
J01CF	Beta-lactamase resistant penicillins	0.105	0.097	0.102	0.103	0.105	0.105	0.104	0.103	0.097	0.116	0.108
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	0.187	0.186	0.171	0.159	0.153	0.128	0.109	0.098	0.078	0.083	0.083
J01DB	First-generation cephalosporins	0.052	0.055	0.053	0.065	0.070	0.070	0.066	0.056	0.061	0.060	0.064
J01DC	Second-generation cephalosporins	0.058	0.065	0.066	0.067	0.070	0.070	0.077	0.073	0.063	0.062	0.071
J01DD	Third-generation cephalosporins	0.066	0.067	0.068	0.067	0.072	0.072	0.074	0.085	0.093	0.093	0.091
J01DH	Carbapenems	0.019	0.021	0.020	0.021	0.020	0.014	0.014	0.013	0.014	0.013	0.014
J01EA	Trimethoprim and derivatives	0.003	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.002	0.002	0.001
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.022	0.021	0.024	0.023	0.022	0.022	0.022	0.024	0.021	0.023	0.026
J01FA	Macrolides	0.034	0.034	0.034	0.030	0.030	0.030	0.026	0.027	0.021	0.022	0.023
J01FF	Lincosamides	0.028	0.030	0.028	0.027	0.026	0.026	0.024	0.022	0.018	0.022	0.026
J01GB	Aminoglycosides	0.044	0.046	0.043	0.037	0.037	0.037	0.033	0.027	0.025	0.024	0.024
J01MA	Fluoroquinolones	0.112	0.112	0.106	0.097	0.087	0.079	0.071	0.066	0.057	0.061	0.063
J01XA	Glycopeptides	0.018	0.019	0.019	0.019	0.018	0.018	0.018	0.019	0.020	0.020	0.019
J01XB	Polymyxins	0.002	0.003	0.002	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.002
J01XD	Imidazole derivatives	0.030	0.032	0.032	0.034	0.033	0.033	0.033	0.031	0.031	0.030	0.031
J01XE	Nitrofuran derivatives	0.018	0.018	0.018	0.019	0.017	0.017	0.015	0.016	0.014	0.017	0.014
J01XX	Other antibacterials*	0.001	0.002	0.002	0.003	0.003	0.003	0.003	0.003	0.003	0.002	0.003
	Others**	0.000	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**0.953**	**0.982**	**0.968**	**0.942**	**0.934**	**0.836**	**0.799**	**0.760**	**0.700**	**0.747**	**0.766**

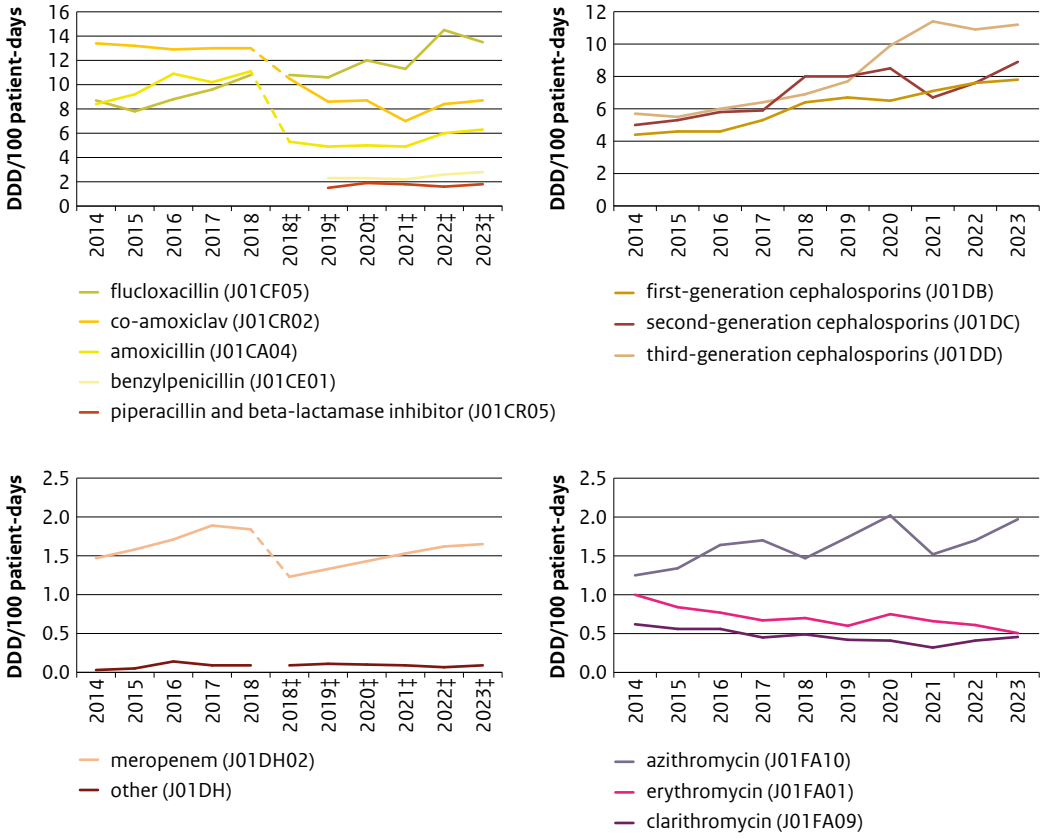
* *fosfomycin, methenamine, linezolid, daptomycin*
** *J01BA, J01DE, J01DF, J01DI, J01EC and J01XC*
‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.2.1 Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days) at ATC-4 level, 2014-2023 (source: SWAB)



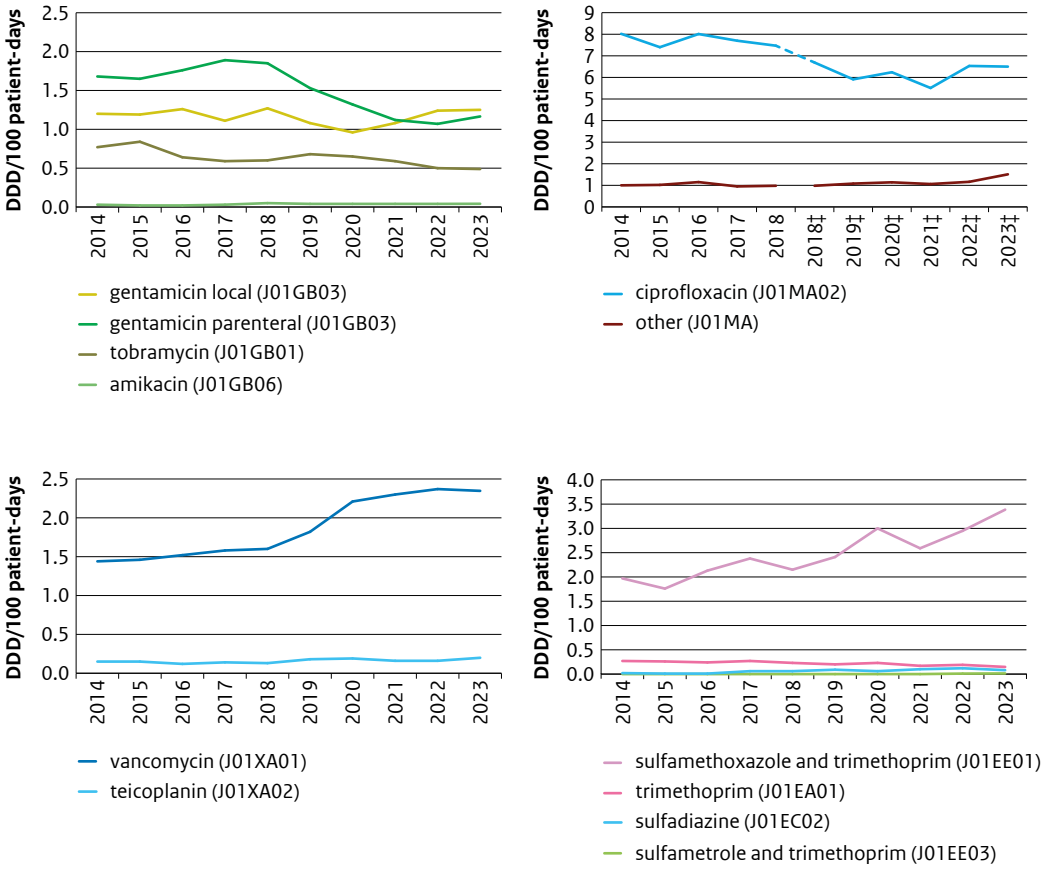
- J01AA tetracyclines
- J01CA penicillins with extended spectrum
- J01CE beta-lactamase sensitive penicillins
- J01CF beta-lactamase resistant penicillins
- J01CR combinations of penicillins, incl. beta-lactamase-inhibitors
- J01DB first-generation cephalosporins
- J01DC second-generation cephalosporins
- J01DD third-generation cephalosporins
- J01DH carbapenems
- J01EE combinations of sulfonamides and trimethoprim, incl. derivatives
- J01FA macrolides
- J01FF lincosamides
- J01GB aminoglycosides
- J01MA fluoroquinolones
- J01XA glycopeptides
- J01XD imidazole derivatives
- J01XE nitrofuran derivatives

Figure 3.2.2 Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days 2014-2023 (source: SWAB)



For antibiotics where the DDD was changed by the WHO in 2019, a dashed line is depicted from the DDD/100 patient-days in 2018 calculated using the DDD until 2018 to the DDD/100 patient-days in 2018 calculated using the DDD from 2019.
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.2.2 (continued) Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days 2014-2023 (source: SWAB)



For antibiotics where the DDD was changed by the WHO in 2019, a dashed line is depicted from the DDD/100 patient-days in 2018 calculated using the DDD until 2018 to the DDD/100 patient-days in 2018 calculated using the DDD from 2019.
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.2.3 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2023 (source: SWAB)

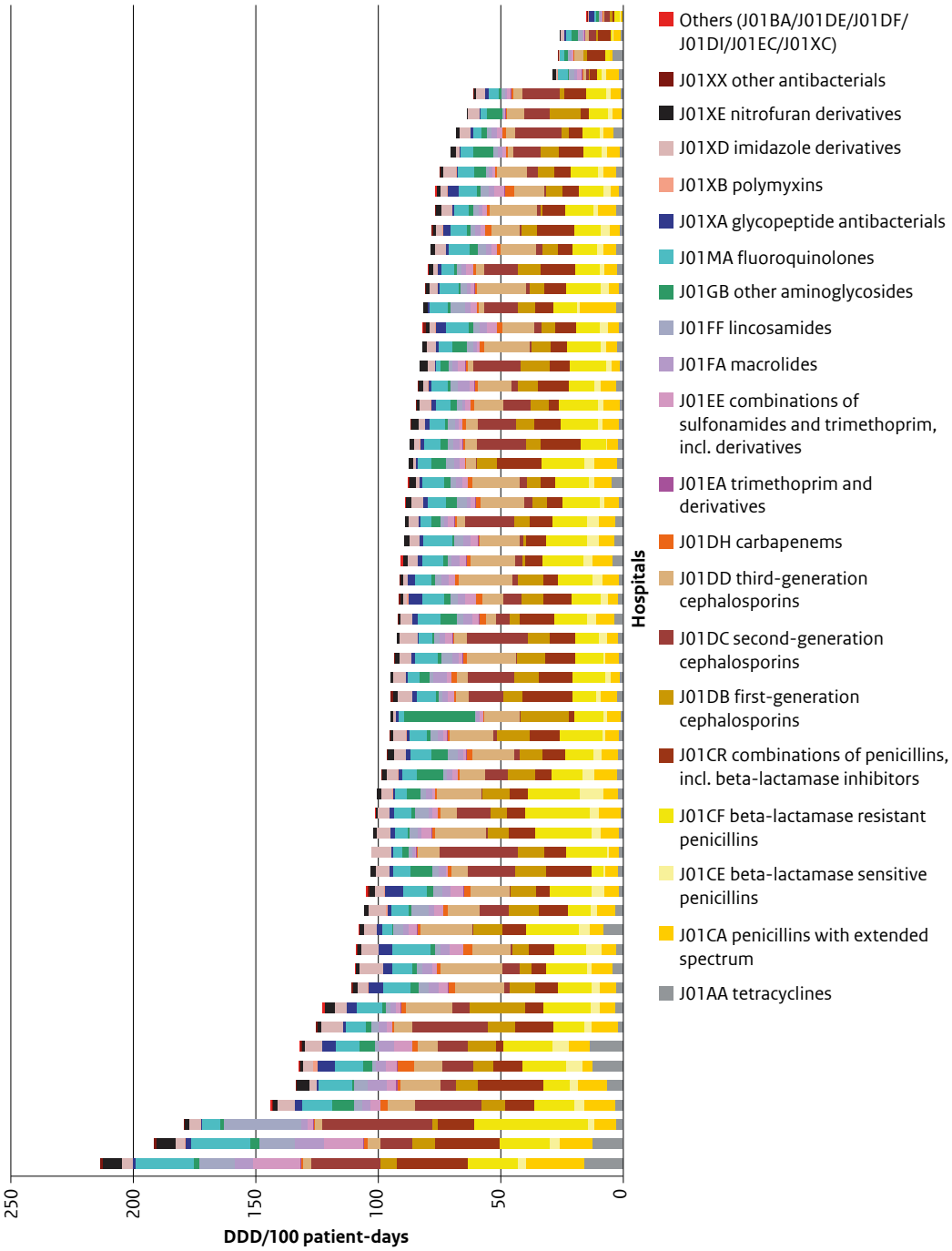
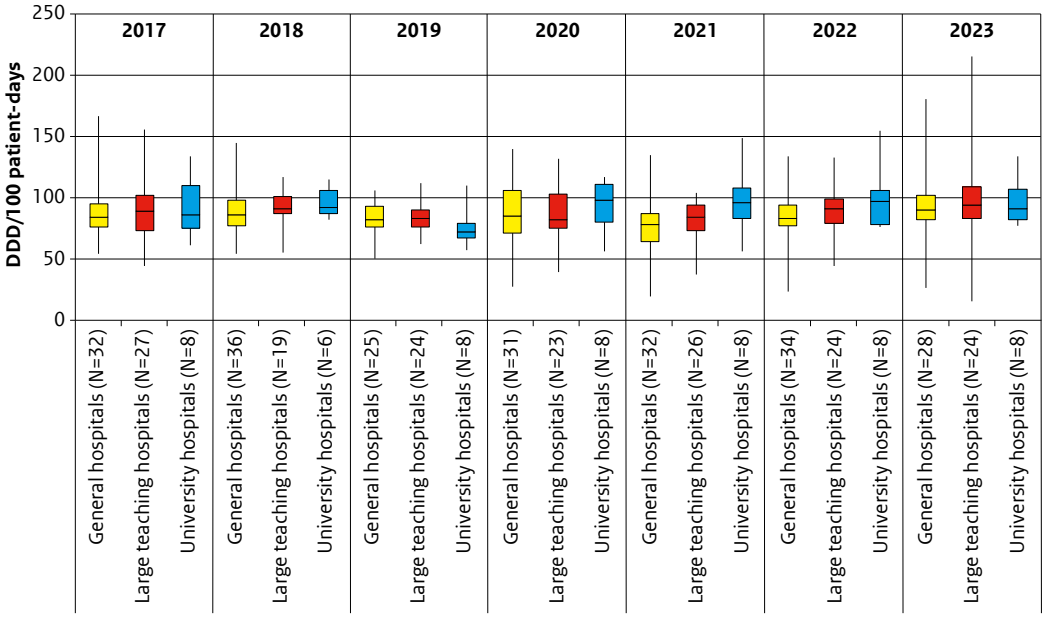


Figure 3.2.4 Seven years use of total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals (source: SWAB)



boxplot shows minimum - P25 - median - P75 - maximum

Figure 3.2.5 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2023 (source: SWAB)

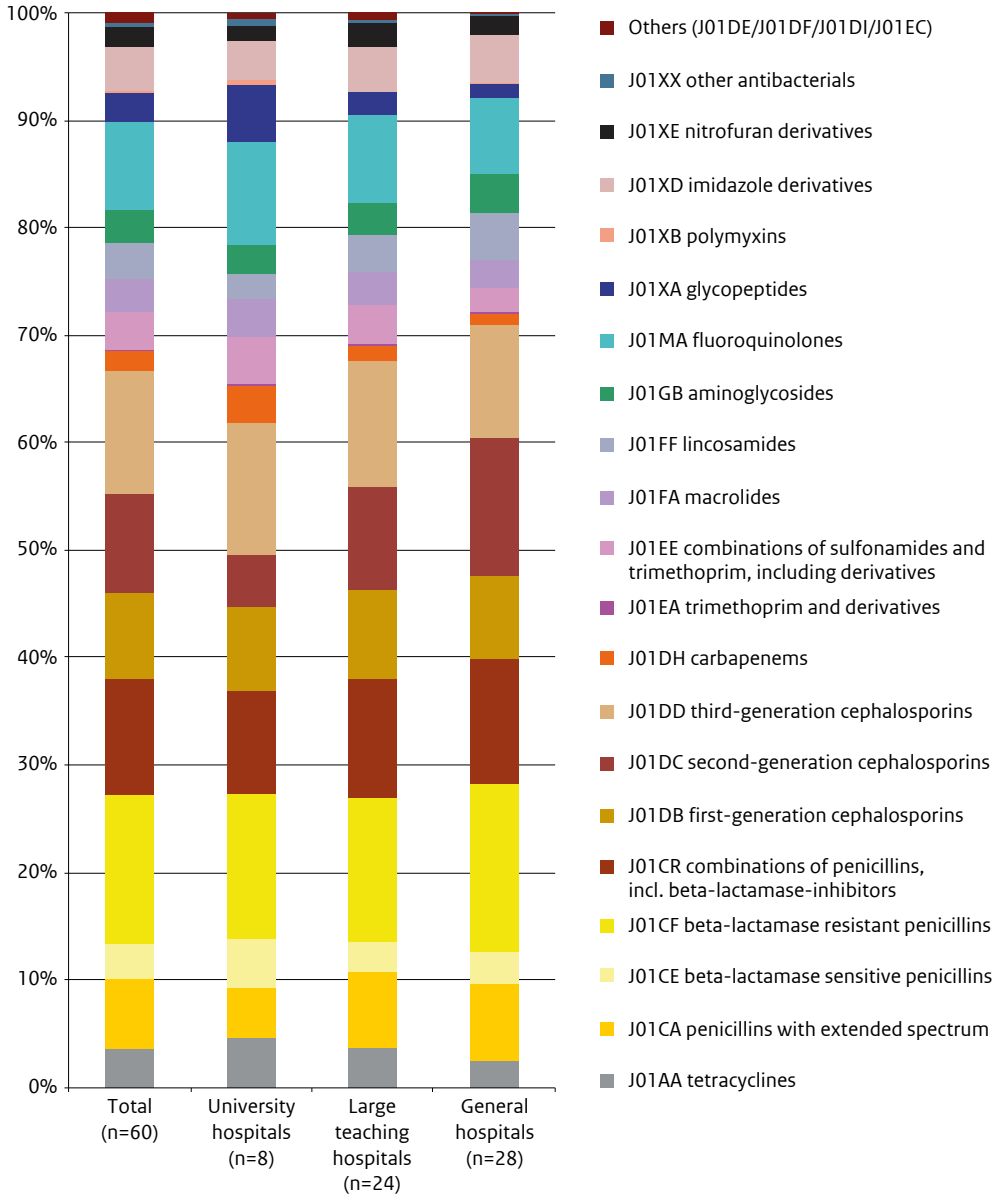


Figure 3.2.6 Use of 1st, 2nd and 3rd generation cephalosporins in university, large teaching and general hospitals at ATC-5 level (2014-2023) (source: SWAB)

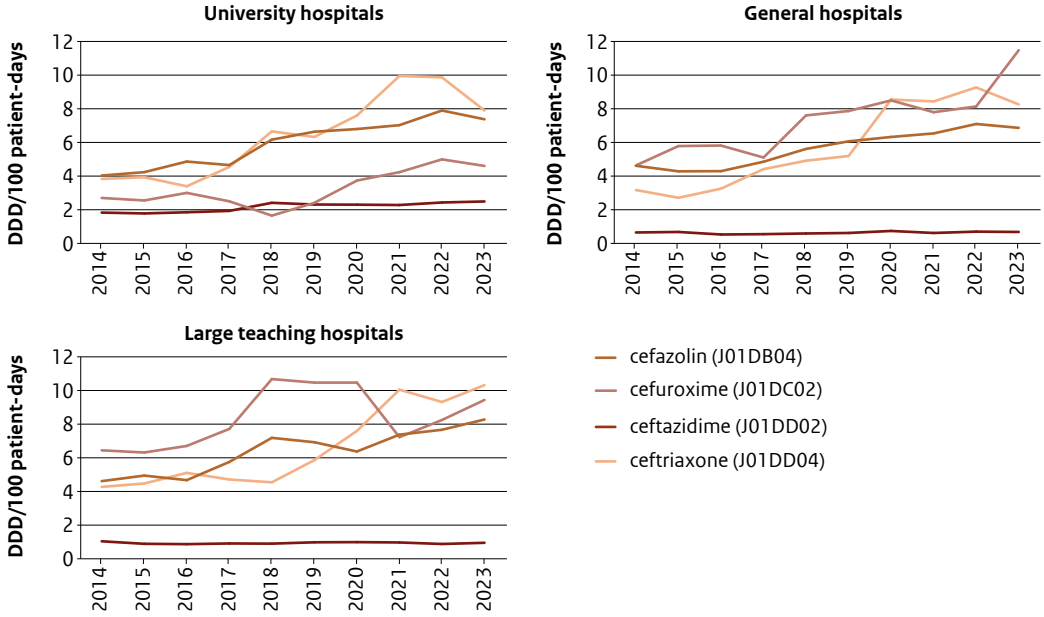


Table 3.2.3 Use of antimycotics (J02) in hospitals (DDD/100 patient-days), 2019-2023 (source: SWAB)

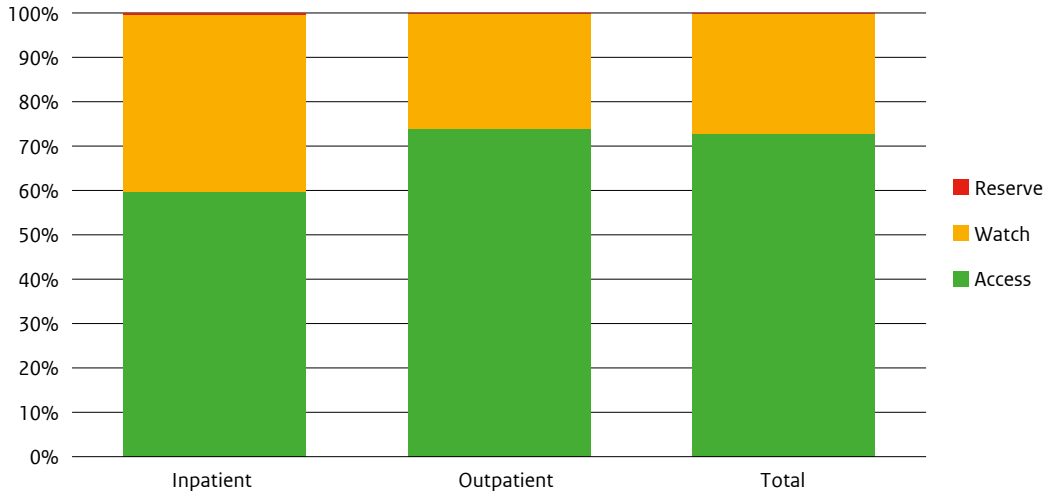
		2019				2020				2021			
ATC group	Therapeutic group	total N=57	academic hospitals N=8	large teaching hospitals N=24	general hospitals N=25	total N=62	academic hospitals N=8	large teaching hospitals N=31	general hospitals N=23	total N=66	academic hospitals N=8	large teaching hospitals N=26	general hospitals N=32
J02AA01	Antibiotics (amphotericin B)	1.12	3.10	0.68	0.29	1.36	3.42	0.95	0.33	1.23	3.03	0.90	0.41
J02AB02	Imidazole derivatives (ketoconazole)	0.03	0.06	0.04	0.01	0.01	0.03	0.00	0.01	0.02	0.05	0.01	0.01
J02AC	Triazole derivatives	3.26	7.12	2.42	1.63	3.72	8.16	2.77	1.63	3.64	7.00	3.09	1.95
J02AX	Other antimycotics for systemic use	0.53	1.08	0.43	0.27	0.63	1.03	0.54	0.44	0.74	1.08	0.71	0.54
J02	**Antimycotics for systemic use (total)**	**4.95**	**11.4**	**3.57**	**2.20**	**5.71**	**12.6**	**4.27**	**2.42**	**5.63**	**11.2**	**4.71**	**2.91**

		2022				2023			
ATC group	Therapeutic group	total N=63	academic hospitals N=7	large teaching hospitals N=23	general hospitals N=33	total N=60	academic hospitals N=8	large teaching hospitals N=24	general hospitals N=28
J02AA01	Antibiotics (amphotericin B)	1.00	3.40	0.55	0.30	1.07	2.94	0.69	0.20
J02AB02	Imidazole derivatives (ketoconazole)	0.02	0.07	0.00	0.00	0.02	0.03	0.02	0.00
J02AC	Triazole derivatives	3.27	7.49	2.63	1.79	3.73	7.34	3.39	1.25
J02AX	Other antimycotics for systemic use	0.68	1.73	0.49	0.37	0.78	1.77	0.55	0.36
J02	**Antimycotics for systemic use (total)**	**4.97**	**12.7**	**3.67**	**2.46**	**5.60**	**12.1**	**4.65**	**1.81**

3.3 AWaRe classification

The AWaRe classification is an internationally used system that serves as a tool for tracking antibiotic consumption, setting targets, and assessing the impact of stewardship policies aimed at optimizing antibiotic use and reducing antimicrobial resistance. The goal at the national level is for at least 60% of total antibiotic consumption to consist of Access group antibiotics.
Applying the WHO AWaRe classification[4], 73.8% of antibiotics used by outpatients in the Netherlands fell into the Access category, 25.9% into the Watch category, and 0.2% into the Reserve category (Figure 3.3.1). For inpatient use, these percentages were 59.7%, 39.8%, and 0.5%, respectively. Overall, 72.1% of antibiotics used in the Netherlands were in the Access category. The most frequently used reserve antibiotics were colistin, linezolid, daptomycin, ceftazidime/avibactam, and aztreonam.

Figure 3.3.1 Antimicrobial consumption according to AWaRe classification in 2023



3.4 Long-term care facilities

Methods

All hospital pharmacists participating in the SWAB surveillance were asked to provide antibiotic consumption data for the long-term care facilities (LTCFs) served by their pharmacies. For each facility, the DDD/1,000 residents/day was calculated, assuming 100% occupancy, with a weighted mean based on capacity.

Results

Data were obtained from 15 LTCF serving 9,637 residents. The LTCFs had a mean of 642 residents, varying from 54 to 2,000 per facility or organization. Antibiotic use in LTCFs increased from 43.9 in 2022 to 52.0 DDD/1,000 residents/day in 2023. The most commonly used antibiotics were combinations of penicillins (amoxicillin with clavulanic acid), fluoroquinolones, and nitrofurantoin (Table 3.4.1). Among the LTCFs, the DDD/1,000 residents/day ranged from 9.2 to 111.

Discussion

In 2023, the total use of antibiotics and their distribution across classes in LTCFs was similar to pre-pandemic levels. The frequent use of amoxicillin with clavulanic acid, fluoroquinolones, and nitrofurantoin is not surprising, as urinary tract infections are one of the most common infections among elderly patients. However, the high use of broad-spectrum fluoroquinolones remains concerning.

Table 3.4.1 Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, (expressed as weighted mean) DDD/1,000 residents/day, 2014-2023 (source: SNIV & SWAB)

ATC group	Therapeutic group	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡	2023‡
J01AA	Tetracyclines	4.7	3.9	4.9	4.0	5.0	5.0	3.7	2.9	2.6	2.3	2.4
J01CA	Penicillins with extended spectrum	5.1	5.0	5.6	4.6	3.8	2.4	2.6	4.8	2.4	2.9	3.6
J01CE	Beta-lactamase sensitive penicillins	0.5	0.7	0.3	0.6	0.4	0.4	0.5	0.4	0.3	0.3	0.5
J01CF	Beta-lactamase resistant penicillins	1.4	2.3	1.8	2.2	3.3	3.3	3.0	2.5	2.4	2.9	3.0
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	16.3	17.9	16.1	15.5	18.0	12.1	12.0	10.2	7.7	10.7	10.9
J01DB	First-generation cephalosporins	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.2	0.3	0.1	0.1
J01DC	Second-generation cephalosporins	0.1	0.2	0.1	0.3	0.1	0.1	0.2	1.0	0.1	0.1	0.1
J01DD	Third-generation cephalosporins	0.6	0.8	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.6	0.4
J01DH	Carbapenems	0.0	0.1	0.0	0.1	0.1	0.1	0.3	0.1	0.0	0.1	0.1
J01EA	Trimethoprim and derivatives	1.9	1.4	1.6	1.6	1.2	1.2	0.8	1.2	1.0	0.7	0.9
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.5	1.6	1.1	1.2	1.9	1.9	3.0	2.6	1.9	1.7	2.0
J01FA	Macrolides	1.8	2.1	2.4	2.8	2.7	2.7	2.7	3.0	2.3	3.3	3.7
J01FF	Lincosamides	2.0	2.6	3.7	2.9	3.0	3.0	2.9	2.2	1.8	2.2	2.8
J01GB	Aminoglycosides	0.2	0.2	0.1	0.3	0.1	0.1	0.0	0.0	0.0	0.1	0.1
J01MA	Fluoroquinolones	8.4	8.9	8.2	6.9	8.7	8.7	7.3	9.1	5.5	6.7	7.8
J01XA	Glycopeptides	0.1	0.2	0.1	0.2	0.2	0.2	0.4	0.1	0.2	0.1	0.1
J01XB	Polymyxins	0.0	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.1	0.0
J01XD	Imidazole derivatives	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
J01XE	Nitrofuran derivatives	10.4	11.4	9.6	8.3	11.3	11.3	9.5	8.2	8.1	8.0	7.6
J01XX	other antibacterials*	0.2	0.5	0.8	0.8	0.7	0.7	0.9	1.4	0.8	0.8	0.9
	others**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
J01	**Antibiotics for systemic use (total)**	**55.3**	**60.0**	**57.2**	**52.9**	**61.4**	**53.9**	**50.4**	**50.4**	**38.1**	**43.9**	**52.0**

* *fosfomycin, methenamine, linezolid, daptomycin*
** *J01DF, J01DI, J01EC and J01XC*
‡ *DDD including changes as of 2019 (source: WHO)*

References

1 WHO Collaborating Centre for Drug Statistics Methodology. DDD alterations from 2005-2024. Available from: https://www.whocc.no/atc_ddd_alterations__cumulative/ddd_alterations/ [Accessed September 27th, 2024]

2 Toename pneumonieën, atypische verwekker. Available from: https://www.nhg.org/actueel/toename-pneumonieen/ [Accessed September 27th, 2024]

3 WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2020. WHO Collaborating Centre; Oslo, Norway, 2020.

4 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
Surveillance of resistance

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

4.1.1 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 2023, 48 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 48 laboratories, 37 provided complete data on the last five years (2019 to 2023). Five of these 37 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 37 laboratories to avoid bias in time trends due to incomplete data.

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 4.2) and long-term care facilities (section 4.4), 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 urine isolates in general practice patients were calculated separately for patients aged ≤12 years and patients aged >12 years. For analyses on data from outpatient departments (section 4.3.1), inpatient departments (excl. intensive care units, section 4.3.2), and intensive care units (section 4.3.3), 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 4.3.4). For urology departments (section 4.3.5), we selected only urine isolates. In section 4.5, we show resistance levels for respiratory pathogens (*Streptococcus 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. Finally, in section 4.6 we show resistance levels for *Helicobacter pylori*, for which we selected isolates from gastro-intestinal mucosa, pus, and normally sterile tissue or liquid as we could not distinguish gastric specimens specifically.

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 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 4.2 through 4.4, *S. argenteus* comprised 0 to 0.06% of the isolates from this complex. *S. schweitzeri*, the third member of the *S. aureus* complex, was found 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 according to EUCAST breakpoints version 13.1 (2023). 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 13.1, including the test values that were within the ATU, trusting that laboratories conducted and reported re-tests if indicated. Nevertheless, 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. However, 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 13.1 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 4.2 through 4.4, 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. 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 approach 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).

Because data on tests for inducible clindamycin were often not available in ISIS-AR, we calculated resistance levels for clindamycin including inducible resistance in *Staphylococcus* spp. and *Streptococcus* spp. 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 considered the isolate as resistant to clindamycin. If no data on a test for inducible clindamycin test were available, but the laboratory reported the isolate resistant we assumed that results of the inducible resistance test was taken into account and considered the isolate as resistant.

We estimated 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 laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/ oxacillin.

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 4.1.1.1. 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 4.1.1.2.

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 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, 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 because *S. aureus* can be susceptible for doxycyclin while being resistant to tetracyclin, the resistance to doxycyclin/tetracyclin can not be used as a proxy for the resistance to doxycyclin, since that might be an overestimation.

For Gram-negative bacteria except *Enterobacter cloacae* complex and *Acinetobacter* spp., we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (for Enterobacterales: co-amoxiclav + gentamicin, cefuroxime + gentamicin, cefotaxime/ceftriaxone + gentamicin, co-amoxiclav + ciprofloxacin, cefuroxime + ciprofloxacin, and cefotaxime/ceftriaxone + ciprofloxacin; for *P. aeruginosa*: ceftazidime + tobramycin and ciprofloxacin + tobramycin; for *H. pylori* clarithromycin + metronidazole, amoxicillin/ampicillin + clarithromycin, and amoxicillin/ampicillin + 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 for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole combined.

Figure 4.1.1.1 Flowchart depicting the algorithm used to calculate resistance to β-lactam antibiotics in *S. pneumoniae*

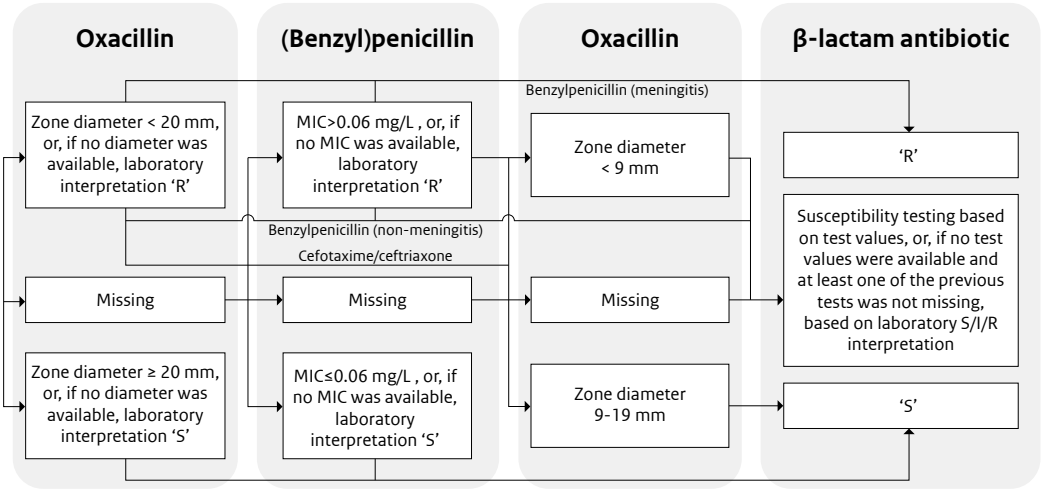
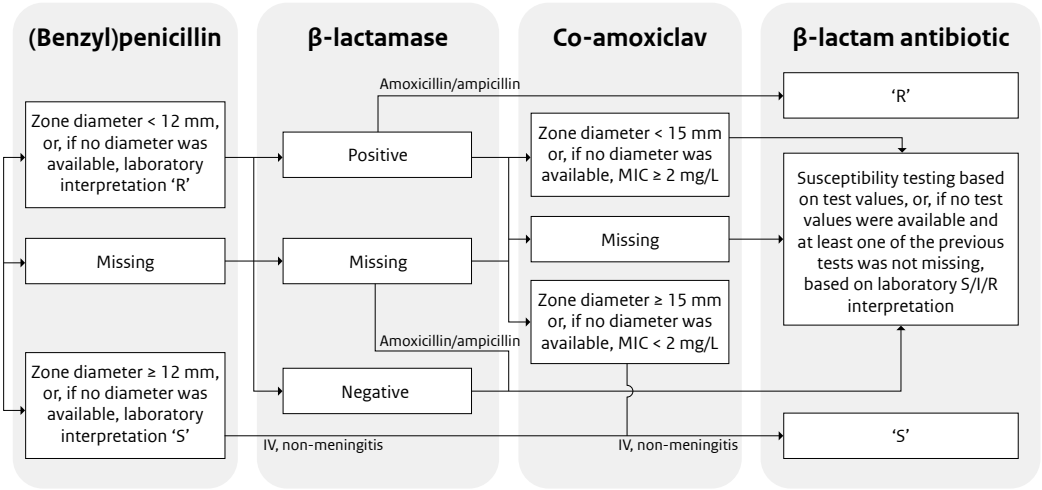


Figure 4.1.1.2 Flowchart depicting the algorithm used to calculate resistance to β-lactam antibiotics in *H. influenzae*



Calculation of time trends

In addition to resistance levels in 2023, we calculated for sections 4.2 to 4.6 time trends over the last five years (2019 to 2023) using logistic regression models, except when data in one or more years before 2023 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 13.1. We made an exception for trends in MRSA, resistance to clindamycin including inducible resistance in *S. aureus*, and resistance to β-lactam antibiotics in *S. pneumoniae* and *H. influenzae* (figures 4.1.1.1 and 4.1.1.2), 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 2019 and 2023.

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 2019 and 2023 was larger than the square root of the predicted resistance in 2019, 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 2019 to 2023 are shown in bar charts. Trends that meet the criteria for significant and microbiologically relevant are indicated with an asterisk.

[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 16 March 2022, <https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_-_gradient_for_benzyl_and_pnc_21nov2019.pdf>.

4.1.2 Description of the ISIS-AR data

In this section, several descriptive characteristics of the data from the ISIS-AR antimicrobial resistance surveillance system are presented. In figure 4.1.2.1, 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 4.2 through 4.6, 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 4.2 through 4.6 (see section 4.1.1 for inclusion criteria). In table 4.1.2.1, 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 can not be compared to data from Nethmap 2023.

Figure 4.1.2.1 Geographical distribution of laboratories, by status of connection to ISIS-AR and inclusion in the analyses in sections 4.2 to 4.6, 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 2023

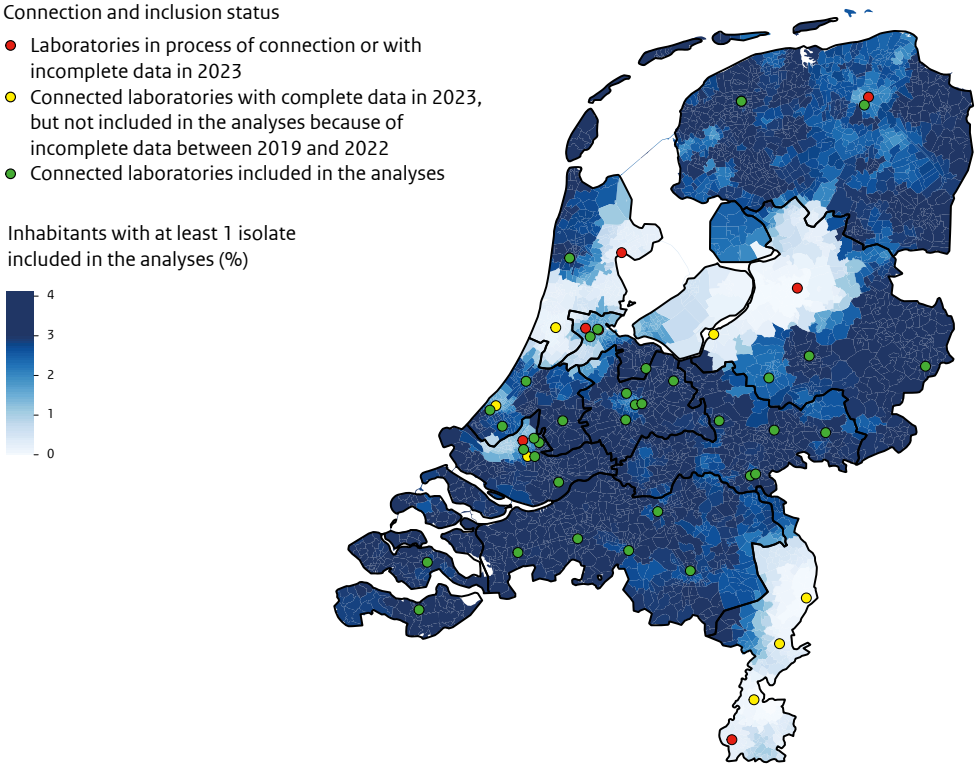


Table 4.1.2.1 Characteristics of 468,022 isolates, sampled in 2023, that were included in the analyses in sections 4.2 through 4.6, by pathogen

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*
Total number of isolates	207,183	34,611	24,082	12,511	26,231	4,391	30,325	6,048	64,522
Sex of patient (%)									
Male	26	33	41	56	53	53	55	52	54
Female	74	67	59	44	47	47	45	48	46
Setting of care (%)									
General practices	65	54	52	38	37	47	46	10	34
Outpatient departments	12	17	16	24	28	25	21	13	36
Inpatient departments (excl. intensive care units)	17	20	19	30	26	22	27	59	25
Intensive care units	1	1	1	4	3	4	2	14	3
Long-term care facilities	5	7	12	4	6	3	5	4	3
Age category of patient in years (%)									
0-4	3	1	3	3	2	4	3	1	5
5-18	5	1	2	2	5	4	2	1	9
19-64	34	27	22	28	30	33	27	31	44
>65	59	71	73	67	63	58	68	68	42
Isolate source (%)									
Blood	3	3	1	4	2	5	3	13	5
Respiratory tract	1	3	2	7	14	9	0	1	14
Urine	91	88	84	61	45	61	85	52	13
Wound or Pus	3	4	10	23	33	20	10	30	57
Genital	1	0	1	0	1	0	0	0	3
Other	1	2	2	5	5	5	1	4	9
Type of hospital (hospital isolates only, %)									
General	37	35	41	32	33	31	34	25	33
Top clinical	49	50	46	47	45	45	53	53	49
University hospital	14	15	13	21	22	24	14	23	19

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

Table 4.1.2.1 (continued) Characteristics of 468,022 isolates, sampled in 2023, that were included in the analyses in sections 4.2 through 4.6, by pathogen

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C and G	*S. anginosus*	*S. mitis*/*S. oralis*	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*	*H. pylori*
Total number of isolates	8,446	22,157	2,777	1,885	997	1,418	355	4,880	11,806	2,979	418
Sex of patient (%)											
Male	42	23	52	51	58	56	57	55	50	50	36
Female	58	77	48	49	42	44	43	45	50	50	64
Setting of care (%)											
General practices	47	56	36	9	11	3	5	8	12	13	1
Outpatient departments	24	23	28	31	23	24	24	29	45	43	69
Inpatient departments (excl. intensive care units)	26	18	33	53	62	65	65	56	38	38	26
Intensive care units	3	1	1	5	3	5	4	6	4	5	0
Long-term care facilities	1	2	2	1	1	2	2	0	1	2	1
Age category of patient in years (%)											
0-4	13	1	1	1	6	1	0	8	9	11	1
5-18	19	3	4	5	5	5	2	3	4	3	6
19-64	51	65	50	54	43	40	34	37	35	27	77
>65	17	31	44	40	46	55	63	51	51	59	17
Isolate source (%)											
Blood	8	2	10	11	38	26	28	29	2	1	0
Respiratory tract	9	1	4	3	1	0	0	54	86	89	0
Urine	7	56	16	14	18	1	1	1	0	0	0
Wound or Pus	50	10	48	60	39	65	59	14	10	10	10
Genital	20	27	14	6	0	1	3	0	2	0	0
Other	6	3	7	5	3	7	9	1	0	0	90
Type of hospital (hospital isolates only, %)											
General	35	36	46	38	39	39	37	34	33	31	35
Top clinical	51	51	40	51	42	48	47	51	50	54	58
University hospital	14	12	14	11	19	13	16	15	17	15	8

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

Key results

- For the 2023 analyses, data of 37 laboratories could be used, resulting in inclusion of data on 468,022 isolates.
- Laboratories from which data could be included in the analyses was relatively low in the regions 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', and 'Euregio-Zwolle', and 'Limburg'. The distribution of included laboratories was reflected in the geographical distribution of isolates.

4.2 Primary care

The distribution of pathogens in diagnostic urine, wound or pus, respiratory, and genital samples from general practitioners' (GP) patients in 2023 is presented in table 4.2.1. The resistance levels in 2023 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.2.2. 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 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 2023 are presented in table 4.2.3, and 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 in table 4.2.4. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), figure 4.2.2 (*S. aureus*) and figure 4.2.3 (β-haemolytic *Streptococcus* spp. group A and group B).

In accordance with the NHG guidelines, GPs usually send urine, wound, or pus samples for culture and susceptibility testing in case of antimicrobial therapy failure or (with regard to urine samples) complicated urinary tract infection. 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 4.2.1 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 2023

Pathogen	Urine		Wound or pus	Respiratory tract	Genital
	Age≤12 N (%)	Age>12 N (%)	N (%)	N (%)	N (%)
E. coli	9,871 (71)	123,414 (55)	820 (3)	96 (2)	583 (8)
K. pneumoniae	265 (2)	18,102 (8)	258 (1)	64 (1)	79 (1)
P. mirabilis	729 (5)	11,122 (5)	635 (2)	33 (1)	80 (1)
Other Enterobacterales [1]	730 (5)	24,812 (11)	2,543 (9)	292 (7)	175 (2)
P. aeruginosa	206 (1)	5,352 (2)	3,661 (13)	251 (6)	98 (1)
Other non-fermenters [2]	153 (1)	2,725 (1)	782 (3)	360 (8)	16 (0)
Other Gram-negatives [3]	4 (0)	22 (0)	462 (2)	1,004 (23)	97 (1)
S. aureus	164 (1)	4,066 (2)	14,627 (51)	1,635 (38)	1,190 (16)
β-haemolytic *Streptococcus* spp. group A	270 (2)	211 (0)	1,878 (7)	258 (6)	1,343 (18)
β-haemolytic *Streptococcus* spp. group B	130 (1)	8,473 (4)	638 (2)	33 (1)	3,119 (42)
Other Gram-positives [4]	1,404 (10)	26,213 (12)	2,343 (8)	299 (7)	599 (8)

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., Cronobacter spp., Yersinia spp., Shigella 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, N. meningitidis, H. pylori.*

4 *In order of frequency: Enterococcus spp., Staphylococcus spp. (non-aureus), A. urinae, S. anginosus, S. dysgalactiae n.n.g., S. pneumoniae, β-haemolytic Streptococcus spp. groups C and G, S. mitis/S. oralis, S. dysgalactiae subsp. equisimilis, C. perfringens, L. monocytogenes.*

Table 4.2.2 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 2023

	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	68	5	74	3	75	3	79
Antibiotic								
amoxicillin/ampicillin	33	34	-	-	14	17	-	-
co-amoxiclav [a]	24	25	23	16	4	5	-	-
piperacillin-tazobactam	-	-	-	-	-	-	0	3
cefuroxime	5	7	5	11	1	1	-	-
cefotaxime/ceftriaxone [b]	3	4	4	5	1	0	-	-
ceftazidime	3	3	5	4	0	0	0	1
meropenem [b]	-	-	-	-	-	-	0	1
imipenem	-	-	-	-	-	-	1	4
ciprofloxacin [b]	5	9	4	11	4	9	1	8
gentamicin	4	4	0	2	2 ↓	5	-	-
tobramycin	4	4	2	3	1	4	0	1
fosfomycin [1]	1	2	-	-	-	-	-	-
trimethoprim	20	21	11	17	18	28	-	-
co-trimoxazole	18	18	7	8	15	21	-	-
nitrofurantoin	0	1	-	-	-	-	-	-
Multidrug resistance								
MDOT [a]	1	3	1	2	0	1	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

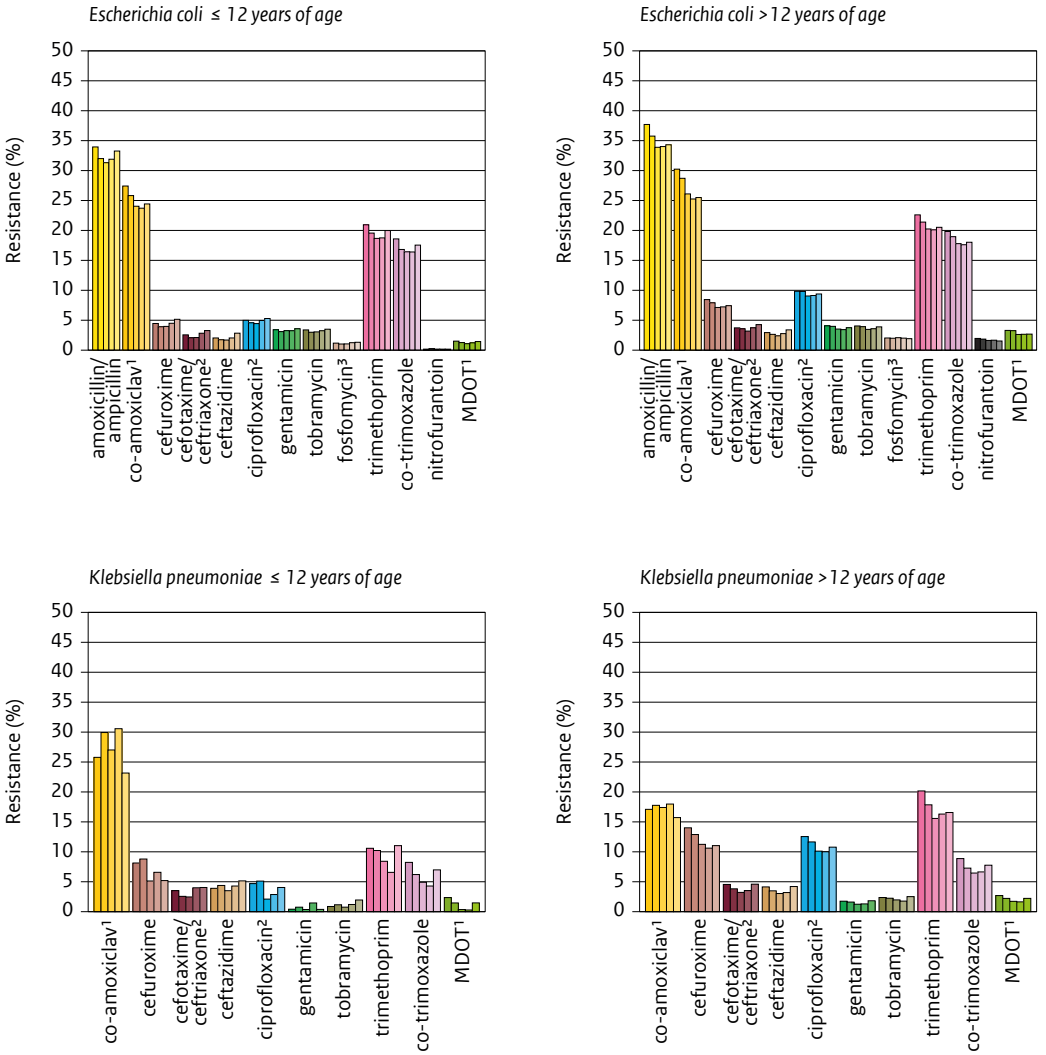
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.1.1.*

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (for ciprofloxacin this applies to E. coli, K. pneumoniae, and P. mirabilis only). For more details see section 4.1.1.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2019 to 2023) 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



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

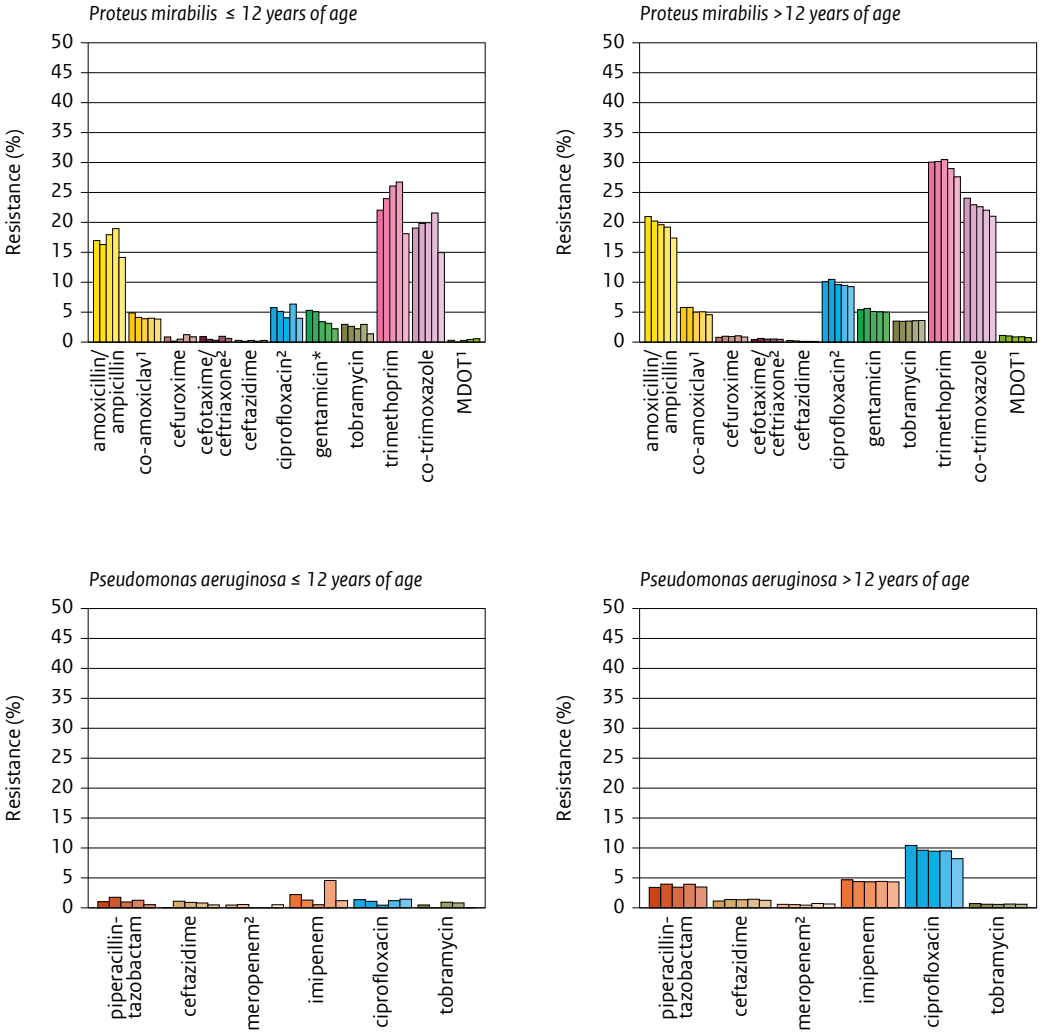
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) 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



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

Table 4.2.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients, ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	4
ciprofloxacin [2]	3
levofloxacin	2
erythromycin	14
clindamycin (including inducible resistance) [3]	13
doxycycline/tetracycline	4
fusidic acid	23
co-trimoxazole	2
mupirocine [a]	1
mupirocine [b]	2

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 0 out of 5 S. argenteus and 0 out of 0 S. schweitzeri were methicillin resistant.*

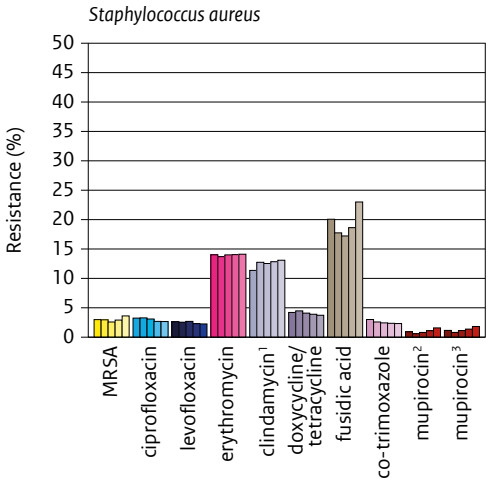
[2] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

[3] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

Figure 4.2.2 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR



MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[3] *According to breakpoint for topical use. For more details see section 4.1.1.*

Table 4.2.4 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 2023

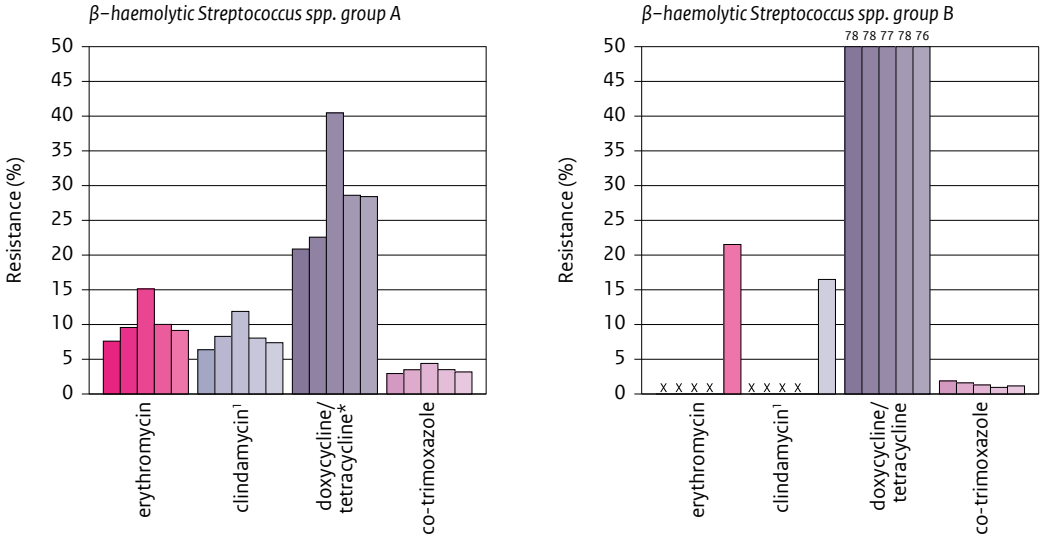
	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B
Antibiotic		
erythromycin	9	22°
clindamycin (including inducible resistance) [1]	7	16°
doxycycline/tetracycline	28 ↑	76
co-trimoxazole	3	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Figure 4.2.3 Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Key results

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 uncomplicatied 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 levels decreased slightly over the last 5 years to 20%. For other Enterobacterales, no 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, decreased to 25% in *E. coli* and was stable at 16% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third empirical choice antibiotic for this indication, was 18% in *E. coli* and 8% 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** (14%), **clindamycin** (including inducible resistance, 13%) and **fusidic acid** (23%). Resistance to **clindamycin** (including inducible resistance) and **macrolides** in *S. aureus* remained stable over the last five years but may limit its usefulness in empiric therapy for those infections possibly caused by *S. aureus*.
- **MRSA** was found in 4% of isolates of primary care patients which was stable over the previous 5 years.
- Resistance to **mupirocine** for nasal decontamination in *S. aureus* was low: 1%.

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

- The rise in resistance to **doxycycline/tetracycline** (28%) in β-haemolytic *Streptococcus* spp. group A over the last five years is worrisome. Resistance to **clindamycin** (including inducible resistance, 7%) remained stable after an initial rise.
- Resistance levels for **doxycycline/tetracycline** (76%), **clindamycin** (including inducible resistance, 16%) and **erythromycin** (22%) in β-haemolytic *Streptococcus* spp. group B were high, which might complicate treatment in case of beta-lactam allergy.

4.3 Hospital departments

In this section, resistance levels among isolates from patients in outpatient departments (section 4.3.1), inpatient departments (excluding intensive care units, section 4.3.2), and intensive care units (section 4.3.3) are presented. Additionally, resistance levels are shown separately for blood isolates from patients admitted to inpatient hospital departments (including intensive care units) in section 4.3.4 and for urine isolates from patients in urology departments (outpatient and inpatient departments) in section 4.3.5.

4.3.1 Outpatient departments

The distribution of pathogens isolated from diagnostic samples (lower respiratory tract, urine, and wound or pus) from patients attending outpatient departments in 2023 is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2023 are presented in tables 4.3.1.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.1.3 (*S. aureus*). Five-year trends in resistance are shown in figures 4.3.1.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.1.2 (*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 4.3.1.1 Distribution of isolated pathogens in diagnostic samples from patients attending outpatient departments, ISIS-AR 2023

	Lower respiratory tract	Urine	Wound or pus
Pathogen	N (%)	N (%)	N (%)
E. coli	353 (3)	21,150 (41)	2,255 (6)
K. pneumoniae	187 (2)	4,472 (9)	561 (1)
P. mirabilis	118 (1)	2,406 (5)	1,167 (3)
Other Enterobacterales [1]	802 (7)	7,925 (15)	4,315 (11)
P. aeruginosa	1,433 (13)	1,977 (4)	3,519 (9)
Other non-fermenters [2]	1,469 (13)	852 (2)	945 (2)
Other Gram-negatives [3]	4,183 (38)	22 (0)	1,163 (3)
S. aureus	1,552 (14)	1,725 (3)	16,323 (41)
Other Gram-positives [4]	1,047 (9)	11,318 (22)	9,658 (24)

[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., Hafnia spp., Escherichia spp. (non-coli), Salmonella spp., Mixta spp., Cronobacter spp.*

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

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

[4] *In order of frequency: ß-haemolytic Streptococcus spp. group B, S. pneumoniae, S. anginosus, S. dysgalactiae n.n.g., S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group A, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae subsp. equisimilis, Enterococcus spp., Staphylococcus spp. (non-aureus), A. urinae, C. perfringens, L. monocytogenes.*

Table 4.3.1.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments, ISIS-AR 2023

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	40	-	21	-
co-amoxiclav [a]	30	18	6	-
piperacillin-tazobactam	4	12	0	5
cefuroxime	11	13	1	-
cefotaxime/ceftriaxone [b]	6	9	1	-
ceftazidime	5	7	0	3
meropenem/imipenem [b]	0	0 ↑	-	-
meropenem [b]	-	-	0	1
imipenem	-	-	-	5
ciprofloxacin [b]	15	13	12	11
gentamicin	5	3	7	-
tobramycin	5	4	5	2
fosfomycin [1]	3	-	-	-
trimethoprim	25	19	31	-
co-trimoxazole	22	12	24	-
nitrofurantoin	2	-	-	-
Empiric therapy combinations				
co-amoxiclav + gentamicin [a]	4	3	2	-
cefuroxime + gentamicin	1	3	0	-
cefotaxime/ceftriaxone + gentamicin [b]	1	3	0	-
co-amoxiclav + ciprofloxacin [a]	9	6	2	-
cefuroxime + ciprofloxacin [b]	6	8	0	-
cefotaxime/ceftriaxone + ciprofloxacin [b]	4	6	0	-
Multidrug resistance				
MDOT [a]	5	4	2	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

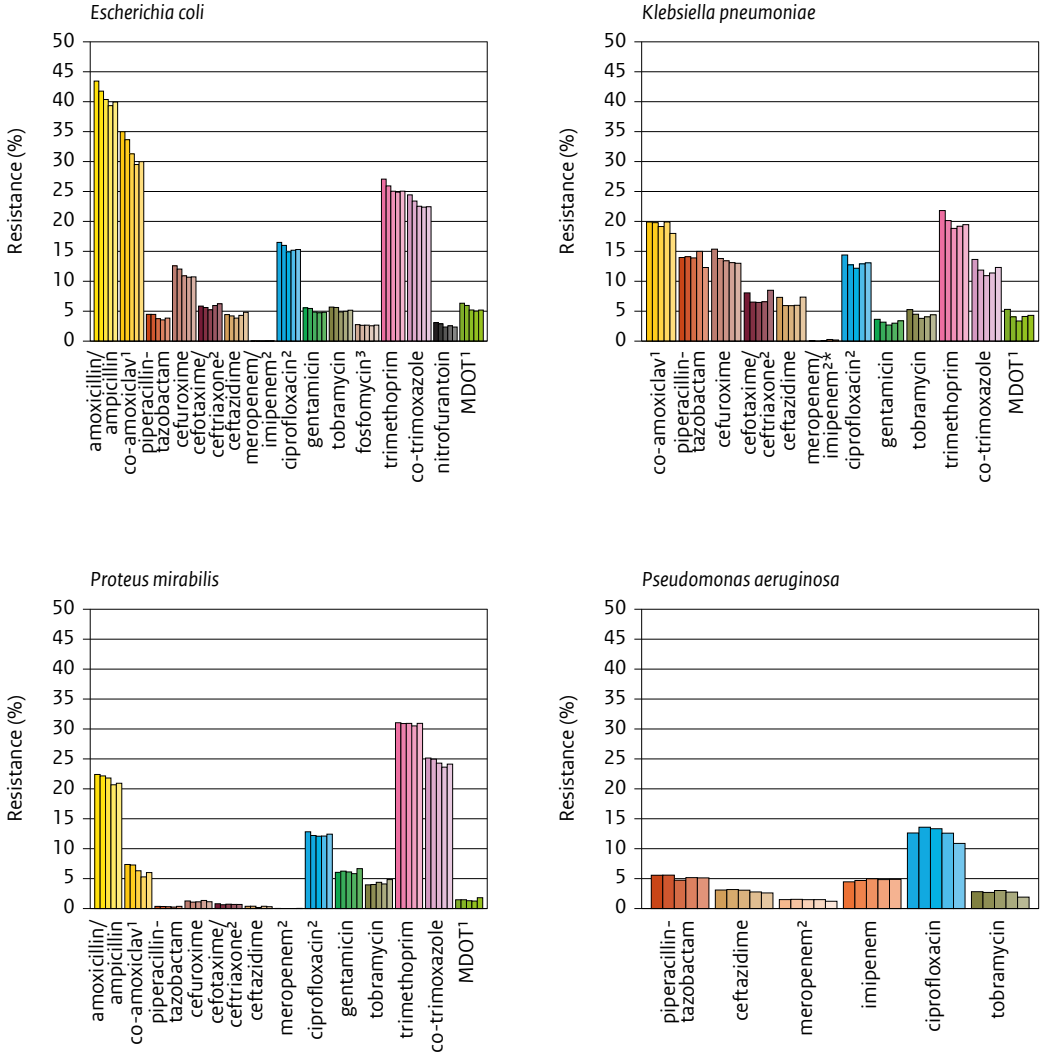
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.1.1.*

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (for ciprofloxacin applies to for E. coli, K. pneumoniae, and P. mirabilis only). For more details see section 4.1.1.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments in ISIS-AR



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

Table 4.3.1.3 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	2
ciprofloxacin [2]	4
levofloxacin	3
gentamicin	1
erythromycin	18
clindamycin (including inducible resistance) [3]	17
doxycycline/tetracycline	4
fusidic acid	9
linezolid	0
co-trimoxazole	2
rifampicin	0
mupirocine [a]	0
mupirocine [b]	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 1 out of 11 S. argenteus and 0 out of 0 S. schweitzeri were methicillin resistant.*

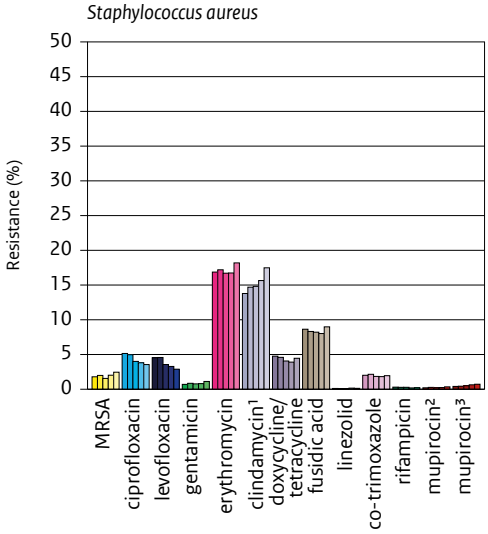
[2] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

[3] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

Figure 4.3.1.2 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR



MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

1 *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
2 *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*
3 *According to breakpoint for topical use. For more details see section 4.1.1.*

Key results

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 or equal 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**, a similar decreasing or stable trend was found as observed in primary care. In *E. coli*, resistance to **co-amoxiclav** decreased to 30% and was stable at 18% in *K. pneumoniae*. Resistance to **co-trimoxazole** was stable at 22% in *E. coli* and 12% in *K. pneumoniae*. Resistance levels for **ciprofloxacin** were 15% in *E. coli*, 13% in *K. pneumoniae* and 11% in *P. aeruginosa*, limiting the probability of succesful treatment when used as empirical treatment. Culture and antibiotic susceptibility testing will in general be required to confirm susceptibility and optimize the chances of successful treatment. Fortunately, in the majority of cases, at least one oral treatment option remains with these three antibiotics, given the relatively low (≤5%) combined resistance rates.
- Although **meropenem** resistance in *K. pneumoniae* was less than 1%, there was a significant increase over the last five years from 0.05% to 0.19%.

Urine, wound/pus and respiratory: S. aureus

- In *S. aureus*, no significant and microbiologically relevant trends in resistance levels were found over the last five years.
- Resistance was generally low with the exception of resistance to **fusidic acid** (9%), **clindamycin** (including inducible resistance, 17%) and **erythromycin** (18%). Resistance to **clindamycin** seems to have slightly increased over the last five years, which limited its usefulness in empiric therapy for those infections possibly caused by *S. aureus*.
- Resistance to **fusidic acid** was much lower in *S. aureus* isolates of OPD patients (9%) than in *S. aureus* isolates of GP patients (23%).
- **MRSA** was found in 2% of isolates of OPD patients which was stable over the previous 5 years.
- Resistance to **mupirocine** for nasal decontamination in *S. aureus* was less than 1%.

4.3.2 Inpatient hospital departments (excl. ICU)

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to inpatient hospital departments (excl. ICU) in 2023 is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2023 are presented in tables 4.3.2.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.3 (*E. faecalis* and *E. faecium*), 4.3.2.4 (*S. aureus*), 4.3.2.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.2.6 (*B. fragilis* complex and *C. perfringens*). Five-year trends in resistance are shown in figures 4.3.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.2 (*E. faecalis* and *E. faecium*), 4.3.2.3 (*S. aureus*), 4.3.2.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus* and *S. mitis/S. oralis*), and 4.3.2.5 (*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 4.3.2.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or pus N (%)
E. coli	5,496 (19)	1,017 (6)	22,447 (42)	3,539 (10)
K. pneumoniae	1,034 (4)	520 (3)	4,278 (8)	747 (2)
P. mirabilis	334 (1)	200 (1)	3,063 (6)	867 (2)
E. cloacae complex	447 (2)	576 (3)	1,428 (3)	1,455 (4)
Other Enterobacterales [1]	1,592 (6)	1,866 (11)	5,867 (11)	3,449 (10)
P. aeruginosa	561 (2)	1,588 (10)	2,754 (5)	1,954 (6)
Acinetobacter spp.	196 (1)	189 (1)	303 (1)	333 (1)
Other non-fermenters [2]	151 (1)	1,732 (10)	234 (0)	415 (1)
B. fragilis complex	359 (1)	1 (0)	7 (0)	596 (2)
Other Gram-negatives [3]	356 (1)	4,749 (28)	2 (0)	244 (1)
E. faecalis	907 (3)	29 (0)	5,390 (10)	1,985 (6)
E. faecium	675 (2)	47 (0)	1,725 (3)	1,362 (4)
S. aureus	2,835 (10)	2,441 (15)	1,502 (3)	9,101 (26)
ß-haemolytic *Streptococcus* spp. group A	665 (2)	109 (1)	64 (0)	1,196 (3)
ß-haemolytic *Streptococcus* spp. group B	413 (1)	121 (1)	1,287 (2)	759 (2)
ß-haemolytic *Streptococcus* spp. groups C and G	256 (1)	29 (0)	73 (0)	389 (1)
S. anginosus	201 (1)	22 (0)	63 (0)	733 (2)
S. mitis/S. oralis	379 (1)	6 (0)	29 (0)	216 (1)
C. perfringens	96 (0)	1 (0)	0 (0)	131 (0)
Other Gram-positives [4]	11,693 (41)	1,427 (9)	2,568 (5)	5,456 (16)

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

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

[3] *In order of frequency: H. influenzae, H. parainfluenzae, N. meningitidis, C. coli, 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 4.3.2.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	39	-	20	-	-	-
co-amoxiclav [a]	29 ↓	18	6	-	-	-
piperacillin-tazobactam	4	13	0	-	6	-
cefuroxime	11	13	1	-	-	-
cefotaxime/ceftriaxone [b]	6	8	1	-	-	-
ceftazidime	5	7	0	-	3	-
meropenem/imipenem [b]	0	0 ↑	-	0	-	2
meropenem [b]	-	-	0	-	1	-
imipenem	-	-	-	-	5	-
ciprofloxacin [b]	13	12	11	4	9	5
gentamicin	4	3	6	2	-	3
tobramycin	5	5	4	3	1	3
fosfomycin [1]	2	-	-	-	-	-
trimethoprim	23	17	30	7	-	-
co-trimoxazole	20	11	23	6	-	4
nitrofurantoin	1	-	-	-	-	-
Empiric therapy combinations						
co-amoxiclav + gentamicin [a]	3	3	2	-	-	-
cefuroxime + gentamicin	1	3	0	-	-	-
cefotaxime/ceftriaxone + gentamicin [b]	1	3	0	-	-	-
ceftazidime + tobramycin	-	-	-	-	0	-
ciprofloxacin + tobramycin	-	-	-	-	1	-
co-amoxiclav + ciprofloxacin [a]	7	6	2	-	-	-
cefuroxime + ciprofloxacin [b]	5	7	0	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin [b]	4	6	0	-	-	-
Multidrug resistance						
MDOT [a]	4	4	1	-	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

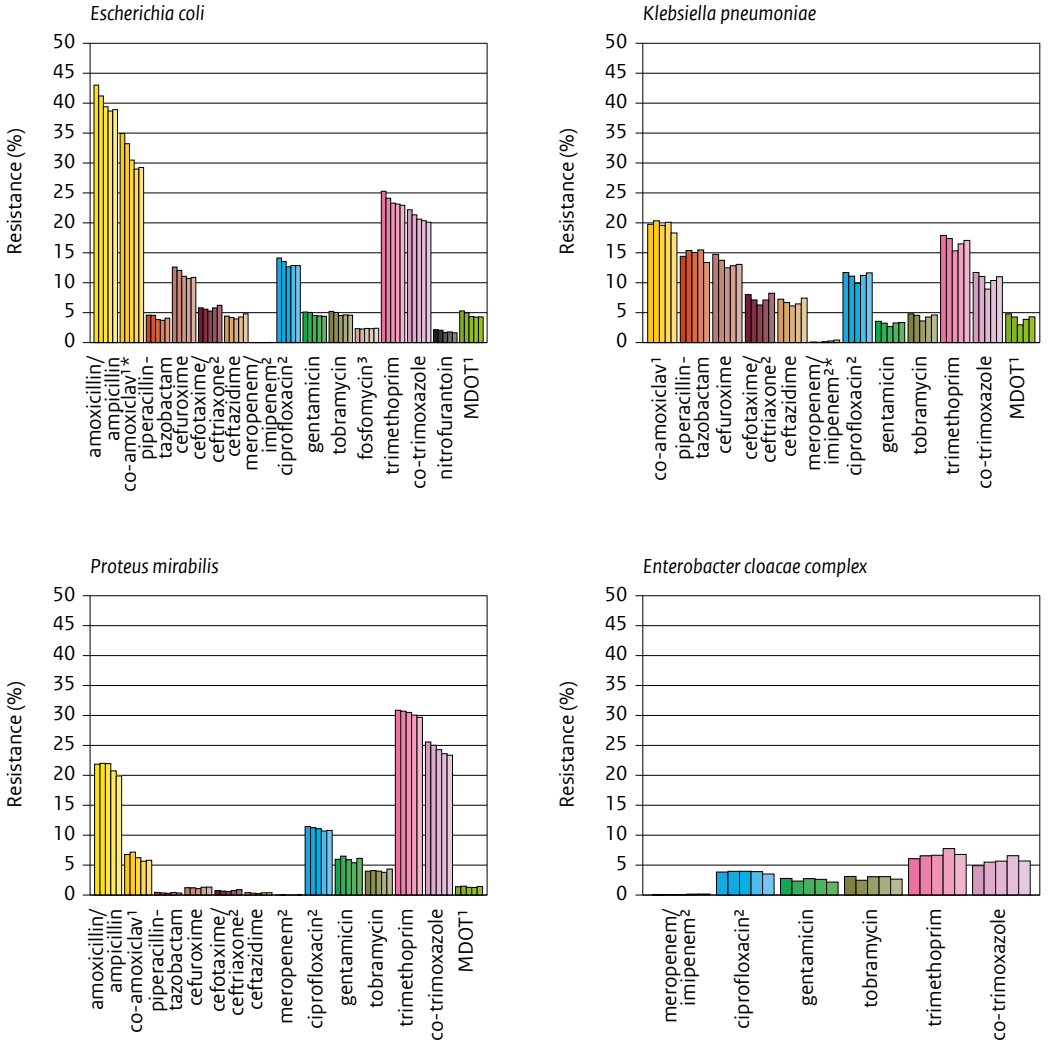
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.1.1.*

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (For ciprofloxacin this applies to E. coli, K. pneumoniae, P. mirabilis, and E. cloacae complex only). For more details see section 4.1.1.*

Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

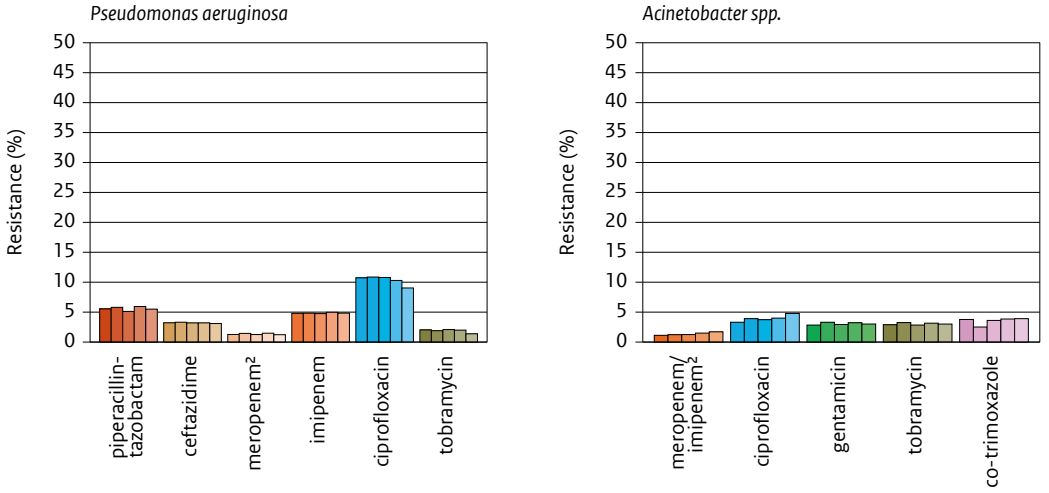
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in other indications than uncomplicated urinary tract infection.*

[2] *According to breakpoint for indications other than meningitis.*

[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 4.1.1.*

Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in other indications than uncomplicated urinary tract infection.*

[2] *According to breakpoint for indications other than meningitis.*

[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 4.1.1.*

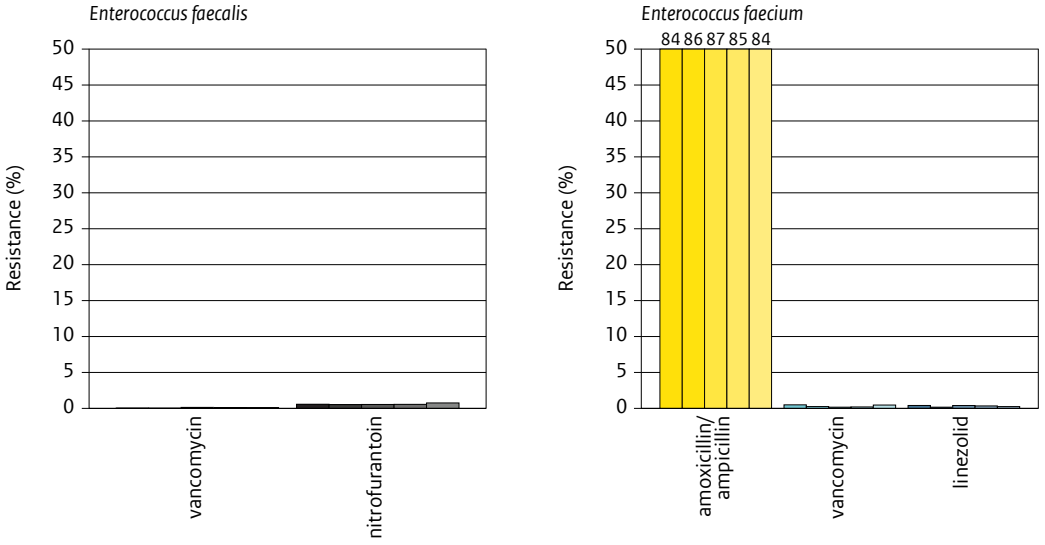
Table 4.3.2.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	84
vancomycin	0	0
linezolid	-	0
nitrofurantoin	1	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).

Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	3
ciprofloxacin [2]	4
levofloxacin	3
gentamicin	1
erythromycin	17
clindamycin (including inducible resistance) [3]	17 ↑
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0
co-trimoxazole	2
rifampicin	0
mupirocine [a]	0
mupirocine [b]	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 2 out of 23 S. argenteus and 0 out of 0 S. schweitzeri were methicillin resistant.*

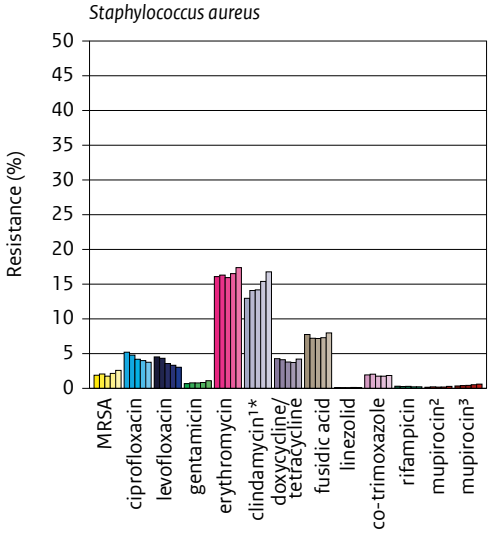
[2] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

[3] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[3] *According to breakpoint for topical use. For more details see section 4.1.1.*

Table 4.3.2.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. groups C and G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic					
(benzyl)penicillin	-	-	-	0	6
amoxicillin/ampicillin [1]	-	-	-	0	7°
erythromycin	8	22°	16	-	-
clindamycin (including inducible resistance) [2]	6	18°	15	11	8
doxycycline/tetracycline	24 ↑	74°	35°	-	-
co-trimoxazole	3	2	0	-	-

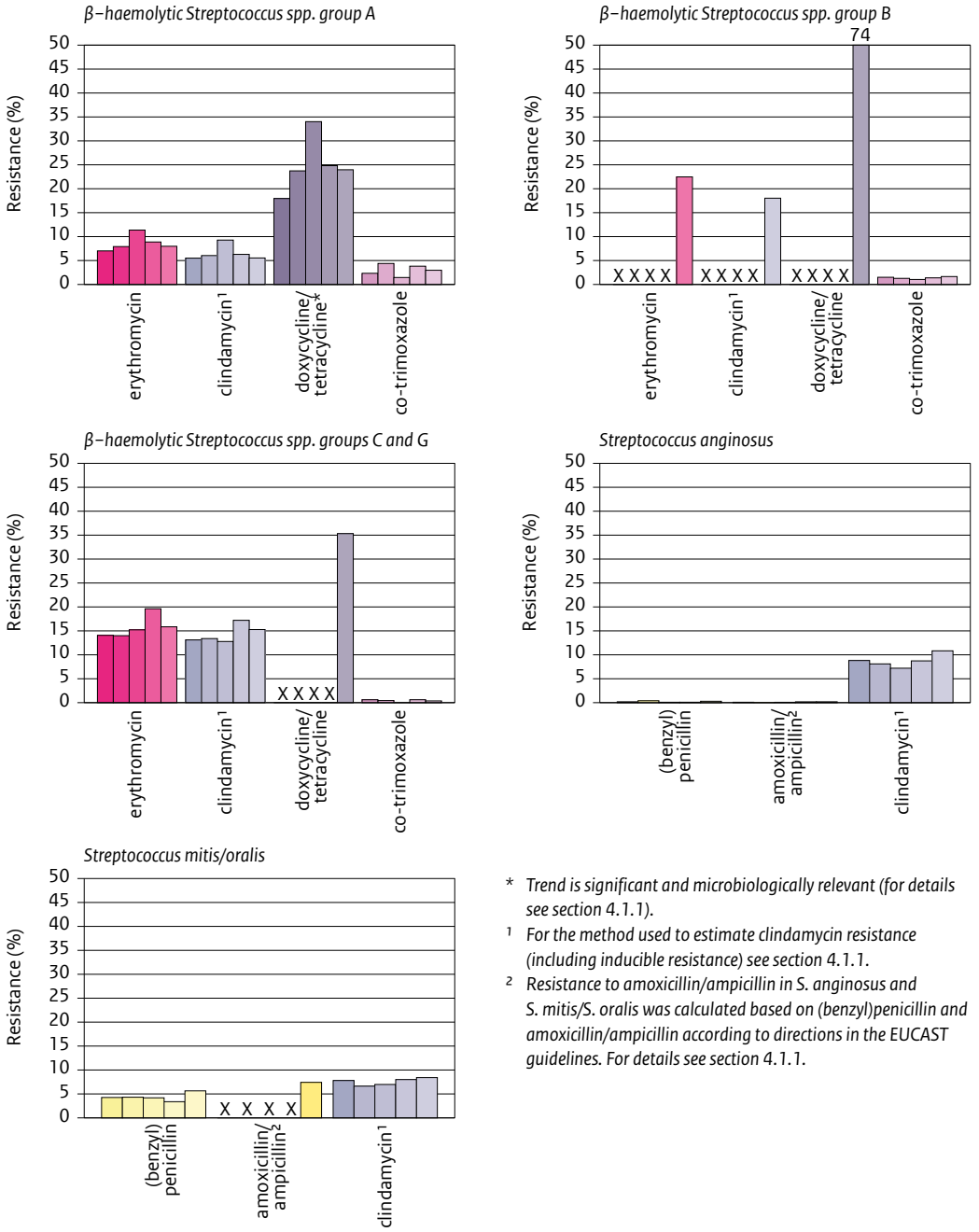
10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

[1] *Resistance to amoxicillin/ampicillin in S. anginosus and S. mitis/S. oralis was calculated based on (benzyl)penicillin and amoxicillin/ ampicillin according to directions in the EUCAST guidelines. For details see section 4.1.1.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Figure 4.3.2.4 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus* and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

¹ *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

² *Resistance to amoxicillin/ampicillin in S. anginosus and S. mitis/S. oralis was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 4.1.1.*

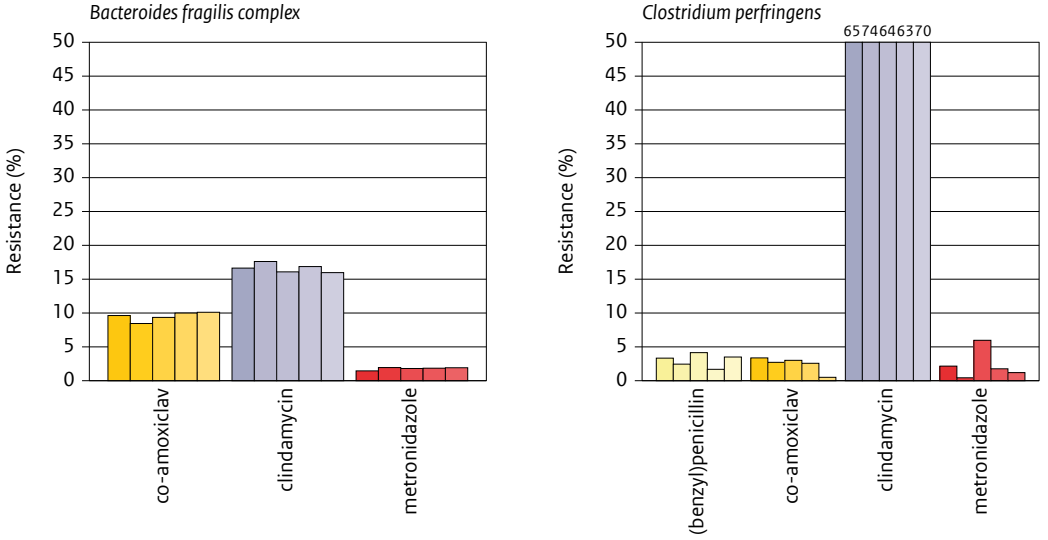
Table 4.3.2.6 Resistance levels (%) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2023

	B. fragilis complex	*C. perfringens*
Antibiotic		
(benzyl)penicillin	-	4
co-amoxiclav	10	0
clindamycin	16	70
metronidazole	2	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.2.5 Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).

Key results

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

- For both Enterobacterales and *P. aeruginosa*, resistance levels for all tested antibiotics were comparable to resistance levels in isolates of OPD patients.
- For all Enterobacterales, resistance to **second and third generation cephalosporins** seemed to have plateaued over the past five years. In 2023, resistance to **cefuroxime** was 11% in *E. coli* and 13% in *K. pneumoniae*. Resistance to **cefotaxime/ceftriaxone** was 6% in *E. coli* and 8% in *K. pneumoniae*. This is encouraging but nevertheless, patients that are infected with *K. pneumoniae* or *E. coli* 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**, reducing likelihood of resistance to 3% or less, might be a suitable option.
- Although **meropenem** resistance in *K. 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**, a similar trend was found as observed in isolates from primary care and OPD patients. In *E. coli*, resistance to **co-amoxiclav** decreased to 29% and was stable at 18% in *K. pneumoniae*. Resistance to **co-trimoxazole** was stable at 20% in *E. coli* and 11% in *K. pneumoniae*. Resistance levels for **ciprofloxacin** were 13% in *E. coli*, 12% in *K. pneumoniae* and 9% in *P. aeruginosa*.
- Resistance to **co-amoxiclav** in Enterobacterales is high. In 2023, the resistance percentage in *E. coli* was 29% and in *K. pneumoniae* 18%. This renders the drug unsuitable for empiric therapy for any infection potentially caused by gram-negative bacteria, unless it is combined with a second drug, preferably an **aminoglycoside**.
- For *P. aeruginosa*, resistance is 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.
- Resistance in *Acinetobacter* spp. for all tested antibiotics remained low at 5% or less.

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

- In *S. aureus* resistance was high for **clindamycin** (including inducible resistance, 17%) and increased significantly over the last five years. This indicates that culture and susceptibility testing are advised before starting treatment with this drug.
- **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 8% for **erythromycin**. Resistance to **doxycycline/tetracycline** increased over the last five years and was 24% in 2023.
- In β-haemolytic *Streptococcus* spp. groups B and C/G, resistance levels for **clindamycin** (including inducible resistance, 18% group B, 15% group C/G), and **erythromycin** (22% group B, 16% group C/G) were higher than for group A. For β-haemolytic *Streptococcus* spp. group C/G, these levels remained stable over the last five years. However, for β-haemolytic *Streptococcus* spp. group B, a time trend could not be calculated due to the low number of isolates tested for these antibiotics in earlier years.

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

- The European Committee on Antimicrobial Susceptibility Testing (EUCAST) publishes clinical minimum inhibitory concentration (MIC) 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 13.1, resistance in *B. fragilis* and *C. perfringens* remained stable and low, except from 70% resistance to **clindamycine** in *C. perfringens*.
- Resistances to **metronidazole** in both *B. fragilis* and *C. perfringens* remained low at 2% and were not influenced by the new species-specific breakpoints.

4.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound infections or pus) from patients admitted to intensive care units in 2023 is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2023 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.3 (*E. faecalis* and *E. faecium*), 4.3.3.4 (*S. aureus*) and 4.3.3.5 (β-haemolytic *Streptococcus* spp. group B). Five-year trends in resistance are shown in figures 4.3.3.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.2 (*E. faecalis* and *E. faecium*), 4.3.3.3 (*S. aureus*) and 4.3.3.4 (β-haemolytic *Streptococcus* spp. group B). For β-haemolytic *Streptococcus* spp. group A, groups C and G, *S. anginosus*, *S. mitis/S. oralis*, *B. fragilis* complex, and *C. perfringens*, resistance levels and trends were not calculated because in 2023 results for the majority of antibiotics were available for less than 100 isolates.

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 unlikely.

Table 4.3.3.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to intensive care units, ISIS-AR 2023

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	222 (8)	336 (8)	515 (41)	326 (12)
K. pneumoniae	44 (2)	135 (3)	98 (8)	67 (3)
P. mirabilis	16 (1)	53 (1)	83 (7)	49 (2)
E. cloacae complex	42 (1)	199 (5)	21 (2)	108 (4)
Other Enterobacterales [1]	97 (3)	689 (17)	123 (10)	283 (11)
P. aeruginosa	65 (2)	308 (8)	70 (6)	131 (5)
Acinetobacter spp.	16 (1)	89 (2)	8 (1)	28 (1)
Other non-fermenters [2]	17 (1)	372 (9)	6 (0)	40 (2)
Other Gram-negatives [3]	39 (1)	477 (12)	0 (0)	63 (2)
E. faecalis	110 (4)	19 (0)	122 (10)	243 (9)
E. faecium	237 (8)	40 (1)	88 (7)	295 (11)
S. aureus	230 (8)	985 (24)	44 (4)	321 (12)
ß-haemolytic *Streptococcus* spp. group B	16 (1)	47 (1)	16 (1)	36 (1)
Other Gram-positives [4]	1,735 (60)	330 (8)	52 (4)	648 (25)

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

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

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

[4] *In order of frequency: Staphylococcus spp. (non-aureus), ß-haemolytic Streptococcus spp. groups C and G, S. pneumoniae, S. dysgalactiae n.n.g., S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group A, S. anginosus, S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, C. perfringens, L. monocytogenes.*

Table 4.3.3.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units, ISIS-AR 2023

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	41 ↓	-	21	-	-	-
co-amoxiclav [a]	30 ↓	18	7	-	-	-
piperacillin-tazobactam	6	15	0	-	13	-
cefuroxime	16	18	2	-	-	-
cefotaxime/ceftriaxone [b]	10	11	1	-	-	-
ceftazidime	8	9	1	-	9	-
meropenem/imipenem [b]	0	1 ↑	-	0	-	3
meropenem [b]	-	-	0	-	3	-
imipenem	-	-	-	-	8	-
ciprofloxacin [b]	13	13	8	4	10	6
gentamicin	5	6	7	5	-	6
tobramycin	5	8	6	5	2	6
co-trimoxazole	20	10	23	5	-	5
Empiric therapy combinations						
co-amoxiclav + gentamicin [a]	4	4	2	-	-	-
cefuroxime + gentamicin	3	6	2	-	-	-
cefotaxime/ceftriaxone + gentamicin [b]	2	5	2	-	-	-
ceftazidime + tobramycin	-	-	-	-	2	-
ciprofloxacin + tobramycin	-	-	-	-	2	-
co-amoxiclav + ciprofloxacin [a]	8	7	1	-	-	-
cefuroxime + ciprofloxacin [b]	7	9	1	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin [b]	6	8	1	-	-	-
Multidrug resistance						
MDOT [a]	5	5 ↓	1	-	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

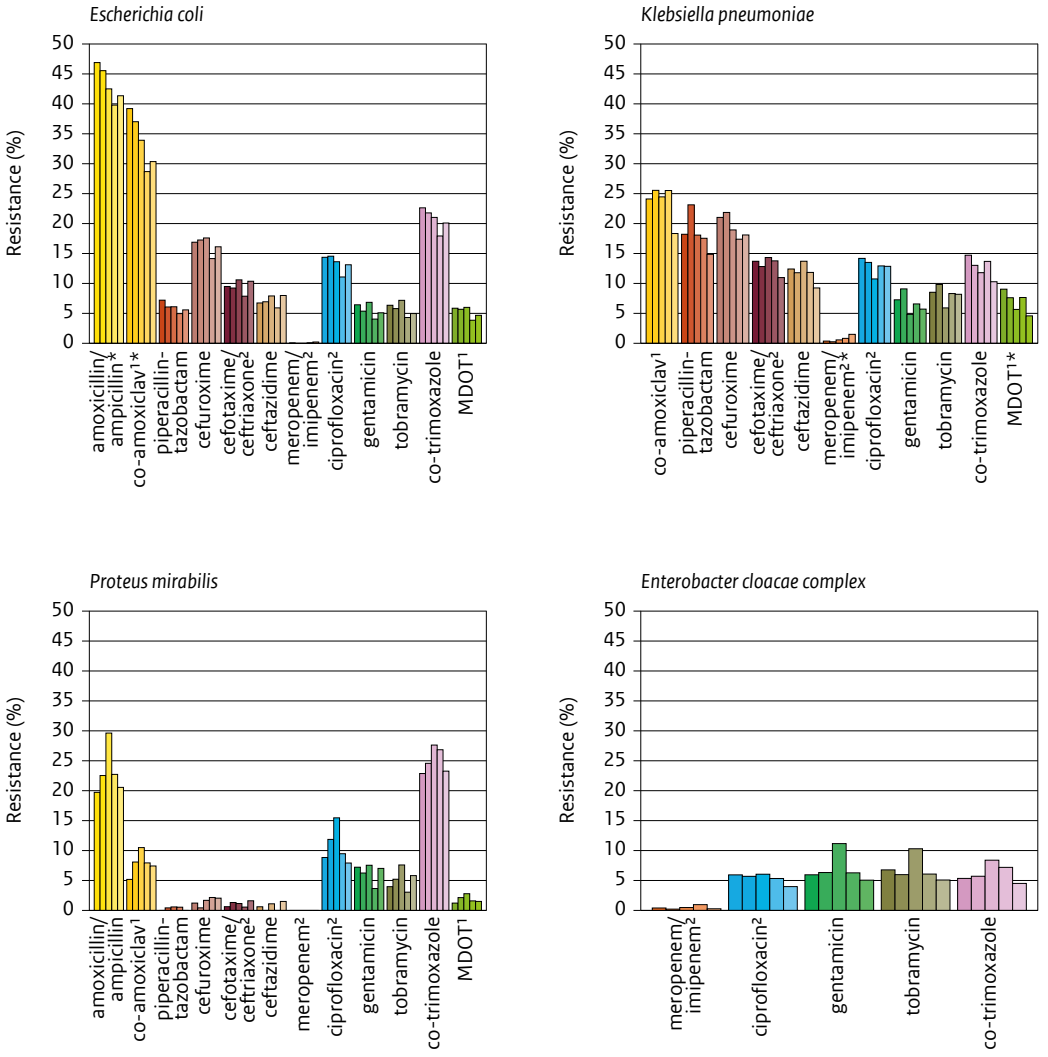
- = Resistance not calculated.

MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (For ciprofloxacin this applies to E. coli, K. pneumoniae, P. mirabilis, and E. cloacae complex only). For more details see section 4.1.1.*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR



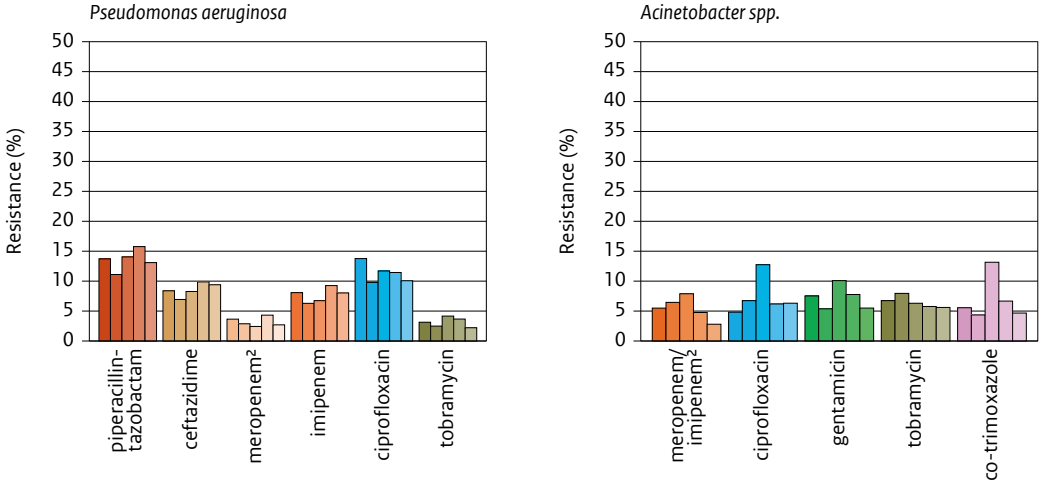
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Table 4.3.3.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to intensive care units, ISIS-AR 2023

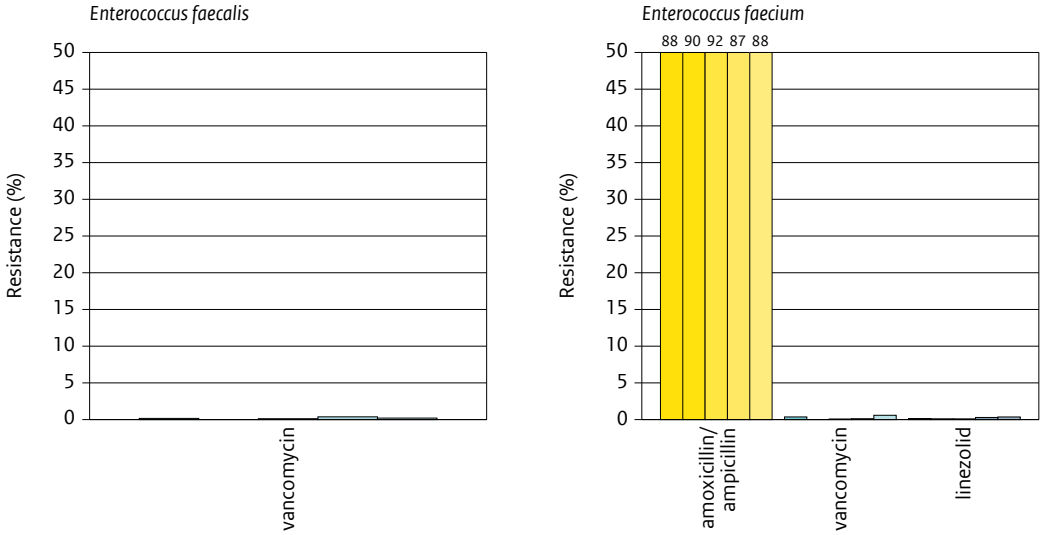
	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	88
vancomycin	0	1
linezolid	-	0

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of *E. faecalis* and *E. faecium* 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 4.1.1).

Table 4.3.3.4 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients admitted to intensive care units, ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	4 ↑
ciprofloxacin [2]	3
levofloxacin	1
gentamicin	2 ↑
erythromycin	15
clindamycin (including inducible resistance) [3]	15 ↑
doxycycline/tetracycline	4
fusidic acid	5
linezolid	0
co-trimoxazole	1
rifampicin	0
mupirocine [a]	0
mupirocine [b]	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 0 out of 0 S. argenteus and 0 out of 0 S. schweitzeri were methicillin resistant.*

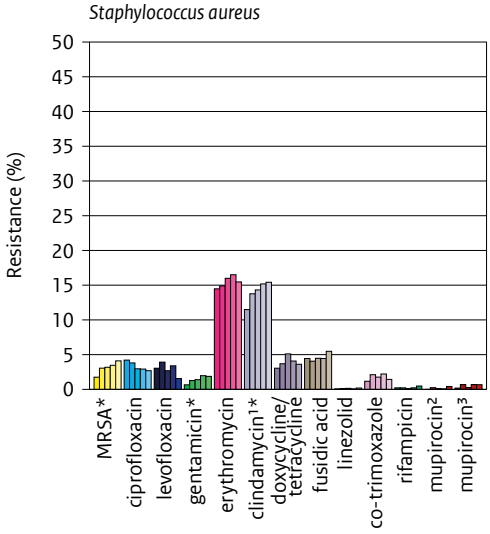
[2] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

[3] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

Figure 4.3.3.3 Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).*

MRSA = Methicillin resistant Staphylococcus aureus.

For the estimation method of MRSA see section 4.1.1.

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[3] *According to breakpoint for topical use. For more details see section 4.1.1.*

Table 4.3.3.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. group B from patients admitted to intensive care units, ISIS-AR 2023

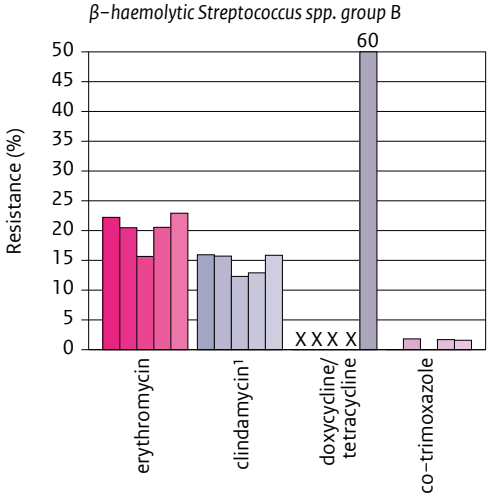
	ß-haemolytic *Streptococcus* spp. group B
Antibiotic	
erythromycin	23
clindamycin (including inducible resistance)[1]	16
doxycycline/tetracycline	60°
co-trimoxazole	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Figure 4.3.3.4 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic isolates of β-haemolytic *Streptococcus* spp. group B 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 4.1.1).

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Key results

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

- For *E. coli*, *K. pneumoniae* and *P. aeruginosa*, resistance levels for **beta-lactam antibiotics** were higher in ICU patients than in isolates from non-Intensive Care patients.
- In *K. pneumoniae*, resistance to **cefuroxime** and **ceftriaxone** was 18% and 11%, respectively and remained stable over the last five years. In *E. coli* resistance to **cefuroxime** and **ceftriaxone** was 16% and 10%, 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, empiric combination therapy with an **aminoglycoside**, reducing likelihood of resistance to 3% in *E. coli* and 6% in *K. pneumoniae*, might be a suitable strategy.
- A worrisome increase in resistance to **meropenem** in *K. pneumoniae* was found. Resistance increased from 0.3% to 1.5% 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 13% 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.
- Resistance in *Acinetobacter* spp. in ICU patients was higher than for non-ICU patients but still remained low for all recommended antibiotics at 6% or less.

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

- **MRSA** percentage in clinical isolates of ICU patients increased to 4% over the last five years.
- *S. aureus* showed an ongoing rise of **clindamycin** resistance to 15%.

4.3.4 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 2023 is presented in table 4.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2023 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp.), 4.3.4.3 (*E. faecalis* and *E. faecium*), 4.3.4.4 (*S. aureus*), 4.3.4.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.4.6 (*B. fragilis* complex). Five-year trends in resistance are presented in figures 4.3.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.4.2 (*E. faecalis* and *E. faecium*), 4.3.4.3 (*S. aureus*), 4.3.4.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.4.5 (*B. fragilis* complex). For *C. perfringens* both resistance levels and trends were not calculated because in 2023 less than 100 isolates were available for analysis.

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 4.3.4.1 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 2023

	Non-ICU	ICU
Pathogen	**N (%)**	**N (%)**
E. coli	6,609 (23)	203 (7)
K. pneumoniae	1,225 (4)	44 (2)
P. mirabilis	427 (1)	17 (1)
E. cloacae complex	482 (2)	45 (2)
Other Enterobacterales [1]	1,712 (6)	115 (4)
P. aeruginosa	616 (2)	73 (3)
Acinetobacter spp.	183 (1)	16 (1)
Other non-fermenters [2]	139 (0)	19 (1)
B. fragilis complex	343 (1)	22 (1)
Other Gram-negatives [3]	333 (1)	13 (0)
E. faecalis	950 (3)	110 (4)
E. faecium	532 (2)	260 (9)
S. aureus	3,063 (11)	186 (7)
ß-haemolytic *Streptococcus* spp. group A	676 (2)	28 (1)
ß-haemolytic *Streptococcus* spp. group B	421 (1)	13 (0)
ß-haemolytic *Streptococcus* spp. groups C and G	264 (1)	1 (0)
S. anginosus	190 (1)	18 (1)
S. mitis/S. oralis	366 (1)	10 (0)
Other Gram-positives [4]	10,106 (35)	1,620 (58)

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

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

[3] *In order of frequency: H. influenzae, H. parainfluenzae, C. coli, C. jejuni, N. meningitidis.*

[4] *In order of frequency: Staphylococcus spp. (non-aureus), S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, S. pneumoniae, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, C. perfringens, L. monocytogenes.*

Table 4.3.4.2 Resistance levels (%) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2023

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	40	-	19	-	-	-
co-amoxiclav [a]	30 ↓	17	4	-	-	-
piperacillin-tazobactam	4	12	0	-	5	-
cefuroxime	11	12	1	-	-	-
cefotaxime/ceftriaxone [b]	7	8	0	-	-	-
ceftazidime	6	7	0	-	3	-
meropenem/imipenem [b]	0	0	-	0	-	1
meropenem [b]	-	-	0	-	3 ↑	-
imipenem	-	-	-	-	7	-
ciprofloxacin [b]	13	10	10	4	8	2
gentamicin	4	4	5	2	-	5
tobramycin	5	5	3	2 ↓	1	5
co-trimoxazole	22	11	21	7	-	5
Empiric therapy combinations						
co-amoxiclav + gentamicin [a]	3	3	1	-	-	-
cefuroxime + gentamicin	2	3	0	-	-	-
cefotaxime/ceftriaxone + gentamicin [b]	2	3	0	-	-	-
ceftazidime + tobramycin	-	-	-	-	0	-
ciprofloxacin + tobramycin	-	-	-	-	1	-
co-amoxiclav + ciprofloxacin [a]	7	6	1	-	-	-
cefuroxime + ciprofloxacin [b]	6	7	0	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin [b]	5	6	0	-	-	-
Multidrug resistance						
MDOT [a]	4	5	1	-	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

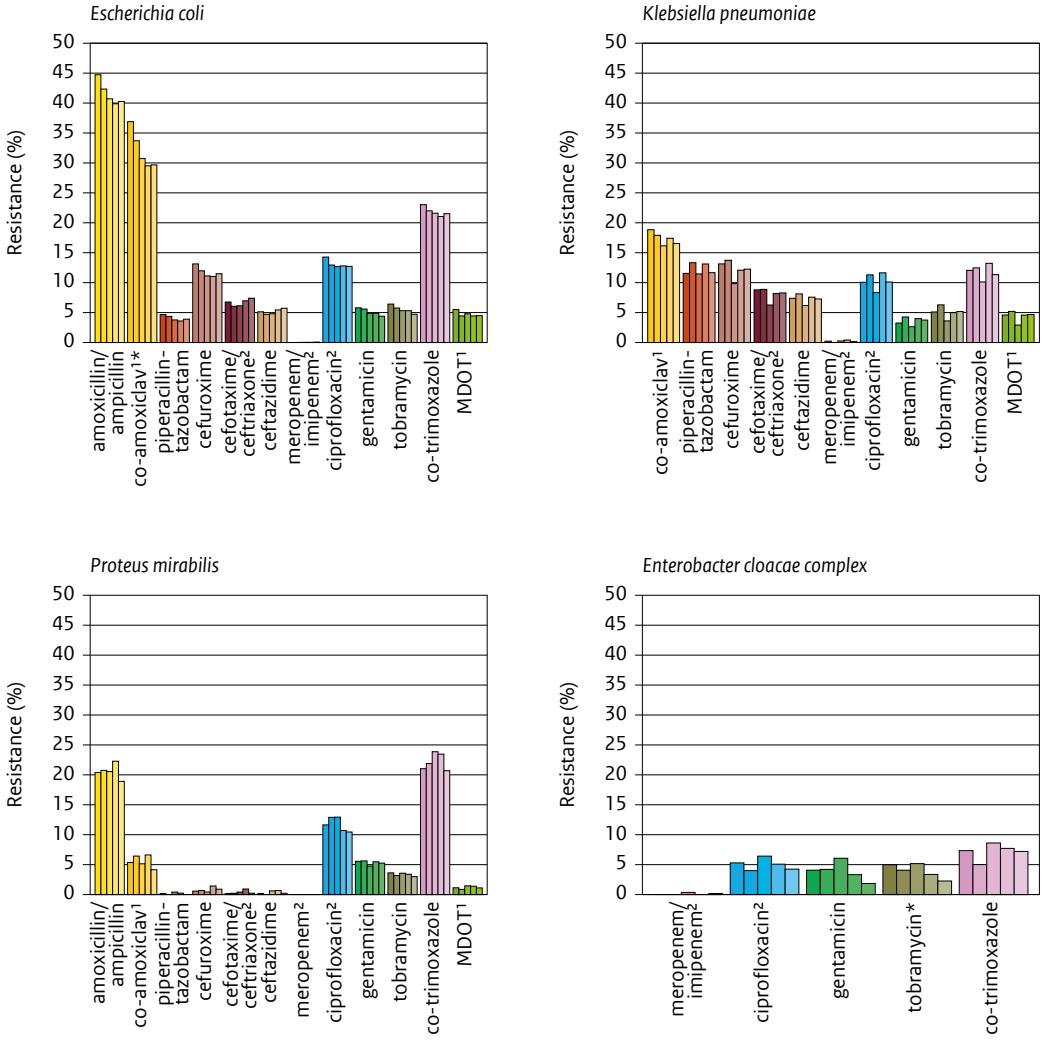
- = Resistance not calculated.

MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (For ciprofloxacin this applies to E. coli, K. pneumoniae, P. mirabilis, and E. cloacae complex only). For more details see section 4.1.1.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



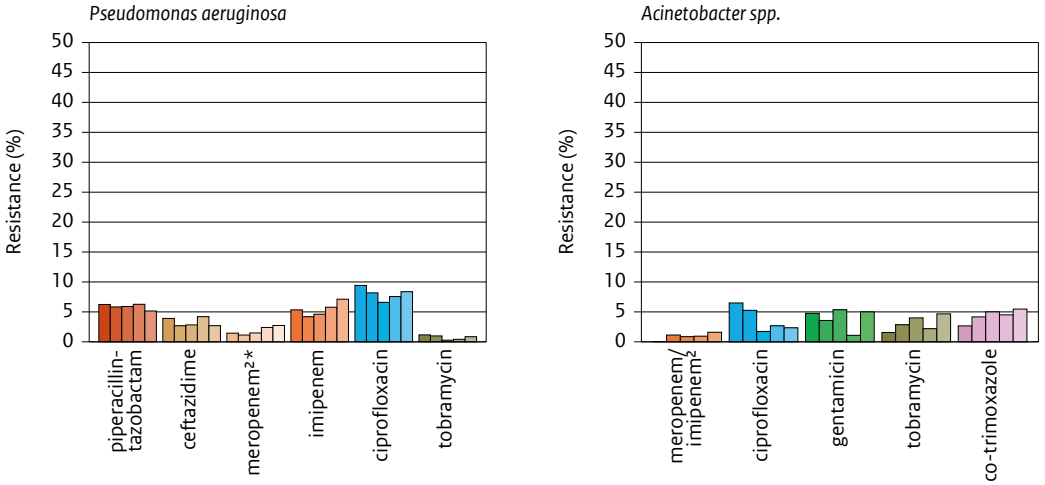
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

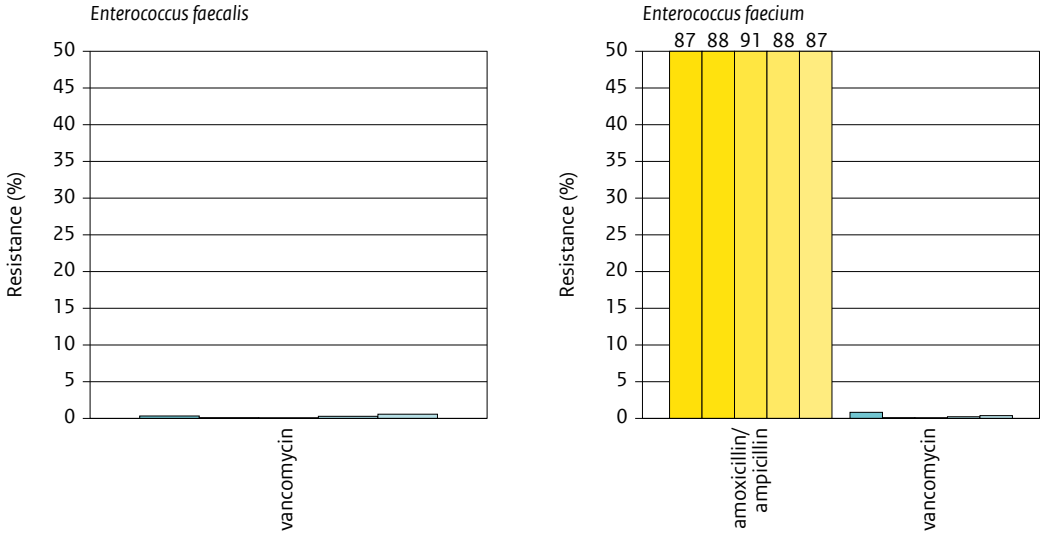
Table 4.3.4.3 Resistance levels (%) among diagnostic blood isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2023

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	87
vancomycin	1	0

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of *E. faecalis* and *E. faecium* 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 4.1.1).

Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	2
ciprofloxacin [2]	4
levofloxacin	3 ↓
gentamicin	1
erythromycin	15
clindamycin (including inducible resistance) [3]	14 ↑
doxycycline/tetracycline	3
linezolid	0
co-trimoxazole	1
rifampicin	0

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

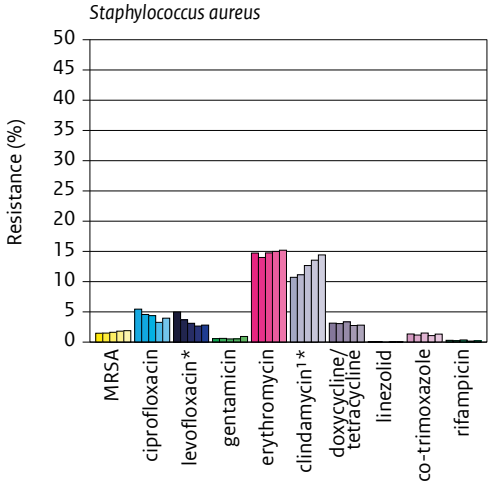
MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 0 out of 5 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 4.1.1.*

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Table 4.3.4.5 Resistance levels (%) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2023

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. groups C and G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic					
(benzyl)penicillin	-	-	-	1	4
amoxicillin/ampicillin [1]	-	-	-	0	7°
erythromycin	6	22	13	-	-
clindamycin (including inducible resistance) [2]	4	18	15	7	6
doxycycline/tetracycline	17	78	30	-	-
co-trimoxazole	3°	1	0	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

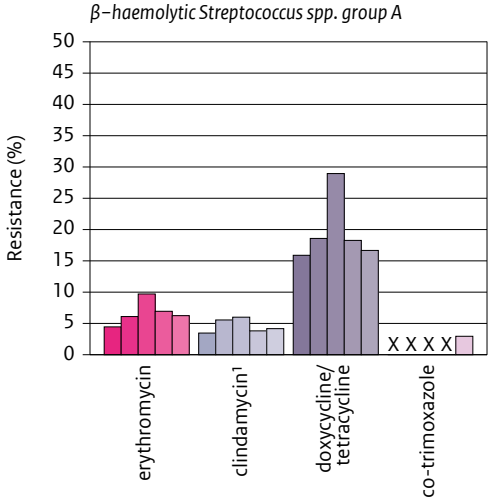
(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = *Resistance not calculated.*

[1] *Resistance to amoxicillin/ampicillin in S. anginosus and S. mitis/S. oralis was calculated based on (benzyl)penicillin and amoxicillin/ ampicillin according to directions in the EUCAST guidelines. For details see section 4.1.1.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Figure 4.3.4.4 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR

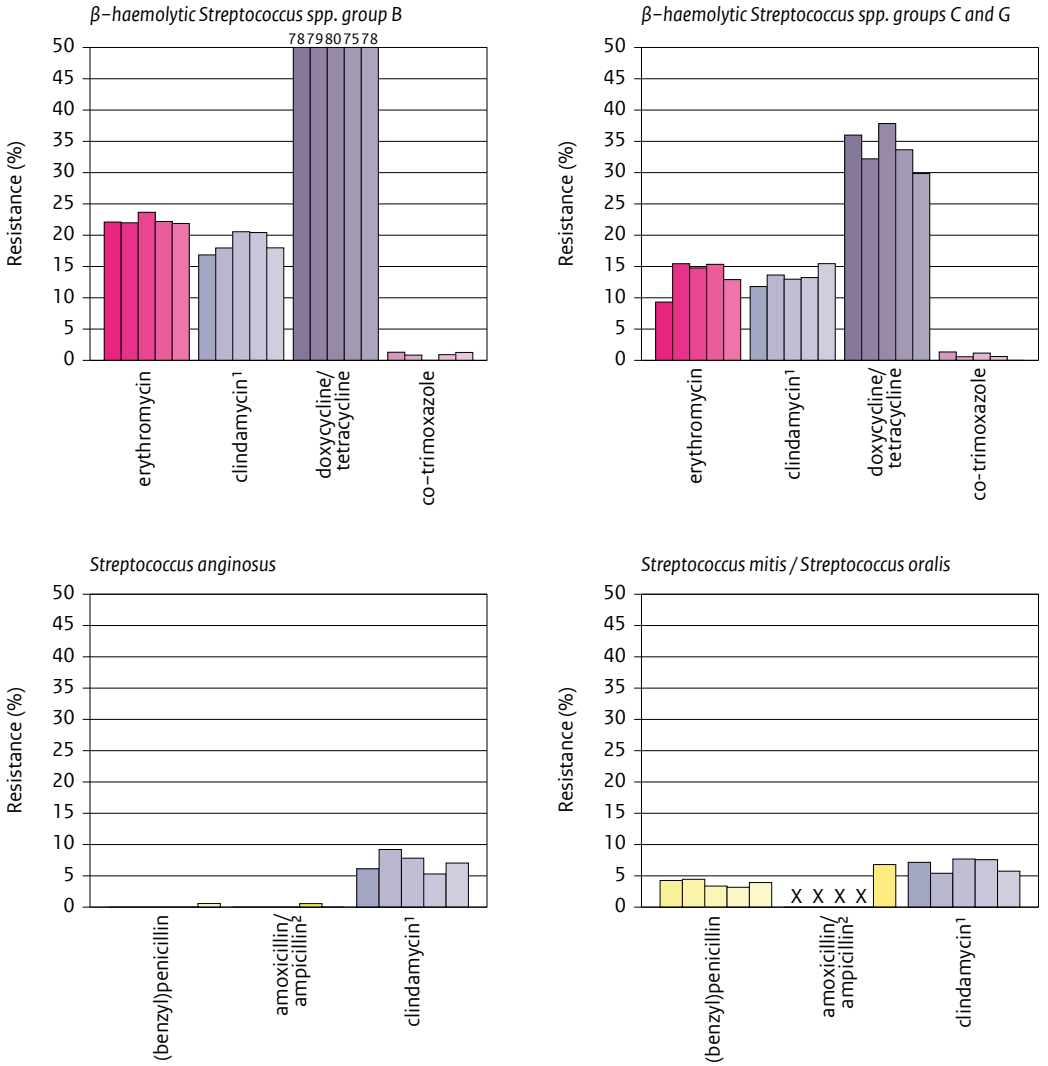


* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *Resistance to amoxicillin/ampicillin in S. anginosus and S. mitis/S. oralis was calculated based on (benzyl)penicillin and amoxicillin/ampicillin according to directions in the EUCAST guidelines. For details see section 4.1.1.*

Figure 4.3.4.4 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *Resistance to amoxicillin/ampicillin in S. anginosus and S. mitis/S. oralis was calculated based on (benzyl)penicillin and amoxicillin/ ampicillin according to directions in the EUCAST guidelines. For details see section 4.1.1.*

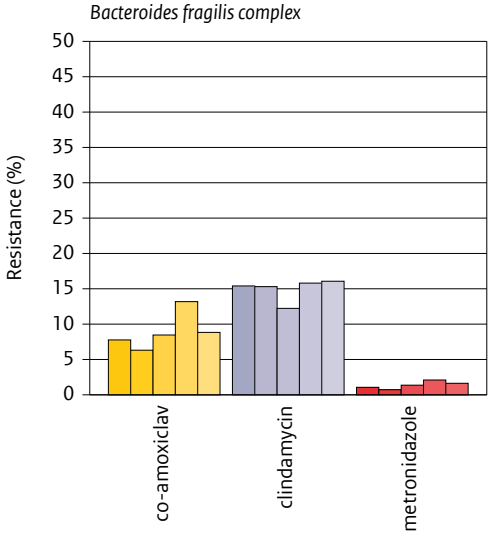
Table 4.3.4.6 Resistance levels (%) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2023

	B. fragilis complex
Antibiotic	
co-amoxiclav	9
clindamycin	16
metronidazole	1

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

Figure 4.3.4.5 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic blood isolates of *B. fragilis* complex 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 4.1.1).

Key results

Enterobacterales and P. aeruginosa in blood cultures

- For *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. in blood isolates no significant and microbiologically relevant trends were found, except for a decrease in resistance to **co-amoxiclav** in *E. coli* (to 30%).
- Resistance levels to **second and third generation cephalosporins** in 2023 were comparable in *E. coli* and *K. pneumoniae* and remained stable over the last five years. This is encouraging but nevertheless, patients with a bloodstream infection with *K. pneumoniae* or *E. coli* 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 3% or less, might be a suitable option.
- After initial iv treatment, a switch to either **ciprofloxacin**, **co-trimoxazole**, or **co-amoxiclav** was most often possible given the relatively low (≤5%) combined resistance rates for these oral agents.
- 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 (≤8%). However, resistance to **meropenem** in *P. aeruginosa* increased significantly over the last years to 3%.

S. aureus in blood cultures

- **MRSA** was found in 2% of *S. aureus* isolates in blood cultures which remained stable over the previous 5 years.

4.3.5 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 2023 is presented in table 4.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2023 are presented by type of department in tables 4.3.5.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.5.3 (*E. faecalis* and *E. faecium*). Five-year trends in resistance are shown in figure 4.3.5.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.5.2 (*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 4.3.5.1 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 2023

	OPD	IPD
Pathogen	N (%)	N (%)
E. coli	11,852 (37)	1,794 (32)
K. pneumoniae	2,790 (9)	398 (7)
P. mirabilis	1,489 (5)	286 (5)
Other Enterobacterales [1]	5,652 (17)	1,035 (18)
P. aeruginosa	1,296 (4)	387 (7)
Other non-fermenters [2]	631 (2)	128 (2)
Other Gram-negatives [3]	14 (0)	4 (0)
E. faecalis	3,486 (11)	716 (13)
E. faecium	249 (1)	155 (3)
Other Gram-positives [4]	4,912 (15)	717 (13)

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

[4] *In order of frequency: Staphylococcus spp., A. urinae, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, ß-haemolytic Streptococcus spp. group A, S. anginosus, ß-haemolytic Streptococcus spp. group B, S. pneumoniae, S. mitis/S. oralis, Enterococcus spp. (non-faecalis, non-faecium), C. perfringens.*

Table 4.3.5.2 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 2023

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	41	45	-	-	22	19 ↓	-	-
co-amoxiclav [a]	30 ↓	34 ↓	18	23	6	5 ↓	-	-
piperacillin-tazobactam	4	6	12	15	0	1 ↑	4	5
cefuroxime	12	17	13	18	1	2	-	-
cefotaxime/ceftriaxone [b]	7	11	8	13	1	0	-	-
ceftazidime	5	9	6	12	0	0	2	3
meropenem/imipenem [b]	0	0	0	0	-	-	-	-
meropenem [b]	-	-	-	-	0	0	1	2
imipenem	-	-	-	-	-	-	5	7
ciprofloxacin [b]	19	23	14	15	15	15	12	16 ↑
gentamicin	5	7	3	6	8	7	-	-
tobramycin	6	7	4	8	6	4	1	1
fosfomycin [1]	3	2	-	-	-	-	-	-
trimethoprim	27	29	20	22	34	34	-	-
co-trimoxazole	24	27	12	15	26	25	-	-
nitrofurantoin	3	2	-	-	-	-	-	-
Empiric therapy combinations								
co-amoxiclav + gentamicin [a]	4	5	3	5	2	4	-	-
cefuroxime + gentamicin	2	4	2	5	0	0	-	-
cefotaxime/ceftriaxone + gentamicin [b]	1	3	3	5	0	0	-	-
ceftazidime + tobramycin	-	-	-	-	-	-	0	0
ciprofloxacin + tobramycin	-	-	-	-	-	-	0	1
co-amoxiclav + ciprofloxacin [a]	10	13	5	9	3	3	-	-
cefuroxime + ciprofloxacin [b]	7	11	7 ↓	11	0	1	-	-
cefotaxime/ceftriaxone + ciprofloxacin [b]	5	8	5	9	0	0	-	-
Multidrug resistance								
MDOT [a]	6	9	4	7	3	3	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

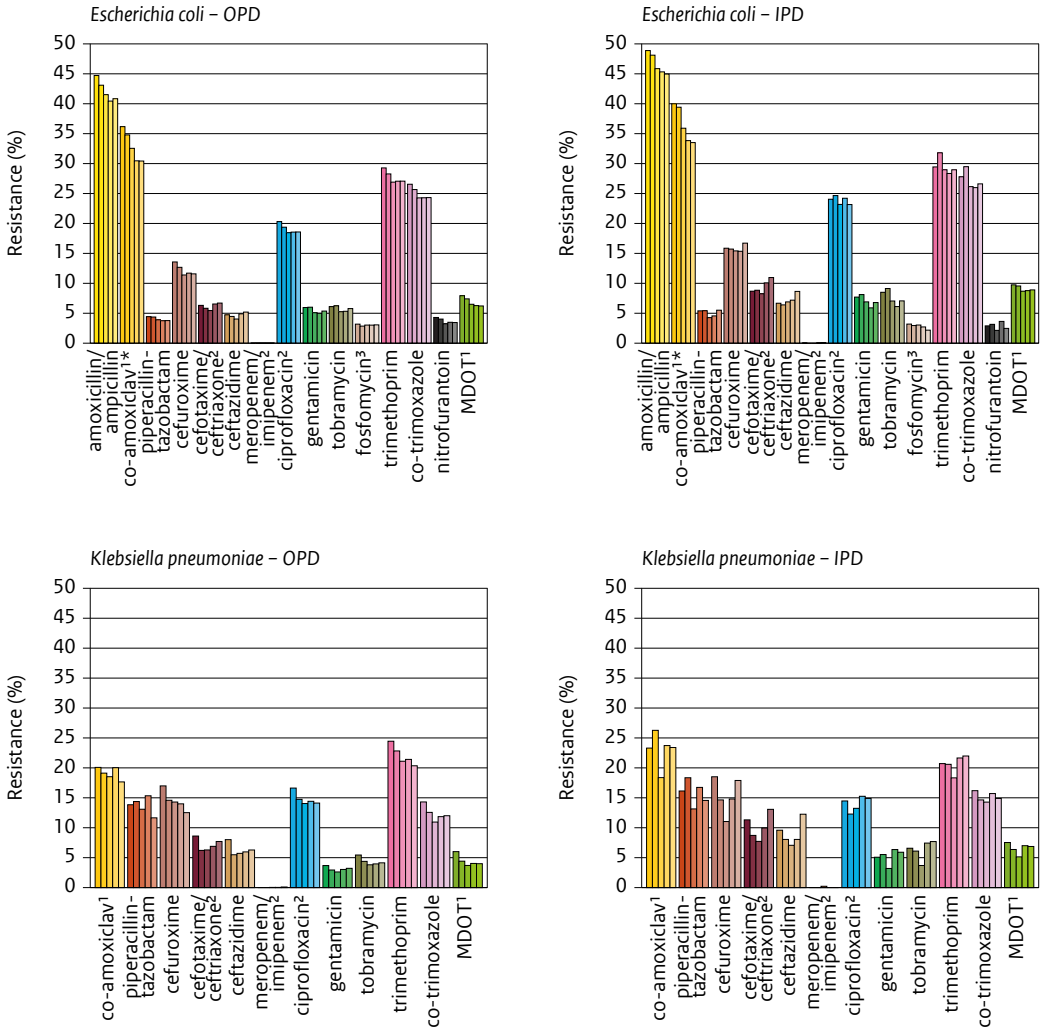
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.1.1.*

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (For ciprofloxacin this applies to E. coli, K. pneumoniae, and P. mirabilis only). For more details see section 4.1.1.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2019 to 2023) 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



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

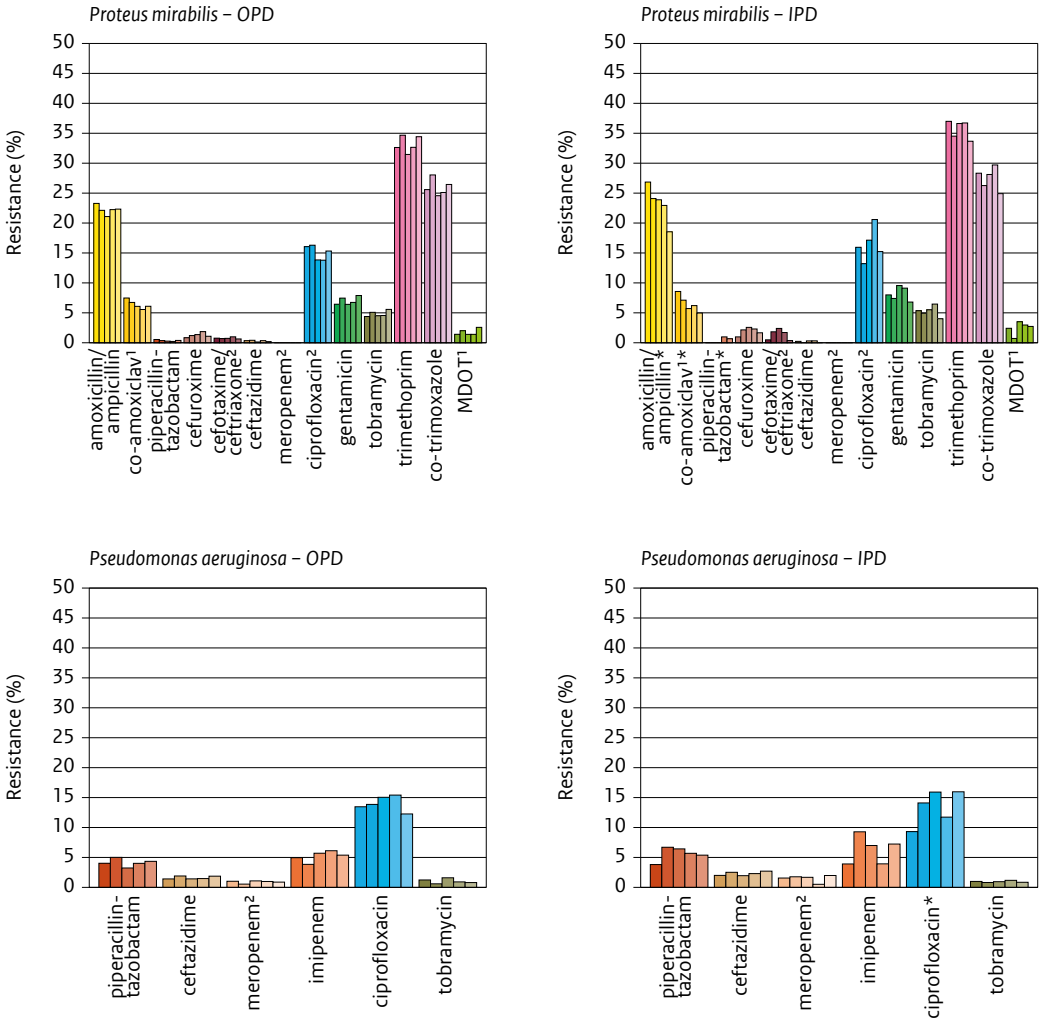
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

2 *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

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 4.1.1.*

Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) 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



MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for oral administration in infections originating from the urinary tract), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

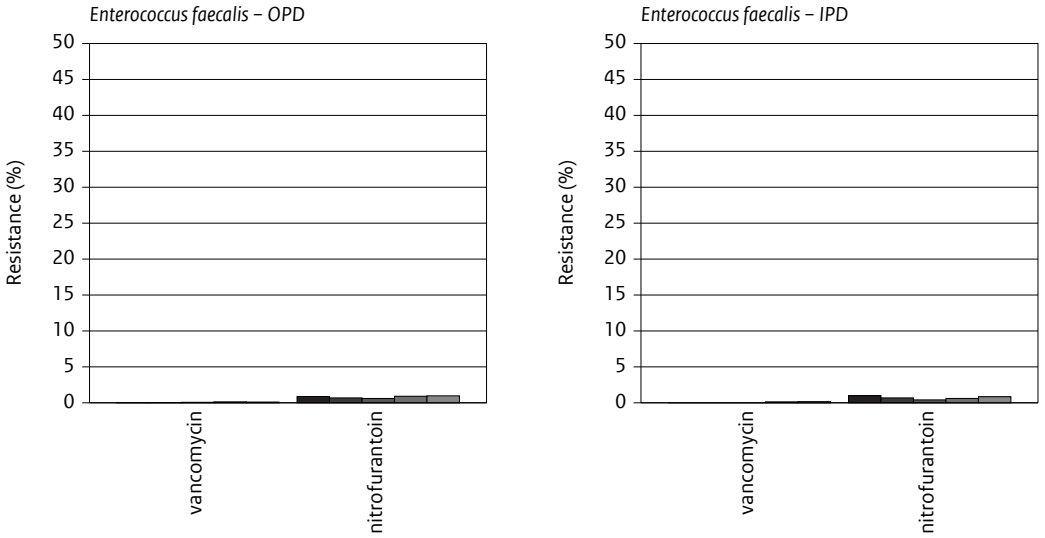
Table 4.3.5.3 Resistance levels (%) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2023

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	79	87
vancomycin	0	0	0	0
nitrofurantoin	1	1	-	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.
	(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

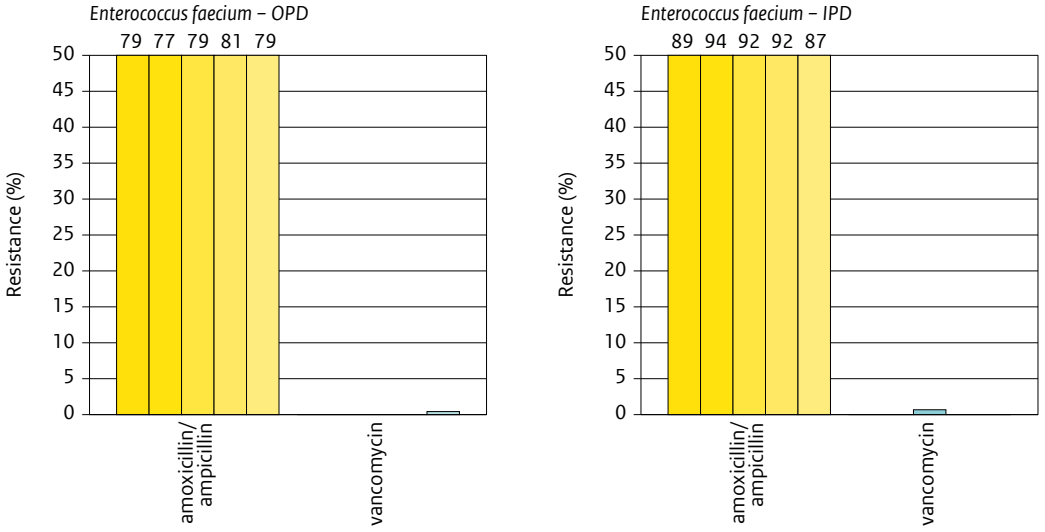
- = Resistance not calculated.

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).

Figure 4.3.5.2 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) 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 4.1.1).

Key results

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** (27%) in *E. coli* from admitted patients remains a problem.
- Resistance to **cephalosporines** in *E. coli* and *K. pneumoniae* isolates from admitted patients is stable but higher than in other departments (17% and 11% to **cefuroxime** and **ceftriaxone** in *E. coli* and 18% and 13% to **cefuroxime** and **ceftriaxone** in *K. pneumoniae*, respectively). This means that patients with a bloodstream infection due to *E. coli* or *K. pneumoniae* had 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 either **cefuroxime** plus **gentamicin** or with **ceftriaxone** plus **gentamicin**, reduced the likelihood of resistance to 5% or less.
- Resistance to **ciprofloxacin** in *P. aeruginosa* in admitted patients has increased from less than 10% to 16% over the last five years. This is a problem as **ciprofloxacin** is the only available oral agent to treat *P. aeruginosa* infections.

4.4 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 2023 is presented in table 4.4.1. The resistance levels in 2023 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.4.2 and for *S. aureus* isolates from wound or pus samples in table 4.4.3. Five-year trends in resistance are shown in figures 4.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), and 4.4.2 (*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 4.4.1 Distribution of isolated pathogens in diagnostic urine and wound or pus samples from selected residents of long-term care facilities, ISIS-AR 2023

Pathogen	Urine N (%)	Wound or pus N (%)
E. coli	9,905 (42)	139 (7)
K. pneumoniae	2,394 (10)	49 (2)
P. mirabilis	2,564 (11)	182 (9)
Other Enterobacterales [1]	2,746 (12)	159 (7)
P. aeruginosa	1,283 (5)	278 (13)
Other non-fermenters [2]	172 (1)	33 (2)
Other Gram-negatives [3]	0 (0)	27 (1)
S. aureus	879 (4)	945 (45)
Other Gram-positives [4]	3,587 (15)	311 (15)

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

[4] *In order of frequency: Enterococcus spp., A. urinae, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, ß-haemolytic Streptococcus spp. group A, S. anginosus, ß-haemolytic Streptococcus spp. group B, S. pneumoniae, S. mitis/S. oralis, Staphylococcus spp. (non-aureus), C. perfringens.*

Table 4.4.2 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 2023

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	40 ↓	-	19	-
co-amoxiclav [a]	30 ↓	17 ↓	6	-
piperacillin-tazobactam	5	14 ↓	0	5
cefuroxime	12 ↓	11 ↓	1	-
cefotaxime/ceftriaxone [b]	5	6	0	-
ceftazidime	4	6	0	2
meropenem/imipenem [b]	0	0	-	-
meropenem [b]	-	-	0	1
imipenem	-	-	-	4
ciprofloxacin [b]	14 ↓	11	12	9
gentamicin	5	2	5	-
tobramycin	5	4	3	0
fosfomycin [1]	3	-	-	-
trimethoprim	20	15	33	-
co-trimoxazole	18	9	24	-
nitrofurantoin	3	-	-	-
Multidrug resistance				
MDOT [a]	4	3	1	-

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

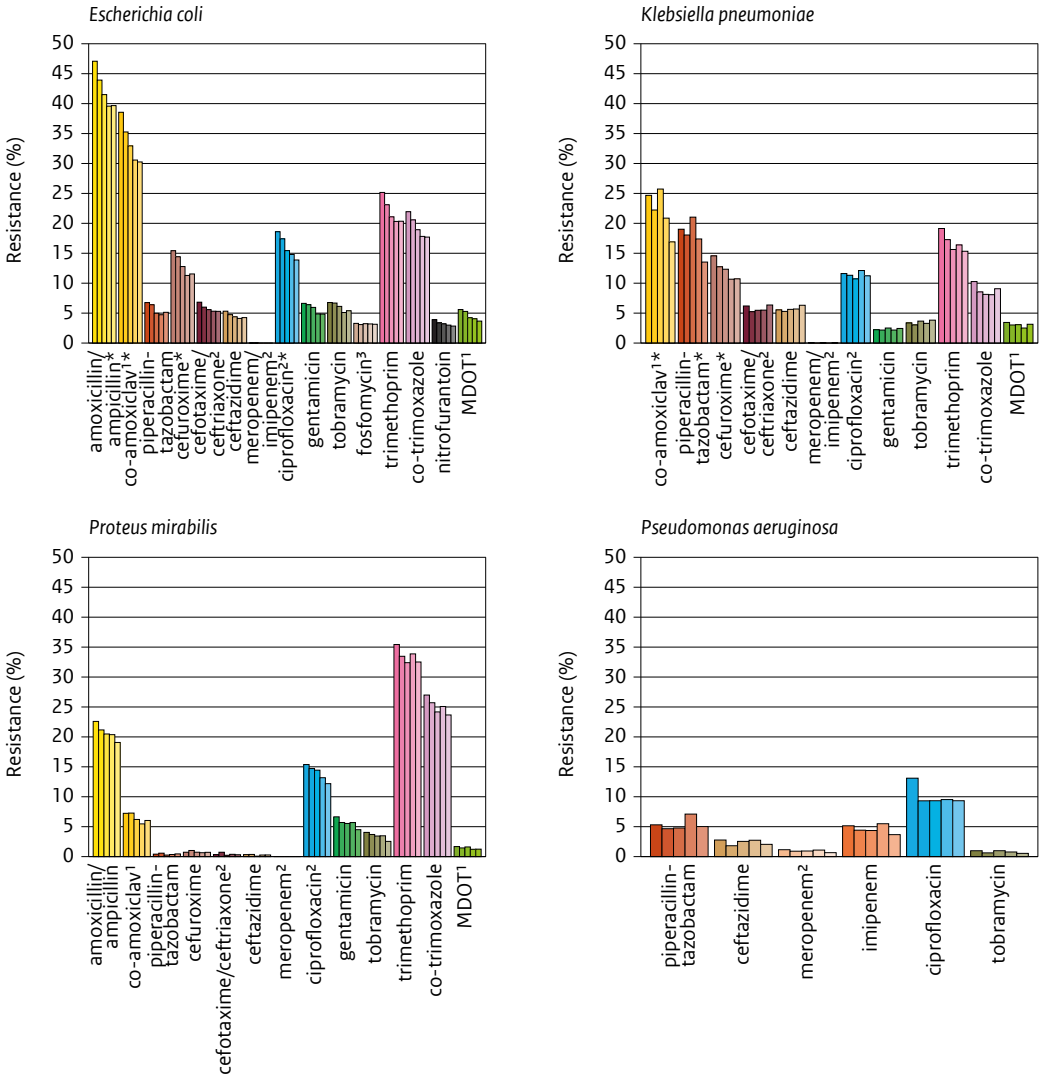
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.1.1.*

[a] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis (For ciprofloxacin this applies to E. coli, K. pneumoniae, and P. mirabilis only). For more details see section 4.1.1.*

Figure 4.4.1 Trends in antibiotic resistance (from left to right 2019 to 2023) 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



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), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for oral administration in infections originating from the urinary tract. For more details see section 4.1.1.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

Table 4.4.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2023

	S. aureus
Antibiotic	
MRSA [1]	2
ciprofloxacin [2]	17
levofloxacin	18
erythromycin	14
clindamycin (including inducible resistance) [3]	15
doxycycline/tetracycline	4
fusidic acid	7
co-trimoxazole	1
mupirocine [a]	0
mupirocine [b]	0

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

MRSA = Methicillin resistant S. aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Within the S. aureus complex 0 out of 0 S. argenteus and 0 out of 0 S. schweitzeri were methicillin resistant.*

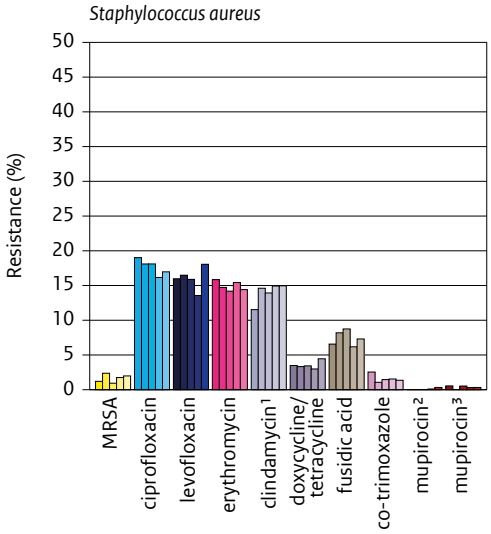
[2] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

[3] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

Figure 4.4.2 Trends in antibiotic resistance (from left to right 2019 to 2023) among diagnostic wound or pus isolates of *S. aureus* from selected residents of long-term care facilities in ISIS-AR



MRSA = Methicillin resistant S. aureus.
For the estimation method of MRSA see section 4.1.1.
[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
[2] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*
[3] *According to breakpoint for topical use. For more details see section 4.1.1.*

Key results

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, has significantly decreased to 14% in *E. coli* and was 12% in *K. pneumoniae* and 9% 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 17% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third choice antibiotic for this indication, was 18% in *E. coli* and 9% 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** (18%), which was much higher in *S. aureus* from selected LTCF residents than in *S. aureus* from OPD 3%), hospital (3%) and ICU patients (1%), which might be the result of either selective sampling or intensive use of **fluoroquinolones** in this setting.

4.5 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 2023 is presented in table 4.5.1. Resistance levels for respiratory pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) in 2023 are presented by patient group in table 4.5.2. Five-year trends in resistance are shown in figure 4.5.1.

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 4.5.1 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 2023

	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	187 (8)	11 (1)	1,480 (5)	2,230 (8)	129 (2)
Other Gram-positives [1]	265 (12)	1,762 (86)	17,816 (55)	4,608 (17)	3,863 (63)
H. influenzae	876 (38)	68 (3)	223 (1)	8,187 (29)	529 (9)
M. catarrhalis	243 (11)	36 (2)	18 (0)	2,140 (8)	151 (2)
Other non-fermenters [2]	301 (13)	31 (2)	1,017 (3)	4,204 (15)	405 (7)
Enterobacterales [3]	356 (16)	129 (6)	11,148 (35)	5,813 (21)	1,027 (17)
Other Gram-negatives [4]	54 (2)	6 (0)	510 (2)	650 (2)	59 (1)

[1] *In order of frequency: Staphylococcus spp., ß-haemolytic Streptococcus spp. groups C and G, ß-haemolytic Streptococcus spp. group A, S. dysgalactiae n.n.g., S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group B, S. anginosus, S. dysgalactiae subsp. equisimilis, Enterococcus spp., A. urinae, C. perfringens, L. monocytogenes.*

[2] *In order of frequency: Pseudomonas spp., S. maltophilia, Acinetobacter spp.*

[3] *In order of frequency: Escherichia spp., Klebsiella spp., Enterobacter spp., Serratia spp., Proteus spp., Citrobacter spp., Morganella spp., Raoultella spp., Salmonella spp., Pantoea spp., Hafnia spp., Providencia spp., Cronobacter spp., Yersinia spp., Mixta spp.*

[4] *In order of frequency: H. parainfluenzae, B. fragilis complex, N. meningitidis, C. jejuni, C. coli.*

Table 4.5.2 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 2023

	S. pneumoniae		*H. influenzae*		*M. catarrhalis*	
	GP	Hospital	GP	Hospital	GP	Hospital
Antibiotic						
(benzyl)penicillin (R) [1 a]	1	0	-	-	-	-
(benzyl)penicillin (I) [1 a]	9	9 ↑	-	-	-	-
amoxicillin/ampicillin [1 b]	-	-	31	29	-	-
co-amoxiclav [1 b]	-	-	12	11	1	3 ↑
cefuroxime [1 b]	-	-	8	11°	-	-
ciprofloxacin [a]	-	-	3	3	4 ↓	6°
levofloxacin/moxifloxacin [2]	0°	0°	-	-	-	-
erythromycin	15	10	-	-	6	5
doxycycline/tetracycline	17	10	2	2	1	1
co-trimoxazole	14	10	19 ↓	20	4	8 ↑

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

- = Resistance not calculated.

In 2021, the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in 2021 may therefore be lower.

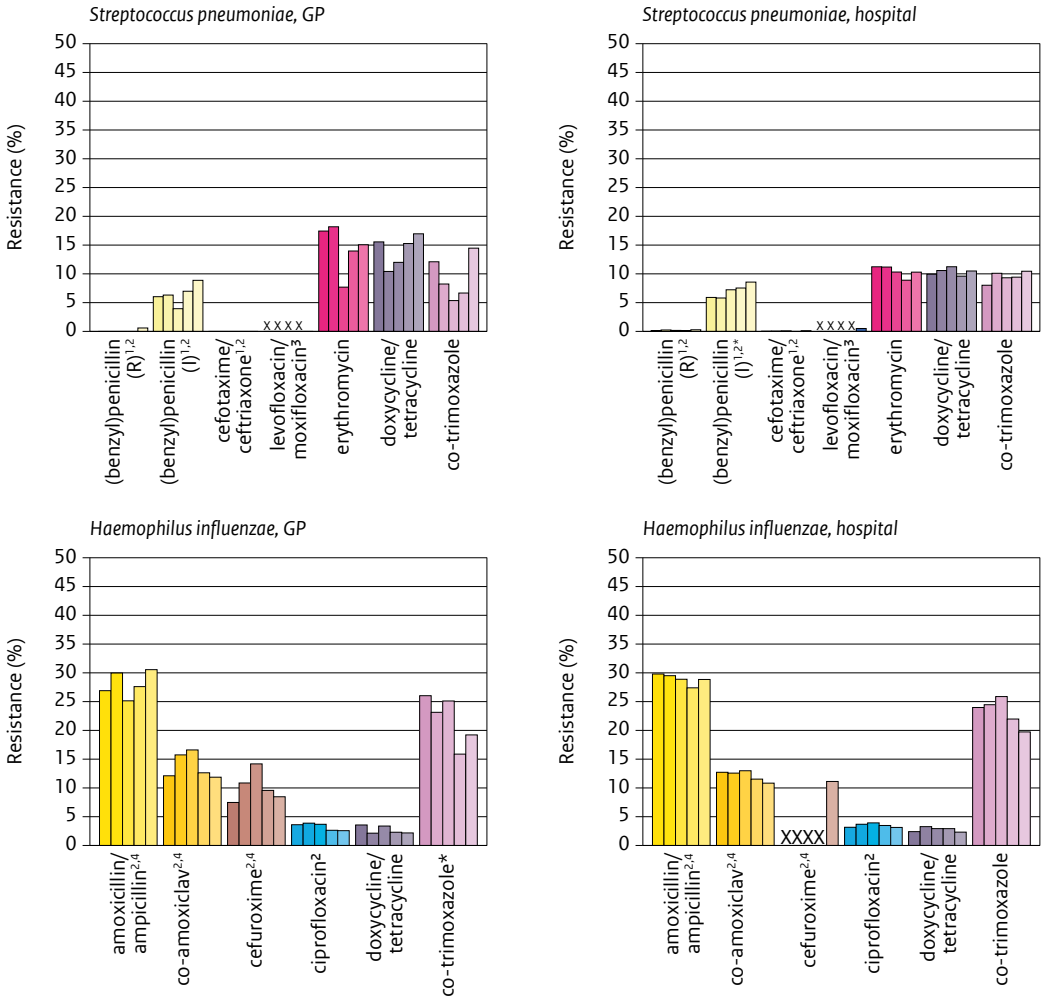
[1] *Resistance to beta-lactam antibiotics in S. pneumoniae and H. influenzae was calculated based on a flow chart according to directions in the EUCAST guidelines (for details see section 4.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). 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 4.1.1.*

[a] *According to breakpoint for indications other than meningitis (for ciprofloxacin this only applies to H. influenzae). For more details see section 4.1.1.*

[b] *According to breakpoint for intravenous administrations (for co-amoxiclav this only applies to H. influenzae). For more details see section 4.1.1.*

Figure 4.5.1 Trends in antibiotic resistance (from left to right 2019 to 2023) 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



In 2021 the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in these years may be lower.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

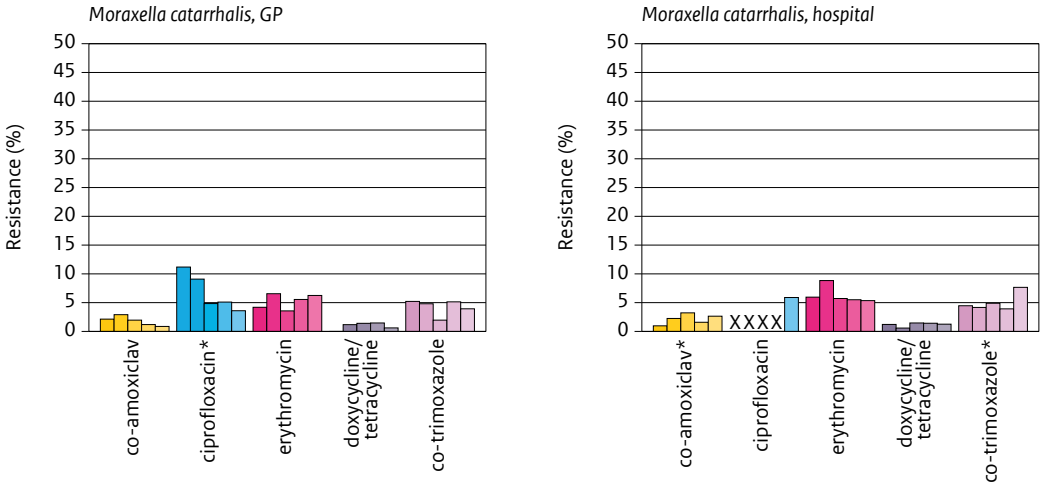
1 *Resistance to beta-lactam antibiotics in S. pneumoniae and H. influenzae was calculated based on a flow chart according to directions in the EUCAST guidelines (for details see section 4.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

2 *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

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 4.1.1.*

4 *According to breakpoint for intravenous administration. For more details see section 4.1.1.*

Figure 4.5.1 (continued) Trends in antibiotic resistance (from left to right 2019 to 2023) 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



In 2021 the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in these years may be lower.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[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 details see section 4.1.1). Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

[2] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

[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 4.1.1.*

[4] *According to breakpoint for intravenous administration. For more details see section 4.1.1.*

Key results

- *H. influenzae* was the pathogen most often cultured from lower respiratory tract samples in both GP and hospitalized patients.

S. pneumoniae

- In *S. pneumoniae*, the percentages I+R results for **(benzyl)penicillin** were 9% in both GP patients and hospital patients and increased over the last five years in hospital patients.
- Resistance to **doxycycline/tetracycline** in *S. pneumoniae* was higher in GP patients (17%) than in hospital patients (10%).

H. influenzae

- In *H. influenzae* isolates, resistance to **amoxicillin/ampicillin** was 31% in GP and 29% in isolates of hospitalized patients. Resistance to **co-amoxiclav** was 12% in GP patients and 11% in hospital patients. Resistance to **cefuroxime** was 8% in both GP and hospital patients. These resistance levels remained stable over the last five years except for **co-trimoxazole**, of which resistance levels decreased to 19% and 20% in both GP and hospitalized patients. Lowest resistance levels in *H. influenzae* were seen for **ciprofloxacin** (3%) and **doxycycline/tetracycline** (2%).

4.6 *Helicobacter pylori*

For *Helicobacter pylori* isolates from gastro-intestinal mucosa, pus and normally sterile tissue or liquid samples resistance levels in 2023 are presented in table 4.6.1. Five-year trends in resistance are shown in figure 4.6.1.

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. Nonetheless, 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.

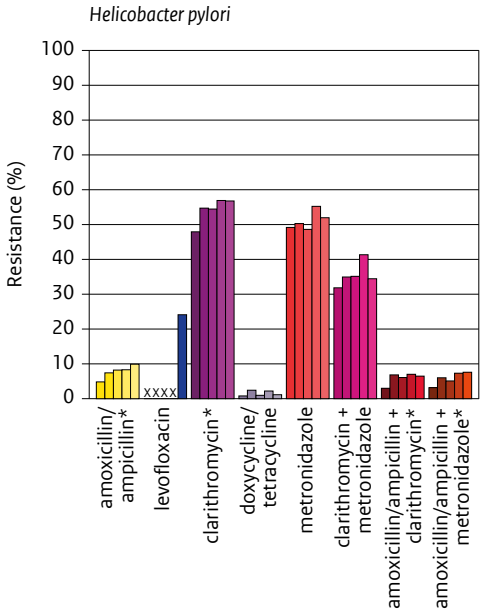
Table 4.6.1 Resistance levels (%) among 354 isolates sampled from gastro-intestinal mucosa, pus and normally sterile tissue or liquid of *H. pylori*, ISIS-AR 2023

	Helicobacter pylori
Antibiotic	
amoxicillin/ampicillin	10 ↑
levofloxacin	24°
clarithromycin	57 ↑
doxycycline/tetracycline	1
metronidazole	52
clarithromycin + metronidazole	34
amoxicillin/ampicillin + clarithromycin	6 ↑
amoxicillin/ampicillin + metronidazole	8 ↑

10 ↑	Significant and microbiologically relevant increasing trend since 2019.
10 ↓	Significant and microbiologically relevant decreasing trend since 2019.
10°	Trend not calculated because data from the years before 2023 did not meet the criteria for trend analysis.
10	No significant and microbiologically relevant time trend.

(For the criteria for trend analysis and the definition of a microbiologically relevant trend see section 4.1.1).

Figure 4.6.1 Trends in antibiotic resistance (from left to right 2019 to 2023) 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%.

** Trend is significant and microbiologically relevant (for details see section 4.1.1).*

Key results

H. pylori

- 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 to **amoxicillin/ampicillin** traditionally has been low but has increased substantially to 10% over the last five years. Resistance to **doxycycline/tetracycline** remained low (1%). Resistance to **claritromycine** (57%) and **metronidazole** (52%) remain high.

For the treatment of *H. pylori* infections, first choice combination treatment consists of **amoxicillin** and **clarithromycin**, of which combined resistance was 6% in 2023. If treatment fails, a combination of **tetracycline** plus **metronidazole** or **amoxicillin** plus **metronidazole** is recommended. Both have combined resistance levels of less than 10%.

4.7 Food-borne pathogens

Data on resistance amongst food-borne bacterial pathogens in humans are described in the report MARAN 2024, which can be found in the following chapters:

3.1.1 *Salmonella*

3.1.2 *Campylobacter*

3.1.3 Shiga-toxin producing *E. coli* and enteropathogenic *E. coli*

4.8 Highly resistant microorganisms

4.8.1 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 2019-2023 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 2023 clinical resistance breakpoints.

Results

In table 4.8.1.1 and 4.8.1.2, 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 2023. In figure 4.8.1.1, trends in ESBL percentages (from left to right 2019 to 2023) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown. Overall, the percentages of ESBL for *E. coli* and *K. pneumoniae* have been stable in the previous five years, with a slight decrease from 2019 to 2021 followed by a small increase to 2023 in most healthcare settings. The ESBL percentage for *K. pneumoniae* isolated from patients in ICUs varied over the consecutive years; the proportion increased from around 13% in 2019 to 15% in 2021, and decreased to 11% in 2023.

Table 4.8.1.1 Extended spectrum β-lactamase (ESBL) producing *E. coli* in the Netherlands in 2023, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL, number (%)[1]
General practitioners	130,821	5,439 (4)
Outpatient departments	23,796	1,440 (6)
Inpatient departments excl. intensive care units	31,633	2,054 (6)
Intensive care units	1,372	129 (9)
Total	**187,622**	**9,062 (5)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic E. coli isolate per patient was selected.

[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, based on re-interpretation of testvalues according to EUCAST 2023.*

Table 4.8.1.2 Extended spectrum β-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2023, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL, number (%)[1]
General practitioners	18,140	826 (5)
Outpatient departments	5,193	419 (8)
Inpatient departments excl. intensive care units	6,317	537 (9)
Intensive care units	330	37 (11)
Total	**29,980**	**1,819 (6)**

Numbers are based on a selection of 37 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, based on re-interpretation of testvalues according to EUCAST 2023.*

Figure 4.8.1.1 Trends in extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data

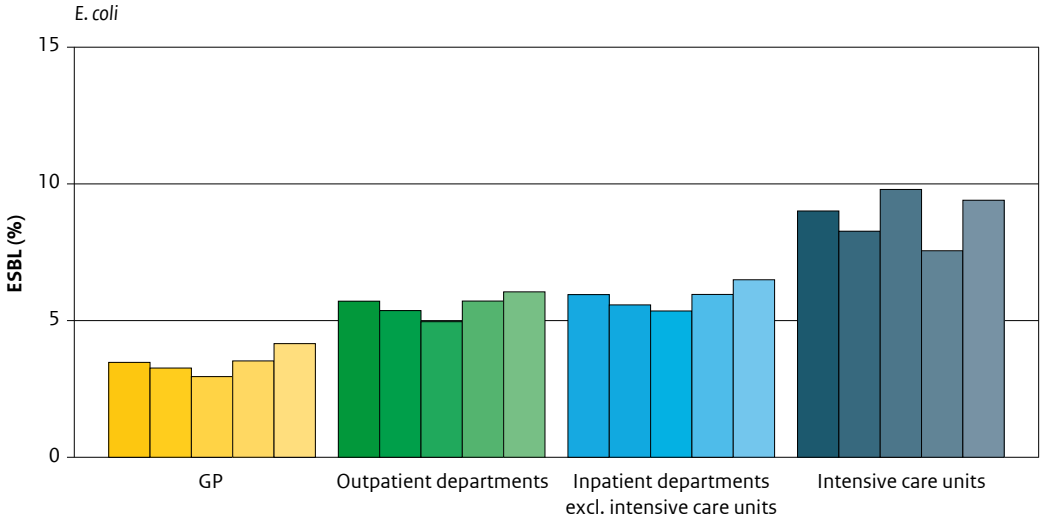
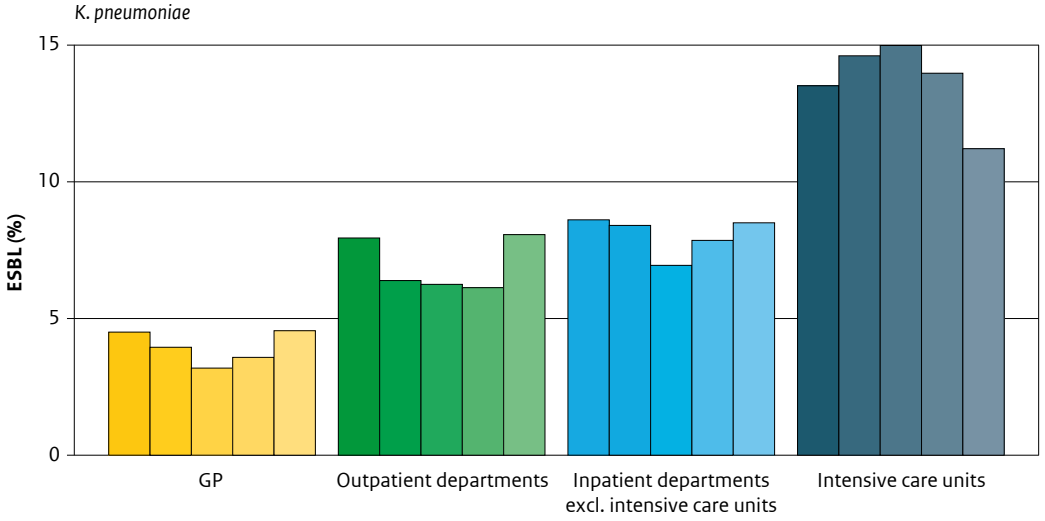


Figure 4.8.1.1 (continued) Trends in extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data



Numbers are based on a selection of 37 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.

[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, based on re-interpretation of testvalues according to EUCAST 2023.*

Discussion

Over the past 5 years, ESBL-producing *E. coli* remained a stable proportion of all isolates. In hospitalised patients, ESBL *E. coli* proportions are slightly higher than in the community, but still below 10%, indicating that we are able to control ESBL in the hospital, despite the emergence of hospital-adapted *E. coli* lineages such as MLST ST131 elsewhere in Europe.[2] ESBL-producing *K. pneumoniae* proportions on ICUs showed a peak in 2020-2021, which also has been observed in other countries during the COVID pandemic.[3] Together with the Scandinavian countries, ESBL *K. pneumoniae* proportions in invasive infections in the Netherlands are among the lowest.[4]

The current strategies applying surveillance and contact barrier precautions in hospitalized patients carrying ESBL-producing Enterobacterales seem to be successful. The continuation of this strategy is recommended in the upcoming SRI guidelines to prevent the transmission of highly resistant microorganisms.[5]

Conclusions

- From 2019 to 2023, the proportion of ESBL *E. coli* was stable in GP, outpatient departments and inpatient departments and ICUs.
- In the ICUs, ESBL *K. pneumoniae* showed a peak in proportions during the COVID-19 years and is decreasing in 2022-2023.

References

1 Surveillance of antimicrobial resistance in Europe, 2022 data.

2 Becerra-Aparicio F, Gómez-Zorrilla S, Hernández-García M, Gijón D, Siverio A, Berbel D, Sánchez-Carrillo C, Cercenado E, Rivera A, de Malet A, Xercavins M, Ruiz de Gopegui E, Canoura-Fernández L, Martínez JA, Seral C, Del Pozo JL, Cotarelo M, Díaz-Regañón J, Cantón R, Oliver A, Horcajada JP, Ruiz-Garbajosa P. Significant increase of CTX-M-15-ST131 and emergence of CTX-M-27-ST131 *Escherichia coli* high-risk clones causing healthcare-associated bacteraemia of urinary origin in Spain (ITUBRAS-2 project). J Antimicrob Chemother. 2023 Sep 5;78(9):2291-2296. doi: 10.1093/jac/dkad234. PMID: 37533351.

3 Mai HTT, Espinoza JL. The Impact of COVID-19 Pandemic on ESBL-Producing Enterobacterales Infections: A Scoping Review. Antibiotics (Basel). 2023 Jun 16;12(6):1064. doi: 10.3390/antibiotics12061064. PMID: 37370383; PMCID: PMC10294973.

4 ECDC Surveillance Atlas of Infectious Diseases

5 Samenwerkingsverband Richtlijnen Infectiepreventie: https://www.sri-richtlijnen.nl/

4.8.2 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 since increased.[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 a problem in hospitals 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 and confirmatory testing 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]

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. bugandensis*, *E. roggenkampii*, and *E. cloacae* complex not further specified. We searched the ISIS-AR database (years 2019-2023) for diagnostic (infection-related) isolates that were tested for meropenem and/or imipenem by an automated system. For *Proteus* spp., *Providencia* spp., *Serratia* spp. and *Morganella morganii*, only meropenem test results were included and analysed because of intrinsic imipenem resistance.[6] 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 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.

Categories ii, iii and iv are together referred to as elevated MIC. Subsequently, for all isolates, we searched the ISIS-AR and Type-Ned database for data on confirmatory tests (i.e., gradient strip tests and tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic)). We included only one

isolate per patient per micro-organism species or complex per year: an isolate with data on confirmatory tests (further referred to as CRE (by gradient strip testing)/CPE (by phenotypic and/or genotypic testing confirmed) was prioritized over an isolate with an automated test result only. If, subsequently, multiple isolates were eligible for inclusion, we prioritized the most resistant isolate. Based on data of isolates from 42 laboratories, we calculated numbers of isolates with automated MIC in the respective categories in 2023, the number of isolates with available data on confirmatory tests and the number of isolates that were CRE/CPE confirmed per micro-organism species or complex. Based on data from 36 laboratories that continuously submitted data to ISIS-AR from 2019 to 2023, we assessed the percentage of isolates i) with an elevated MIC based on automated testing, ii) with elevated automated MIC that underwent confirmatory testing, and iii) that are CRE/CPE confirmed, by year.

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=50). 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).[8] 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). Next-generation sequencing (NGS) and Nanopore long-read sequencing is performed for all isolates that are CIM positive.[9]

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 2019-2023. This includes all isolates belonging to genetic clusters. In 2023, due to the Russia-Ukraine war, a substantial number of CPE isolates were cultured from persons 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). Here, to include these isolates for NGS, CPE from persons without a person ID (n=88 CPE from 61 persons) were included for further analysis if it represented a unique person, based on sex, 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 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 wgMLST analysis. Except for the first isolate, clusters solely consisting of multiple isolates from the same patient, including over different years and/or submitted by different laboratories, were not counted. Since the end of 2019, genetic cluster numbers for CPE are reported to the submitting laboratory in Type-Ned.

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 collected by Municipal Health Services (MHS) and entered into the national web-based system for notifiable diseases (OSIRIS). Only notifications with a sampling date between 1 January and 31 December 2023 that are approved by the RIVM are included in this chapter. Notifications are reported to the RIVM and stratified into persons with diagnostic and screening isolates.

Outbreaks based on SO-ZI/AMR

The SO-ZI/AMR database (see chapter 4.8.7 for more details) was interrogated for CPE outbreaks that were reported in 2023.

Results

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

Absolute numbers of isolates and categorization according to automated MICs in 2023 are presented in Table 4.8.2.1. Of a total number of 334,729 isolates with an automated test value for meropenem or imipenem (214,575 *E. coli*, 36,507 *K. pneumoniae*, 13,221 *E. cloacae* complex, and 70,426 other Enterobacterales species), an elevated MIC on automated testing was found in 4.4% of isolates (14,736). CRE/CPE confirmed isolates were mostly found in *E. cloacae* complex (0.85%) followed by *K. pneumoniae* (0.41%), other Enterobacterales (0.28%), and *E. coli* (0.05%). Most CRE/CPE confirmation tests were performed for species in the category other Enterobacterales (6.9% of all isolates in the category), followed by *E. cloacae* complex (3.7%), *K. pneumoniae* (1.2%), and *E. coli* (0.34%).

Figure 4.8.2.1 shows automated and confirmatory carbapenem susceptibility testing results of the past 5 years. The overall prevalence of *E. coli* strains with CRE/CPE confirmation has been fluctuating around an average of 0.04% (Figure 4.8.2.1a). For *K. pneumoniae*, the prevalence of strains with CRE/CPE confirmation has decreased between 2019 and 2020 from 0.31% to 0.19% and increased between 2021 and 2023 from 0.25% to 0.41% (Figure 4.8.2.1a). The use of gradient strip tests fluctuates around an average of 70.5% for *E. coli* and 67.6% for *K. pneumoniae* with an elevated automated MIC. The use of phenotypical and genotypical carbapenemase tests as well as gradient strip tests to confirm elevated automated carbapenem MIC values decreased between 2019 and 2021 but increased in 2022 and 2023 for both species.

As for *E. cloacae* complex, the overall prevalence of strains with CRE/CPE confirmation decreased between 2019 and 2021 from 0.94% to 0.78% and increased in 2023 to 0.96% (Figure 4.8.2.1b). For the other Enterobacterales species, the prevalence of strains with CRE/CPE was on average 0.25% between 2019 and 2021, and increased in 2022 and 2023 from 0.29% to 0.34% (Figure 4.8.2.1b). The use of gradient strip tests fluctuates around an average of 70.5% for *E. cloacae* complex species and 40.0% for the other Enterobacterales species with elevated automated MIC. The use of phenotypical carbapenemase confirmation tests also fluctuates around an average of 16.2% for *E. cloacae* complex species and 6.4% for the other Enterobacterales species with elevated automated MIC. The use of genotypical carbapenemase confirmation tests decreased for both organism categories between 2019 and 2023 (Figure 4.8.2.1b).

Table 4.8.2.1 Results of automated and confirmatory carbapenem susceptibility testing among diagnostic (infection-related) Enterobacterales isolates in 2023, in 42 laboratories participating in ISIS-AR

	Automated 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)	213,510	867	110	88	214,575
CRE/CPE confirmatory test performed (N (%))	135 (0.06)	453 (52.2)	69 (62.7)	68 (77.3)	725 (0.34)
CRE/CPE confirmed (N (% of total))	11 (0.01)	47 (5.4)	12 (10.9)	33 (37.5)	103 (0.05)
K. pneumoniae					
Total (N)	35,892	437	78	100	36,507
CRE/CPE confirmatory test performed (N (%))	41 (0.11)	245 (56.1)	53 (67.9)	87 (87)	426 (1.2)
CRE/CPE confirmed (N (% of total))	1 (0.00)	53 (12.1)	17 (21.8)	79 (79)	150 (0.41)
***E. cloacae* complex**					
Total (N)	12,548	586	70	17	13,221
CRE/CPE confirmatory test performed (N (%))	49 (0.39)	369 (63)	58 (82.9)	14 (82.4)	490 (3.7)
CRE/CPE confirmed (N (% of total))	4 (0.03)	83 (14.2)	17 (24.3)	8 (47.1)	112 (0.85)
Other Enterobacterales* **					
Total (N)	58,043	12,212	128	43	70,426
CRE/CPE confirmatory test performed (N (%))	410 (0.71)	4,348 (35.6)	95 (74.2)	37 (86)	4,890 (6.9)
CRE/CPE confirmed (N (% of total))	6 (0.01)	151 (1.2)	19 (14.8)	23 (53.5)	199 (0.28)
All isolates					
Total (N)	319,993	14,102	386	248	334,729

CRE/CPE confirmed = confirmation by gradient strip test (>screening breakpoint) and/or phenotypical carbapenemase production test and/or genotypical carbapenemase gene PCR test.

* *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.13.0.*

*** *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 in this category are: Proteus mirabilis, Klebsiella oxytoca, Citrobacter koseri, Serratia marcescens, Morganella morganii.*

Figure 4.8.2.1a Proportion of isolates with elevated MIC, proportion of isolates which are CRE/CPE confirmed, and additional testing of isolates with elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* by year, in 36 laboratories, ISIS-AR 2019-2023

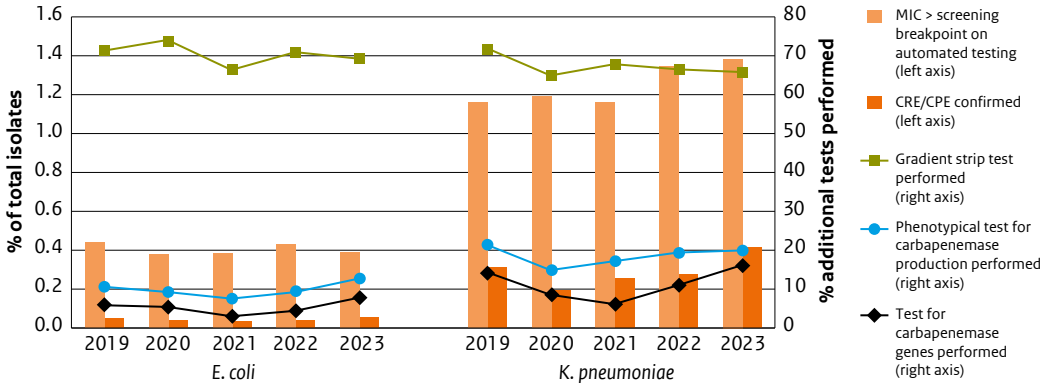
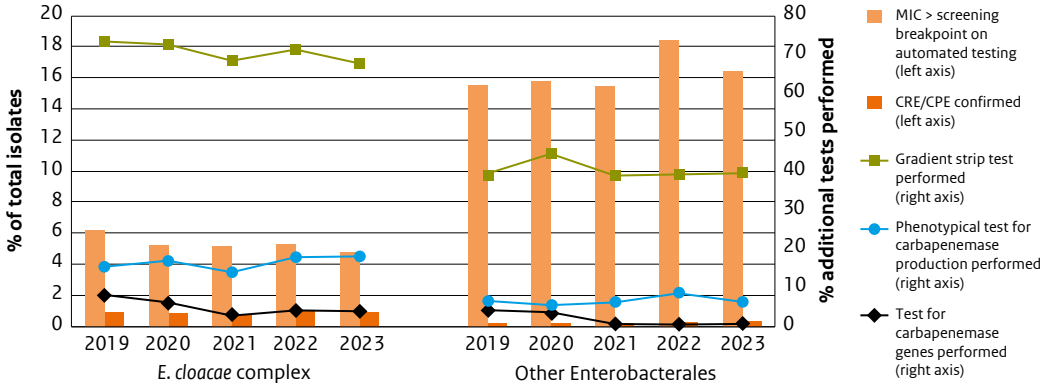


Figure 4.8.2.1b Proportion of isolates with elevated MIC, proportion of isolates which are CRE/CPE confirmed, and additional testing of isolates with elevated carbapenem MIC (%) in *E. cloacae* complex and other Enterobacterales by year, in 36 laboratories, ISIS-AR 2019-2023



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

Carbapenemase-production was confirmed in 579 Enterobacterales isolates (unique species/carbapenemase allele combinations per person) obtained in 2023 from 488 patients with and 91 without a person ID. Fifty-eight (10%) patients without a person ID were from Ukraine, and 33 (6%) 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 50 of the 51 Dutch medical microbiology laboratories. The number of unique CPE isolates submitted to the RIVM were 398 in 2019, decreased to 226 and 245 in 2020 and 2021, respectively, and increased with 19.6% from 484 in 2022 to 579 in 2023 (Figure 4.8.2.2a). Of the 579 CPE isolates in 2023, 236 (41%) were *E. coli*, 225 (39%) *K. pneumoniae*, 46 (8%) *E. cloacae* complex, and 21 (4%) *C. freundii* complex and the remaining 51 (9%) isolates belonged to other species.

As in previous years, the bla_{OXA-48} gene was the most frequently (18%, 107/579) identified carbapenemase-encoding gene in CPE isolates from 2023 (Figure 4.8.2.2b). In addition, of the CPE analysed in 2023, 37% (214/579) carried a bla_{OXA-48} or bla_{OXA-48}-like gene ($bla_{OXA-181}$, $bla_{OXA-232}$, $bla_{OXA-244}$ and $bla_{OXA-1207}$). The bla_{OXA-48} gene was found in equal percentages (18%) in the *K. pneumoniae* and *E. coli* isolates, while the bla_{OXA-48}-like alleles were found in 14% and 28% of the *K. pneumoniae* and *E. coli* isolates, respectively. Despite this, the overall trend in the past 5 years is a decline of the presence of bla_{OXA-48} among CPE from 34% in 2019 to 18% in 2023 (Figure 4.8.2.2c). In 2023, there was an increase in $_{OXA-48}$-like-carrying CPE when compared to previous years (Figure 4.8.2.2b and 4.8.2.2c). In *E. coli*, 32% (75/236) of the isolates carried bla_{NDM-5} and the gene was found in 7% (16/225) of the *K. pneumoniae* isolates. Conversely, bla_{NDM-1} was found predominantly in *K. pneumoniae* isolates (20%, 45/225) and only in 5% (12/236) of the *E. coli* isolates.

Overall, when the EUCAST clinical breakpoints were applied, 259/579 (45%) of the CPE isolates were resistant (MIC >8 mg/L) to meropenem and 248/579 (43%) were susceptible for meropenem (MIC ≤2 mg/L). Twelve percent (72/579) of the CPE were susceptible with increased exposure (MIC >2 and ≤8 mg/L). In 2023, it was the first time in the past 5 years that more resistant than susceptible CPE isolates were submitted to Type-Ned. In general, a larger proportion of the *K. pneumoniae* isolates (70%, 158/225) was meropenem resistant compared to the *E. coli* isolates (32%, 76/236), irrespective of the carbapenemase-encoding genes present. Forty-eight of the 50 (96%) $bla_{OXA-244}$ *E. coli* isolates submitted in 2023 and 32/36 (89%) of the $bla_{OXA-181}$ carrying CPE had MICs for meropenem ≤2 mg/L. Only 25/107 (23%) of the CPE carrying bla_{OXA-48} had a MIC above the clinical breakpoint for meropenem resistance (MIC >8 mg/L), while this was 66/101 (65%) for CPE carrying bla_{NDM-5}. Nine percent (55/579) of the CPE carried two carbapenemase-encoding genes, and in these cases 95% (52/55) of the isolates were resistant for meropenem. In 23/579 (4%) of the isolates no carbapenemase-encoding gene was detected. Of these isolates, 14 (61%) were *Enterobacter* spp. and 9 (39%) 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} (van Gorp *et al.*, in preparation).

Figure 4.8.2.2 Numbers of carbapenemase-producing Enterobacterales (CPE), per species (a), per carbapenemase allele (b), and trend in detected carbapenemase genes (c), based on Type-Ned, 2019-2023

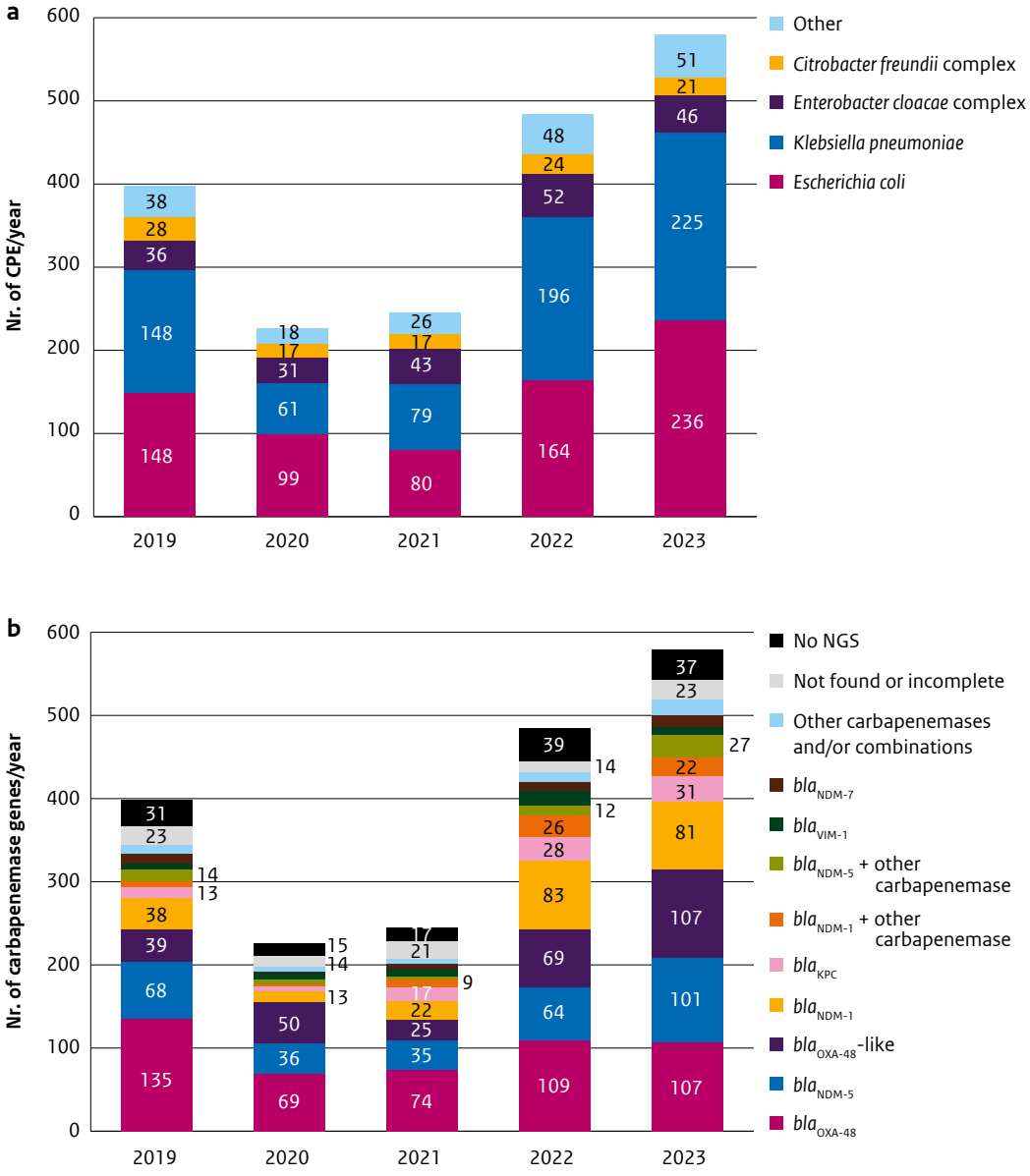
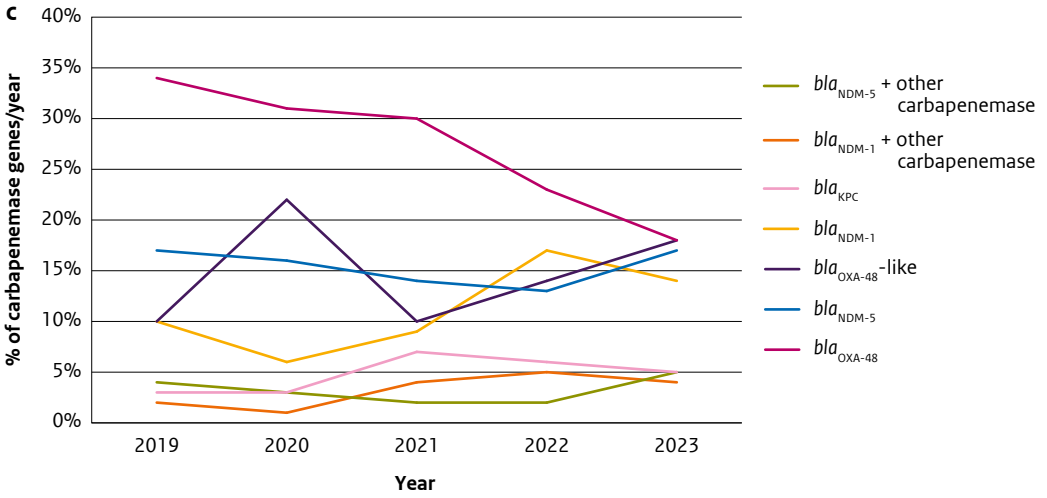


Figure 4.8.2.2 (continued) Numbers of carbapenemase-producing Enterobacterales (CPE), per species (a), per carbapenemase allele (b), and trend in detected carbapenemase genes (c), based on Type-Ned, 2019-2023



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

Between 2019 and 2023, the CPE that could be typed by multilocus sequence typing (MLST) revealed a waxing and waning of the vast majority (n=368 of 393) of distinct MLST sequence types through the years (not shown). However, there was a marked increase in globally epidemic and multidrug-resistant lineages, such as *K. pneumoniae* ST147, ST307, ST395 and *E. coli* ST361, ST405 and ST410 especially in 2022 and 2023 (Table 4.8.2.2). Some CPE lineages persisted in the Netherlands, such as *E. coli* ST38, ST167, ST131, uropathogenic *E. coli* ST127,10 *C. freundii* ST22 and *E. cloacae* complex ST78.

Table 4.8.2.2 Results of multilocus sequence typing (MLST) of 1932 CPE isolates from 2019-2023*

Species	MLST sequence type	2019	2020	2021	2022	2023	Total
K. pneumoniae	147	27	12	6	35	58	138
E. coli	38	24	19	8	13	18	82
K. pneumoniae	307	7	5	10	31	25	78
E. coli	167	20	4	5	12	22	63
K. pneumoniae	395	4	2	3	18	21	48
C. freundii	22	17	7	7	5	7	43
E. coli	410	5	6	2	14	15	42
E. cloacae complex	121	2	8	10	13	6	39
E. coli	10	6	5	3	9	13	36
E. coli	405	8	5	2	5	15	35
K. pneumoniae	16	12	2	4	8	9	35
E. coli	131	6	6	2	7	11	32
K. pneumoniae	11	4	2	7	8	10	31
E. coli	127	8	4	4	4	7	27
E. cloacae complex	78	8	7	5	2	5	27
E. coli	361	4		1	8	14	27
Not shown	various	236	132	166	292	323	1,149
Total		**398**	**226**	**245**	**484**	**579**	**1,932**

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

Based on species-specific whole-genome multilocus sequence typing (wgMLST), 742 of the 1932 (38%) isolates from 2019-2023 fell in one of the detected 217 genetic clusters, including clusters partially containing older isolates (<2019). The species involved were: *K. pneumoniae* (99 clusters), *E. coli* (90 clusters), *E. cloacae* complex (13 clusters), *C. freundii* complex (9 clusters), *Klebsiella aerogenes* (1 cluster), *Proteus mirabilis* (4 clusters), *Providencia stuartii* (3 clusters), *Raoultella ornithinolytica* (1 cluster) and *Serratia marcescens* (1 cluster). The five largest clusters concerned the following: *C. freundii* complex with bla_{NDM-5} with isolates from 56 persons, *E. cloacae* complex carrying bla_{OXA-48} with isolates from 43 persons, *E. coli* $bla_{OXA-244}$ with isolates from 24 persons, *E. coli* bla_{OXA-48} with isolates from 29 persons, and *K. pneumoniae* bla_{NDM-1} and/or bla_{OXA-48}

with isolates from 30 persons. The numbers depicted here include isolates from before and within the study period of 2019-2023. These five clusters spanned 2 to 9 years. The two *E. coli* clusters were multi-institutional,[10,11] as was the *K. pneumoniae* cluster, which consisted largely of isolates from persons from Ukraine.[12] The other two clusters were predominantly from one institute each. MMLs are notified by email if isolates they submitted are part of a genetic cluster if that cluster contains more isolates from within the previous year. Fifty-one new clusters were detected in 2023, 27 involving *K. pneumoniae*, 17 *E. coli*, 2 *C. freundii* complex, 2 *E. cloacae* complex and one each of *P. mirabilis*, *P. stuartii* and *R. ornithinolytica*. The majority (31) of the new clusters contained only two isolates, the largest contained 7 *E. coli* and 44 (86%) clusters concerned multi-institutional genetic clusters. Nineteen clusters involved isolates from 2023 only. Ten of the nineteen clusters, of which 8 were *K. pneumoniae*, involved persons from Ukraine.[12,13] Ten (59%) of the new *E. coli* clusters carried the $bla_{\text{NDM-5}}$ gene. Nineteen (70%) of the *K. pneumoniae* clusters involved at least an NDM-variant, in 11 occasions in combination with a $bla_{\text{OXA-48}}$-like carbapenemase gene.

Clinical/epidemiological characteristics of persons with carbapenemase-producing Enterobacterales based on mandatory notifications in OSIRIS

Additional epidemiological questionnaire data was available in OSIRIS for 483 CPE positive persons with a sampling date in between 1 January 2023 and 31 December 2023 (Table 4.8.2.3). This is much higher than the previous years (n= 179 in 2019 (July-December), n=170 in 2020, n=201 in 2021, n=376 in 2022). The median age of the 483 persons was 62 (range 0 - 96) years and 273 (56%) were male.

A sample taken for diagnostic purposes was the reason for sampling in 24% (118/483) of the notified persons in 2023 (Table 4.8.2.3), compared to 31% (55/179) in 2019 (July-December), 28% in 2020 (48/170), 25% (50/201) in 2021, and 23% (86/376) in 2022. Most patients with a diagnostic isolate had no known risk factor identified (68%) (Figure 4.8.2.3). Hospitalization abroad for at least 24 hours within the previous two months occurred in 10% of patients with a diagnostic isolate, which was comparable to 2020 (10%) and 2021 (8%), but much lower than in 2022 (21%) (Table 4.8.2.3 and Figure 4.8.2.3). Ukraine, Morocco, and Italy (all n=2) were most often reported as countries of hospitalization. The most common reported infection among patients with a diagnostic isolate was urinary tract infection (48%, 57/118), followed by bacteraemia/sepsis (10%, 12/118) and wound infection (7%, 8/118).

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% (359/483) of the persons in 2023, compared to 66% (119/179) in 2019 (July-December), 69% (118/170) in 2020, 73% (146/201) in 2021, and 74% (280/376) in 2023. Hospitalization abroad for at least 24 hours within the previous two months was the most common reported risk factor for the presence of CPE among persons with a screening isolate in 2023 (61%) which was comparable to 2022 (62%), but higher than the years before 2022 (54% in 2019 (July-December), 42% in 2020, and 49% in 2021) (Table 4.8.2.3 and Figure 4.8.2.3). Turkey (n=47), Ukraine (n=35), Egypt (n=18) and Morocco (n=16) were most often reported as countries where hospitalization had occurred. No risk factor could be identified in 19%. For the 6 (1%) remaining persons the reason for sampling was different from diagnostic or screening or was unknown.

Table 4.8.2.3A Epidemiological data of notifications of persons carrying CPE, OSIRIS, 2023

Characteristic	Total[a] N (%)	Diagnostic N (%)	Screening N (%)
N	483	118	359
Location of sampling			
Outpatient/emergency departments or by a general practitioner	197 (40.8)	64 (54.2)	130 (36.2)
Inpatient departments (excl. intensive care units)	240 (49.7)	45 (38.1)	192 (53.5)
Intensive care units	33 (6.8)	6 (5.1)	27 (7.5)
Other/unknown	13 (2.7)	3 (2.5)	10 (2.8)
Residence			
Living independently	349 (72.3)	95 (80.5)	250 (69.6)
Rehabilitation centre	14 (2.9)	1 (0.8)	13 (3.6)
Nursing or elderly home/facilities for small-scale housing for elderly	28 (5.8)	5 (4.2)	22 (6.1)
Asylum seekers centre	28 (5.8)	4 (3.4)	24 (6.7)
Other/unknown	64 (13.3)	13 (11.0)	50 (13.9)
Invasive medical procedure/diagnostics			
No	141 (29.2)	49 (41.5)	90 (25.1)
Surgery	147 (30.4)	29 (24.6)	116 (32.3)
Other (incl. endoscopy, cystoscopy, urinary catheter, renal dialysis)	76 (15.7)	16 (13.6)	60 (16.7)
Yes but unknown which invasive procedure(s)	15 (3.1)	3 (2.5)	12 (3.3)
Unknown	104 (21.5)	21 (17.8)	81 (22.6)
Identified risk factors			
No risk factor known/unknown	154 (31.9)	80 (67.8)	68 (18.9)
Hospitalization abroad >24 hours during the previous two months	232 (48.0)	12 (10.2)	220 (61.3)
Contact with a hospital abroad in the past twelve months in a different way than >24 hours during the previous two months	40 (8.3)	15 (12.7)	25 (7.0)
Travelling abroad in the past twelve months without hospitalization or visiting a hospital	39 (8.1)	9 (7.6)	30 (8.4)
Already known carrier of CPE	10 (2.1)	1 (0.8)	9 (2.5)
Received care in a department of a healthcare facility with an ongoing outbreak of CPE in the previous two months	8 (1.7)	1 (0.8)	7 (1.9)

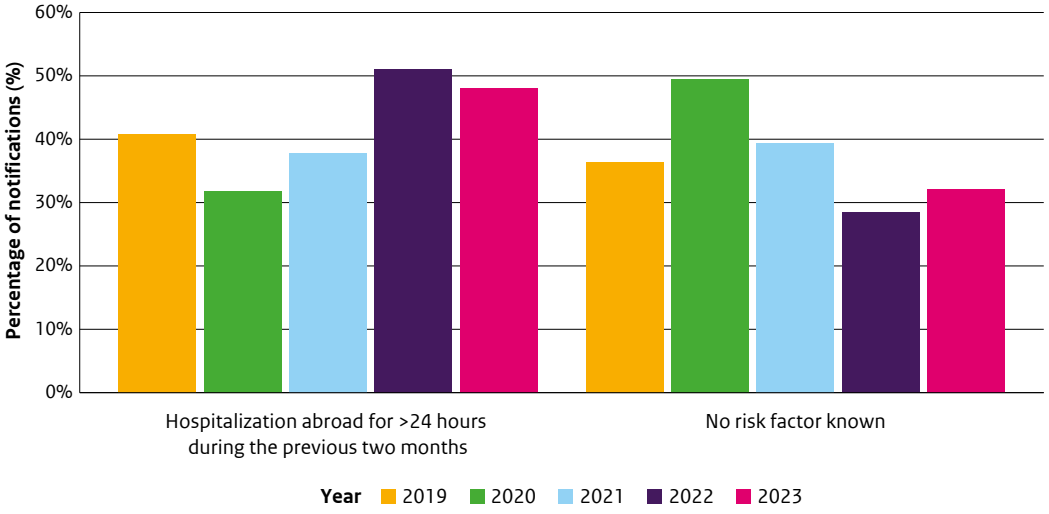
[a] *Including persons for whom the reason for sampling was unknown.*

Figure 4.8.2.3B Hospitalization abroad for at least 24 hours during the previous two months and no risk factor known among CPE positive persons (diagnostic and screening combined), OSIRIS, 2019-2023

Characteristic	Total[a] N (%)	Diagnostic N (%)	Screening N (%)
N	232	12	220
Hospitalized in a country in			
Western Asia	60 (25.9)	1 (8.3)	59 (26.8)
Northern Africa	43 (18.5)	3 (25.0)	40 (18.2)
Eastern Europe	43 (18.5)	2 (16.7)	41 (18.6)
Southern Europe	21 (9.1)	2 (16.7)	19 (8.6)
Southern Asia	18 (7.8)	1 (8.3)	17 (7.7)
Other region of the world/unknown	47 (20.3)	3 (25.0)	44 (20.0)

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

Figure 4.8.2.3 Hospitalization abroad for at least 24 hours during the previous two months and no risk factor known among CPE positive persons (diagnostic and screening combined), OSIRIS, 2019-2023[a]



[a] *Data from 2019 only includes notifications from 1 July-31 December 2019 since mandatory notification started on 1 July 2019.*

Outbreaks based on SO-ZI/AMR

In 2023, two new outbreaks with CPE in hospitals and one in a long-term care facility (LTCF), were reported to SO-ZI/AMR. See chapter 4.8.7 for more details about SO-ZI/AMR.

Table 4.8.2.4 Outbreaks of carbapenemase-producing Enterobacterales reported in 2023 to the Early warning and response meeting for Healthcare-associated Infections and Antimicrobial Resistance (SO-ZI/AMR)

Region	Facility	Microorganism	Gene	No. of patients
West	Hospital	*K. pneumoniae*	bla_{OXA-48} + bla_{NDM}	2
Middle	Hospital	*K. pneumoniae*	bla_{NDM}	8
East	Long-term care facility	*E. coli*	bla_{OXA-48}	7

Discussion

In ISIS-AR, the prevalence of confirmed carbapenem resistance (by demonstrating with gradient strip testing meropenem and/or imipenem MICs above the screening breakpoint and/or demonstrating carbapenemase production) of *E. coli* was 0.05% in 2023 which was comparable to the previous years. Confirmed carbapenem resistance in *K. pneumoniae* is significantly higher compared to *E. coli*, and has slightly increased from 0.2% in 2020 to 0.4% in 2023. Other Enterobacterales show similar patterns as *E. coli*. Since 2021, the revised Dutch national guideline suggests to perform tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic) of isolates with an elevated MIC based on automated tests, and gradient strip tests are not warranted anymore. The percentage of isolates with elevated automated MIC with a gradient strip test performed has already been decreasing since 2017, especially for *E. coli* and *K. pneumoniae*. On the other hand, the percentages of confirmatory carbapenemase tests among *E. coli* and *K. pneumoniae* isolates with elevated automated MIC has been stable or increasing since 2021, in accordance with the new guideline. The percentage of confirmed CRE/CPE isolates differed between the various species categories within Enterobacterales. Even among the isolates with automated MIC > the clinical R breakpoint, the confirmation of carbapenemase production percentage was below 50% for both *E. coli* and *E. cloacae* complex isolates. 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. Furthermore, the data showed that there could be substantial discrepancies between automated MIC values and MICs determined by gradient strip test, regularly leading to an overestimation of phenotypical resistance measured by automated MIC testing.

In 2023, the number of carbapenemase-producing Enterobacterales isolates that was submitted to the RIVM was considerably higher than in previous years and was succeeding pre-COVID totals. 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. The increase in 2022-2023 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,[12] resulting in Ukraine being the second country of all reported countries where recent hospitalization abroad had occurred.

A large number (51) of new genetic clusters were found in 2023, compared to previous years (45 in 2022, five in 2021, 14 in 2020). Most of the clusters, especially with *E. coli*, were small clusters of 2 isolates only, whereas a number of the *K. pneumoniae* clusters involved a larger number of isolates, up to 7.

Forty-four (86%) of the new clusters involved more than one laboratory. 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 direct 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 2023 compared to previous years. The percentage of persons with a diagnostic sample decreased over time and, as a logical consequence, the percentage with a screening isolate increased over time but was comparable to 2022. The increase of screening samples in the last two 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 automated test value for meropenem or imipenem (i.e., > the screening breakpoint) was 5.8% in 2023. The prevalence of CRE/CPE confirmed isolates among *E. coli* was 0.05%, among *K. pneumoniae* 0.41%, *E. cloacae* complex 0.96% and other Enterobacterales 0.34%.
- In 2023, the number of carbapenemase-producing Enterobacterales 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 carbapenemase-producing Enterobacterales species in 2023 were *E. coli*, *K. pneumoniae*, *E. cloacae* complex, and *C. freundii* complex, respectively.
- The most frequently identified carbapenemase encoding genes in Enterobacterales were $bla_{\text{OXA-48}}$, $bla_{\text{OXA-48}}$-like, $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$.
- There is a decrease of OXA-48-producing CPE, and increase of $bla_{\text{OXA-48}}$-like (i.e., $bla_{\text{OXA-181}}$, $bla_{\text{OXA-232}}$, $bla_{\text{OXA-244}}$ and $bla_{\text{OXA-1207}}$) CPE in the Netherlands.
- The MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates.
- There is a marked increase in globally epidemic multidrug-resistant *E. coli* and *K. pneumoniae* lineages.
- Of the isolates found between 2019 and 2023, 38% could be categorized into one of the known 217 genetic clusters.
- Fifty-one new genetic clusters were detected in 2023, 44 of which concerned multi-institutional genetic clusters. Thirty-one of the 51 new genetic clusters comprise two isolates only.
- Twenty-four percent of CPE cases were identified in diagnostic samples, 74% were identified upon routine screening or targeted screening because of suspected CPE carriage, and for 1% a different/unknown reason was reported.
- In 48% of patients, there is a relation with hospitalization abroad for more than 24 hours during the preceding two months (10% and 61% among persons with a diagnostic and screening isolate, respectively), which was lower than in 2022 but comparable to the years before 2022. Turkey, Ukraine, Egypt, and Morocco are the countries that are most often reported.
- In 32% of the CPE positive persons no known CPE risk factor was identified (68% and 19% among persons with a diagnostic and screening isolate, respectively), which is higher than in 2022, but lower than the years before 2022.

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4.8.3 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 two different surveillance systems: 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 2019-2023. 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 (production) and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2023 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 *P. aeruginosa* that was MDR was calculated. Multidrug resistance was defined as resistance to ≥3 antimicrobials or antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam, based on re-interpretation of test-values according to the EUCAST 2023 breakpoints. Only isolates which were tested for all five (groups of) antimicrobials were included in the latter analysis. CPPA strains were also classified as carbapenem-resistant. The numbers were based on a selection of 37 laboratories (out of a total of 50 laboratories in the Netherlands), which provided complete data on the last five years (2019 to 2023).

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=49), however, not all laboratories submit 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). The presence of carbapenemase-encoding genes are assessed by PCR (carba-PCR on bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{OXA}), and next-generation sequencing (NGS) and Nanopore sequencing is performed 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 2020-2023, and all of these isolates collected in 2023 were included. As in 2022, 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. 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

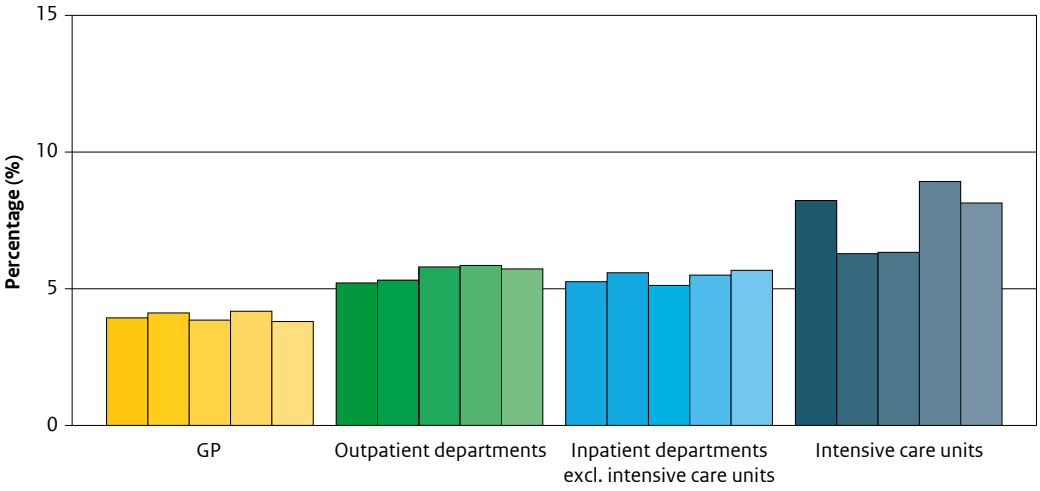
Based on the ISIS-AR database, 5% (921/18,037) of the diagnostic *P. aeruginosa* isolates were carbapenem-resistant in 2023 (Table 4.8.3.1). The percentage of carbapenem-resistant *P. aeruginosa* isolates was higher among *P. aeruginosa* isolates from patients in intensive care units (ICUs) compared to other departments. The observed proportion of resistance appears to be relatively stable over the 2019-2023 time period, except for a decrease in the proportion of carbapenem resistance in *P. aeruginosa* isolates from ICUs during the COVID-years 2020 and 2021 (Figure 4.8.3.1). Of the total number of 921 carbapenem-resistant *P. aeruginosa* isolates, for 123 (13%) isolates, data on tests for carbapenemase production was available, of which 25 (20%) showed a positive result.

Table 4.8.3.1 Carbapenem-resistant *P. aeruginosa* among diagnostic *P. aeruginosa* isolates in the Netherlands in 2023, based on ISIS-AR data

Type of department	Tested isolates, N	Carbapenem-resistant *P. aeruginosa*, N (%)
General practitioners	6,340	241 (4)
Outpatient departments	5,117	293 (6)
Inpatient departments excl. intensive care units	6,027	342 (6)
Intensive care units	553	45 (8)
Total	**18,037**	**921 (5)**

Numbers are based on a selection of 37 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 2023 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 4.8.3.1 Percentages of carbapenem-resistant *P. aeruginosa* among diagnostic *P. aeruginosa* isolates in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data



Numbers are based on a selection of 37 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 2023 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).

Additional analyses in the 2023 ISIS-AR database showed that 2% (n=266) of 17,244 diagnostic *P. aeruginosa* isolates tested for all five (groups of) antimicrobials included in the MDR definition, were MDR (Table 4.8.3.2) and 65% (173/266) of the MDR isolates were carbapenem-resistant.

Table 4.8.3.2 Multidrug resistant (MDR) *P. aeruginosa* among diagnostic *P. aeruginosa* isolates in the Netherlands in 2023, based on ISIS-AR data

Type of department	Tested isolates, N	MDR *P. aeruginosa*, N (%)	Carbapenem-resistant MDR *P. aeruginosa*, N (%)
General practitioners	6,252	36 (1)	22 (61)
Outpatient departments	4,838	109 (2)	64 (59)
Inpatient departments excl. intensive care units	5,651	104 (2)	75 (72)
Intensive care units	503	17 (3)	12 (71)
Total	**17,244**	**266 (2)**	**173 (65)**

Numbers are based on a selection of 37 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 2023;
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 2023 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

In the enhanced surveillance, the RIVM received 57 *P. aeruginosa* isolates from samples collected in 2023 through Type-Ned, all of which produced carbapenemase according to the CIM. The CPPA were from 56 patients and these isolates were submitted by 26 MMLs. One patient carried two unrelated CPPA strains harbouring either bla_{NDM-1} or bla_{IMP-1}. Sixteen of the 57 CPPA isolates (28%) were obtained from Ukrainian patients. From 54 of the 57 isolates whole-genome sequencing data was available upon analysis. Analysis of whole-genome sequencing data of 54/57 CPPA isolates revealed that 22 of the 54 carbapenemase-producing isolates (41%) carried a bla_{NDM-1} allele, seventeen harboured bla_{VIM-2} (31%) and six bla_{IMP-1} (11%). Two isolates carried bla_{VIM-1} (4%), two bla_{VIM-6} (4%) and two bla_{VIM-11} (4%). bla_{IMP-10}, bla_{IMP-13}, or bla_{GES-5} were detected in one single isolate each (6%). Of the sixteen CPPA isolates from Ukrainian patients, seven harboured a bla_{NDM-1} allele, six bla_{IMP-1}, one bla_{VIM-1}, one bla_{VIM-2} and one was unknown.

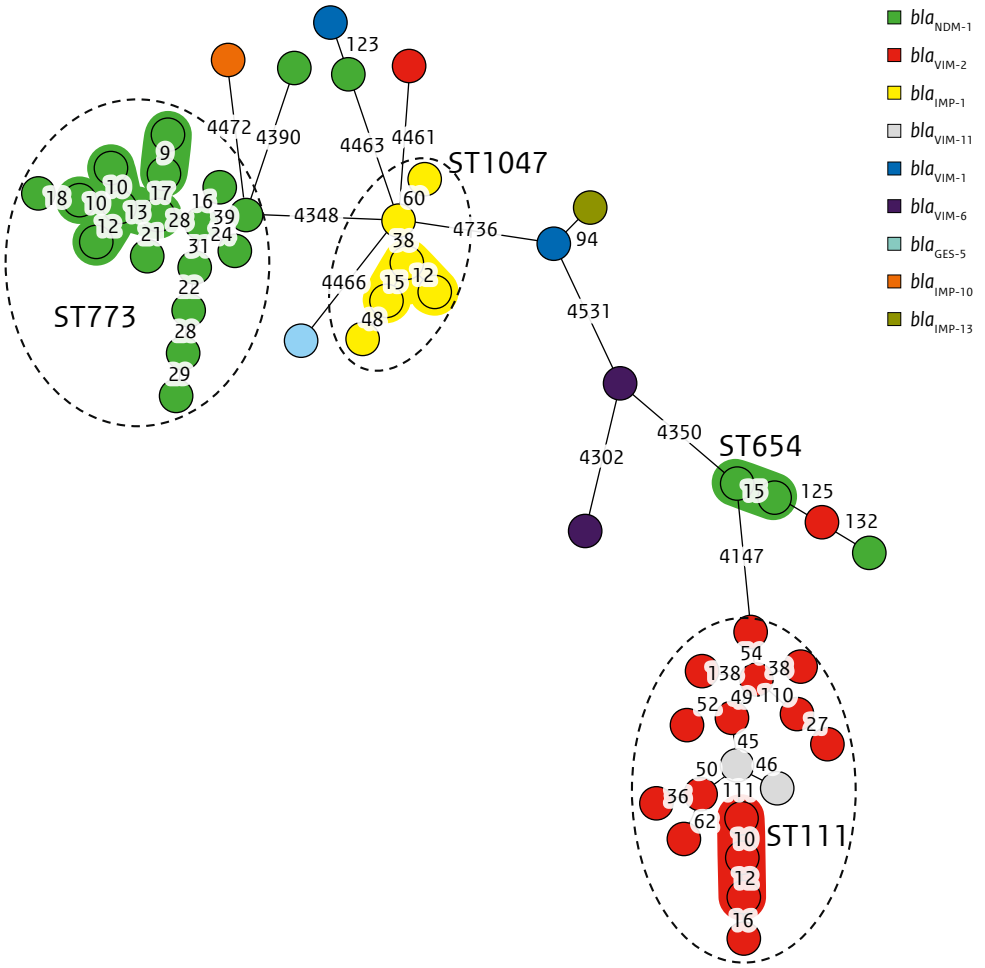
The genetic relations of the 54 sequenced isolates were assessed by performing wgMLST (Figure 4.8.3.2). This revealed that most of the isolates belonged to three distinct groups, generally according to the carbapenemase allele they harbored and MLST sequence type ST111, ST773 and ST1047. One group of seven CPPA ST773 isolates, two groups of three isolates (ST111 and ST1047), and one group of two CPPA ST654 isolates differed in ≤15 wgMLST alleles and could therefore be regarded as genetic clusters. The isolates of the CPPA ST773 and ST1047 clusters were found among Ukrainian (n=9) and Dutch (n=1) patients in nine different hospitals, while for the ST111 and ST654 clusters, isolates were found in patients in the same hospital. Of the CPPA isolates, 89% (51/57) had MICs for meropenem above the clinical EUCAST breakpoint of 8 mg/L (Table 4.8.3.3), whereas five CPPA isolates with a bla_{VIM-2} allele (9%) had an MIC below the clinical breakpoint. The following sample materials were reported: fifteen CPPA were from screening swabs, seven were from wound, ten from urine samples, three from urine catheter, and the remainder from other isolation sites. The majority (54/57) of the CPPA were obtained from materials sent in by hospitals.

Table 4.8.3.3 Distribution of carbapenemase-encoding genes based on whole-genome sequencing and susceptibility for meropenem in carbapenemase-producing *P. aeruginosa* isolates received via the enhanced CPPA surveillance in 2023

MIC meropenem (mg/L)	bla_{GES-5}	bla_{IMP-1}	bla_{IMP-10}	bla_{IMP-13}	bla_{NDM-1}	bla_{VIM-1}	bla_{VIM-2}	bla_{VIM-6}	bla_{VIM-11}	No NGS	Total
>32	1	6	1	1	22	2	12		2	3	50
32								1			1
8								1			1
6							2				2
4							2				2
2							1				1
Total	**1**	**6**	**1**	**1**	**22**	**2**	**17**	**2**	**2**	**3**	**57**

NB: "No NGS" indicates that whole-genome sequencing has not been performed.

Figure 4.8.3.2 wgMLST-based minimum spanning tree of 54/57 CPPA isolates from patients sampled in 2023, 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 31 of the 56 CPPA-carrying persons, of whom 27 were in-hospital patients. Twenty-six patients (46%) were male and the median age was 39 years (range 1-89 years). Five patients (5/31, 16%) were admitted to the ICU at the moment of sampling, 22 (71%) were admitted to a non-ICU hospital department, and three (10%) samples were taken at the outpatient department.

Nine questionnaires came from Ukrainian patients. For 3/9 (33%) of the Ukrainian patients, clinical signs of an infection was mentioned as the reason for taking the sample. Seven of these patients (7/9, 78%) had

been admitted >24 hours in a hospital abroad in the previous two months (all in Ukraine except one for whom the country was unknown).
Twenty-two questionnaires concerned non-Ukrainian patients. In these patients, 6/22 samples (27%) were taken for diagnostic purposes, while for the other sixteen (73%), the reason for sampling was screening. Six patients (6/22, 27%) had been admitted >24 hours in a hospital abroad in the previous two months, namely two in Thailand, and one in Morocco, Greece, Somalia or Tanzania. Four of the non-Ukrainian patients were admitted to the ICU at the moment of sampling, and five patients (including one of the ICU patients) had (severe) comorbidities.

Discussion

In 2023, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 13% of these isolates, data on carbapenemase tests (phenotypically or genotypically) performed by the participating MMLs, were available in the ISIS-AR database. Of the 123 carbapenem-resistant isolates with carbapenemase test results, 25 were positive for carbapenemase production. Not all phenotypically carbapenem-resistant 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 stable in 2023, 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.[4] 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.[2] Consequently, the 2023 results of the enhanced CPPA surveillance submitted via Type-Ned were very different to those of 2021. Twenty-eight percent of the CPPA isolates in the enhanced surveillance in 2023 were from samples of 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 (63%) carbapenemase-encoding allele in CPPA was $bla_{\text{VIM-2}}$, in 2022 this was reduced to 18% and dominated the $bla_{\text{NDM-1}}$ allele in 33% of the isolates, while $bla_{\text{NDM-1}}$ further increased to 41% in 2023. CPPA with $bla_{\text{IMP-1}}$ were only found in Ukrainian patients.
For almost half 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 a third of the patients had been hospitalized abroad and one sixth had severe 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, 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 2023. Therefore, the data as shown here are most likely an underestimation of the numbers present in the Netherlands.

Conclusions

- In 2023, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 13% of these isolates, information was reported on tests for carbapenemase production; of these, 20% produced carbapenemase. The proportion of carbapenem-resistant *P. aeruginosa* in ICUs returned to pre-COVID-19 levels in 2022 and remained stable in 2023, after two years during the COVID-19 pandemic in which this proportion was remarkably lower.
- In 2023, two percent of the total number of *P. aeruginosa* isolates was MDR and 65% of these MDR isolates were carbapenem-resistant.
- Twenty-eight percent of the CPPA isolates in the enhanced CPPA surveillance in 2023 were from samples of Ukrainian patients.
- The predominant (41%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was bla_{NDM-1}. In contrast, in 2021 the dominant carbapenemase encoding allele in CPPA was bla_{VIM-2}.
- Ukrainian patients carried CPPA with bla_{IMP-1} or bla_{NDM-1}, thereby contributing to the changing molecular epidemiology of CPPA in the Netherlands since 2022.
- A total of 89% of the carbapenemase-producing *P. aeruginosa* had MICs for meropenem above the EUCAST defined clinical breakpoint (8 mg/L).
- The majority (78%) of the Ukrainian patients with CPPA had been hospitalized in a hospital abroad >24 hours, while this proportion was 27% 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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4 Routes of transmission of VIM-positive *Pseudomonas aeruginosa* in the adult intensive care unit-analysis of 9 years of surveillance at a university hospital using a mathematical model. Pham TM, Büchler AC et al. Antimicrobial Resistance and Infection Control. 2022;11(1).

4.8.4 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*. 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 classified carbapenem-resistant *A. baumannii* as 'priority 1: critical'.[2]

Methods

Data on carbapenem-resistant *A. baumannii-calcoaceticus* complex (CRAB) were obtained from two different surveillance systems of the RIVM: ISIS-AR and the national enhanced CRAB pilot 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 2019-2023. 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 2023 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 37 laboratories (out of a total of 51 laboratories in the Netherlands), which provided complete data on the past five years (2019 to 2023).

Molecular characteristics of carbapenem-resistant Acinetobacter baumannii-calcoaceticus *complex and patient related characteristics based on Type-Ned*

Since 2022, a pilot CRAB surveillance via Type-Ned is implemented. All but one Dutch medical microbiology laboratories (n=50) participate, however, participation is voluntary, and possibly not all laboratories submit 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 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 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). An *Acinetobacter*-specific OXA-23-like, OXA-51-like and OXA-58-like PCR is not performed. 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 2023, and all of these isolates collected in 2023 were included. Samples without a person ID from persons whom had 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 were excluded from further analysis. Due to the Ukraine/Russia war, Ukrainian patients migrated to multiple European countries and carried highly resistant microorganisms.[4] 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. Assigning genetic cluster numbers in the 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 in the RIVM. 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*

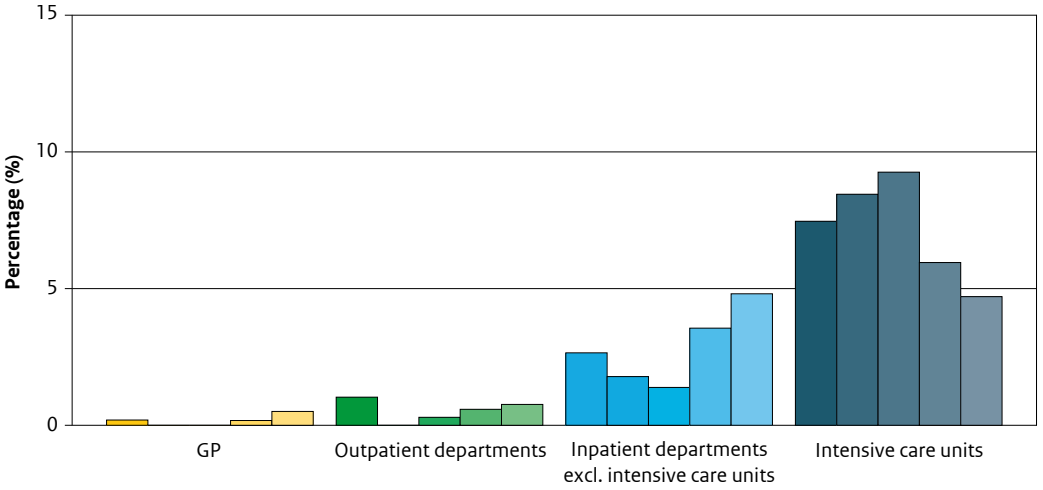
Based on the ISIS-AR database, 2% (29/1,465) of the diagnostic *A. baumannii-calcoaceticus* complex isolates were carbapenem-resistant in 2023 (Table 4.8.4.1). The percentage of carbapenem-resistant *A. baumannii-calcoaceticus* isolates was higher among diagnostic samples from inpatient departments and intensive care units (ICUs) compared to other types of healthcare settings. The observed proportion of resistance changed over the years 2019-2023, which may be influenced by the low absolute numbers of tested and resistant isolates (Figure 4.8.4.1).

Table 4.8.4.1 Carbapenem-resistant *A. baumannii-calcoaceticus* complex in the Netherlands in 2023, based on ISIS-AR data

Sampling location	Tested isolates, N	Carbapenem-resistant *A. baumannii-calcoaceticus* complex, N(%)
General practitioners	592	3 (1)
Outpatient departments	393	3 (1)
Inpatient departments excl. intensive care units	395	19 (5)
Intensive care units	85	4 (5)
Total	**1,465**	**29 (2)**

Numbers are based on a selection of 37 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 2023 EUCAST breakpoints for meropenem (8 mg/L or 15 mm) and/or imipenem (4 mg/L or 21 mm).

Figure 4.8.4.1 Percentages of carbapenem-resistant *A. baumannii-calcoaceticus* complex in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. 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 2023 EUCAST breakpoints for meropenem (8 mg/L or 15 mm) and/or imipenem (4 mg/L or 21 mm).

Molecular characteristics of carbapenem-resistant Acinetobacter baumannii-calcoaceticus complex (CRAB)

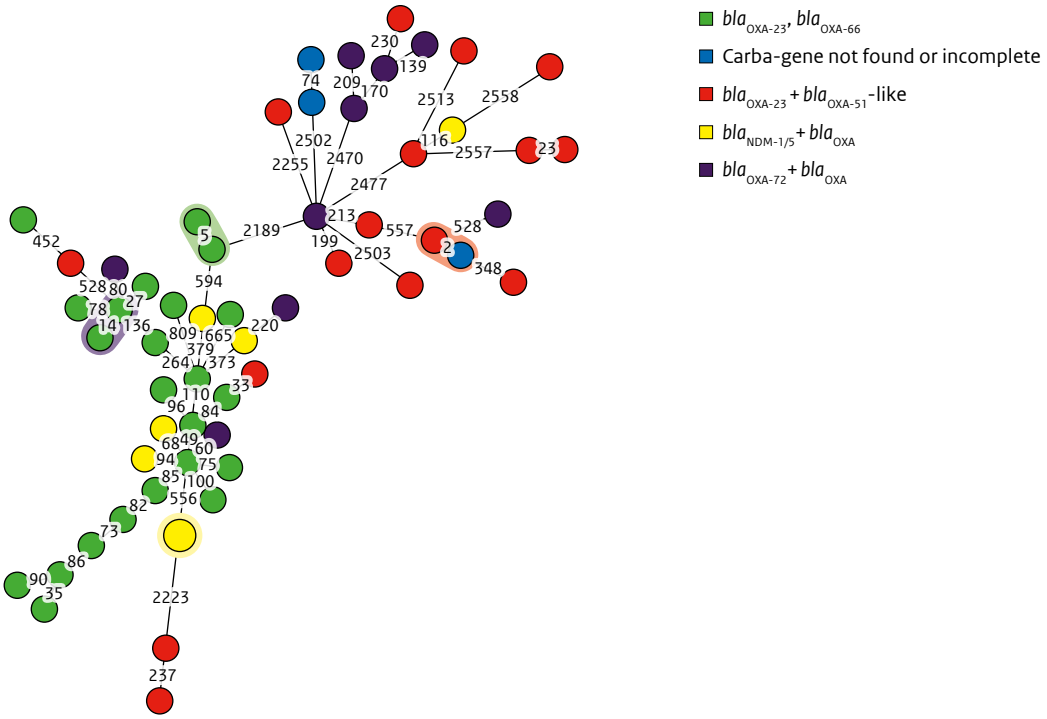
In the enhanced pilot surveillance, the RIVM received 66 suspected carbapenem-resistant *A. baumannii-calcoaceticus* complex isolates from both diagnostic and screening samples collected in 2023 through Type-Ned, of which almost all (65/66) produced carbapenemase according to the CIM. The CIM negative isolate had an MIC for meropenem of 0.38 mg/L and was thus excluded from further analyses. Of the CIM positive *A. baumannii-calcoaceticus* complex, 92% (60/65) had an MIC for meropenem above the clinical EUCAST R breakpoint of 8 mg/L and are designated CRAB. Nevertheless, in this chapter all 65 *A. baumannii-calcoaceticus* complex isolates were analyzed together. The 65 *A. baumannii-calcoaceticus* complex isolates were from 65 patients and these isolates were submitted by 28 MMLs. Fourteen of the 65 isolates (22%) were obtained from Ukrainian patients. From 58 of the 65 isolates whole-genome sequencing data was available upon analysis. Analysis of whole-genome sequencing data of 58/65 *A. baumannii-calcoaceticus* complex isolates revealed that 22 of the 58 *A. baumannii-calcoaceticus* complex isolates (38%) carried the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$ carbapenemase gene combination. Sixteen isolates carried either $bla_{\text{OXA-23}}$ alone (n=3), or in combination with an OXA-51-like carbapenemase allele (n=13, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-69}}$, $bla_{\text{OXA-71}}$, $bla_{\text{OXA-82}}$, $bla_{\text{OXA-90}}$, $bla_{\text{OXA-91}}$, $bla_{\text{OXA-94}}$, $bla_{\text{OXA-132}}$, or $bla_{\text{OXA-378}}$). Six isolates harboured $bla_{\text{NDM-1}}$ in combination with one (n=1) or two other carbapenemase genes (n=5). One isolate carried $bla_{\text{NDM-5}}$ in combination with $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$.

Nine *A. baumannii-calcoaceticus* complex isolates carried $bla_{\text{OXA-72}}$ alone (n=3) or in combination with one (n=4, $bla_{\text{OXA-90}}$) or two other carbapenemases (n=2). Twelve *A. baumannii-calcoaceticus* complex isolates carried other carbapenemase genes alone (n=1), were not found or incomplete (n=3) or were not

sequenced (n=8). Of the fourteen *A. baumannii-calcoaceticus* complex isolates from Ukrainian patients, seven harboured a bla_{OXA-23} allele alone (n=1) or in combination with an OXA-51-like carbapenemase allele (n=6, bla_{OXA-66}, bla_{OXA-69}).

The genetic relations of the 58 sequenced *A. baumannii-calcoaceticus* complex isolates were assessed by performing wgMLST (Figure 4.8.4.2). This revealed that most of the isolates were unrelated. Four groups of two isolates differed in ≤15 wgMLST alleles and could therefore be regarded as genetic clusters. Two of the four clusters were from Ukrainian patients, while the other two clusters were patients from the Netherlands. Furthermore, the isolates were diverse; 47 isolates were assigned to 29 distinct MLST types of which two MLST types, ST195 (n=4) and ST231 (n=4), occurred most often. The carbapenemase genes and combinations hereof were randomly distributed among the isolates in the minimum spanning tree (Figure 4.8.4.2).
The following sample materials were reported: twenty-seven *A. baumannii-calcoaceticus* complex were from screening swabs, seventeen were from wound, seven from urine samples, five from sputum, five from pus, three from blood, and the remainder from other isolation sites. The majority (57/65) of the *A. baumannii-calcoaceticus* complex were obtained from materials sent in by hospitals.

Figure 4.8.4.2 wgMLST-based minimum spanning tree of 58/65 *A. baumannii-calcoaceticus* complex isolates from patients sampled in 2023, based on enhanced CRAB pilot 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 or carbapenemase-producing A. baumannii-calcoaceticus complex

Clinical/epidemiological questionnaire data in Type-Ned were available for 34 of the 65 persons with an *A. baumannii-calcoaceticus* complex isolate in the enhanced pilot surveillance. All 34 samples were acquired within a hospital setting. Twenty-seven patients were male and the median age was 55 years (range 19-81 years). Eight patients (8/34, 24%) were admitted to the ICU at the moment of sampling, 22 (65%) were admitted to a non-ICU hospital department, and four (12%) samples were taken at the outpatient department.

Eleven questionnaires concerned Ukrainian patients. For 1/11 (9%) of the Ukrainian patients, diagnostics of an infection was mentioned as the reason for taking the sample. For one patient though, for whom screening was mentioned as the sampling reason, the isolate was cultured in a blood sample, suggesting a diagnostic sampling reason rather than screening. All of these Ukrainian patients had been admitted >24 hours in a Ukrainian hospital in the previous two months.

Twenty-three questionnaires concerned non-Ukrainian patients. In these patients, 8/23 samples (35%) were taken for diagnostic purposes, but again, screening was mentioned as the sampling reason in one patient with an isolate cultured in a blood sample. Twenty-two patients (22/23, 96%) had been admitted >24 hours in a hospital abroad in the previous two months, namely six in Morocco, four in Greece, three in Turkey, and nine in nine different countries, spread over four different continents. For one patient, no risk-factor for carrying CPAB or CRAB was known. Eight of the non-Ukrainian patients were admitted to the ICU at the moment of sampling, and eight patients (including four of the ICU patients) had (severe) comorbidities.

Discussion

In 2023, in ISIS-AR, 2% 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 change 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, the overall majority of carbapenem-resistant isolates were sampled in hospital settings.

The 2023 results of the enhanced CRAB pilot surveillance submitted via Type-Ned revealed a genetically highly diverse, and highly resistant CRAB population in the Netherlands. Twenty-two percent of the *A. baumannii-calcoaceticus* complex isolates in the enhanced pilot surveillance in 2023 were from samples of Ukrainian patients, and none of these carried bla_{NDM}-like carbapenemases in contrast to isolates from Dutch 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 started in 2022. Also, because not all laboratories routinely perform tests for carbapenemase production. 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 pilot 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. Presumably, 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 2023. Therefore, the data as shown here might be an underestimation of the number 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 2023, in ISIS-AR, 2% 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 29 distinct MLST types of which ST195 (n=4) and ST231 (n=4) occurred most often.
- A total of 90% of the submitted *A. baumannii-calcoaceticus* complex to the enhanced CRAB pilot surveillance (n=60/65) had MICs for meropenem above the EUCAST defined R breakpoint (8 mg/L).
- Twenty-two percent of the *A. baumannii-calcoaceticus* complex isolates in the enhanced CRAB pilot surveillance in 2023 were from clinical or screening samples of Ukrainian patients.
- The predominant (38%, 22/58) carbapenemase-encoding gene combination in *A. baumannii-calcoaceticus* complex was $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$, and 12% (7/58) carried a bla_{NDM}-like carbapenemase.
- All Ukrainian patients with carbapenem-resistant *A. baumannii-calcoaceticus* complex had been hospitalized in a Ukrainian hospital for >24 hours. *S*imilarly, all but one non-Ukrainian patients had been hospitalized in a hospital abroad recently and hospitalization abroad can therefore be considered as a risk factor.

References

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3 van der Zwaluw K et al. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One. 2015 Mar 23;10(3):e0123690. doi: 10.1371/journal.pone.0123690.

4 Zwittink RD et al. Multidrug-resistant organisms in patients from Ukraine in the Netherlands, March to August 2022. Eurosurveillance 2022:27(50):2200896. doi: 10.2807/1560-7917.ES.2022.27.50.2200896.

4.8.5 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) and the relative 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.

Methods

Prevalence of MRSA based on ISIS-AR

From the ISIS-AR database, *S. aureus* isolates, including MRSA, cultured between 2019 to 2023 were selected. Data were originating from 37 laboratories that continuously reported complete data to the ISIS-AR database during the defined period. The first diagnostic *S. aureus* isolate per person per year from blood, cerebrospinal fluid, urine, lower respiratory tract, or wound/pus was selected. 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 S/R interpretation for cefoxitin was R, or, if no data on a cefoxitin test was available, the S/R laboratory interpretation for flucloxacillin/oxacillin was R. An additional prevalence assessment was conducted for *S. aureus* isolates from blood only.

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 that are identified in routine practices using the Type-Ned system for molecular typing 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). Isolates from the MLVA-complex MC0398 are labelled as livestock-associated MRSA (LA-MRSA).

The first MRSA isolate per person sampled in the period 2019 to 2023 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. In contrast to the analyses from previous editions of NethMap, samples without a person ID were not excluded but were included for further analysis, also retrospectively, 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 lacking a *mecA* or *mecC* gene, and isolates that could not be typed by MLVA were also excluded from molecular analysis.

Since 2019, a semi-random selection of MRSA isolates is analysed through whole-genome sequencing (WGS; 150 bp paired-end reads, Illumina NovaSeq 6000). It concerns a random selection of 40 isolates per month that meet the following criteria: one isolate per person, per MLVA type, per laboratory. 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 2023 and compared with the previous four years, for all isolates combined and after stratification into diagnostic- and screening isolates.

Results

Prevalence of MRSA based on ISIS-AR

In ISIS-AR, the overall proportion of diagnostic *S. aureus* isolates in 2023 that was identified as MRSA was 3% (n=1,503/51,549). A higher proportion of 4% was seen for cultures requested by general practitioners (GPs; wound or pus only) and for intensive care units, while a proportion of 2% was observed for outpatient departments (Table 4.8.5.1). In blood isolates only, the prevalence of MRSA in 2023 was 2% (n=70/3,523). Figure 4.8.5.1 shows the trends in MRSA prevalence from 2019 to 2023 in all diagnostic isolates. Percentages MRSA were quite stable, except in intensive care units in which the prevalence increased: 2% in 2019 increasing to ~3% in the period 2020-2022, and 4% in 2023).

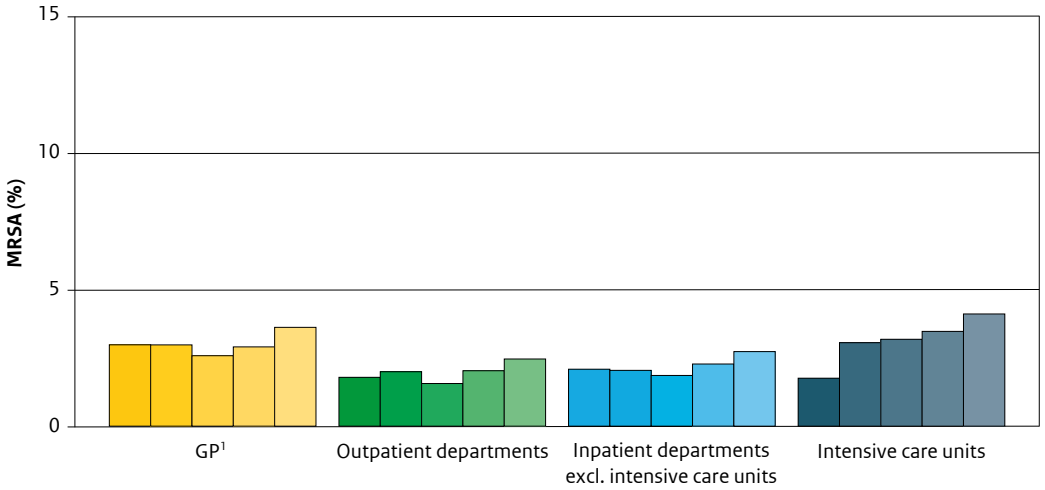
Table 4.8.5.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2023, based on ISIS-AR data

Sampling location	Tested isolates, N	MRSA, N (%)
GP[1]	14,608	528 (4)
Outpatient departments	20,267	498 (2)
Inpatient departments excl. intensive care units	15,066	411 (3)
Intensive care units	1,608	66 (4)
Total	**51,549**	**1,503 (3)**

Numbers are based on a selection of 37 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 laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin.

[1] *From GP patients only wound or pus isolates were selected.*

Figure 4.8.5.1 Trends in Methicillin-resistant *S. aureus* (MRSA) in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data

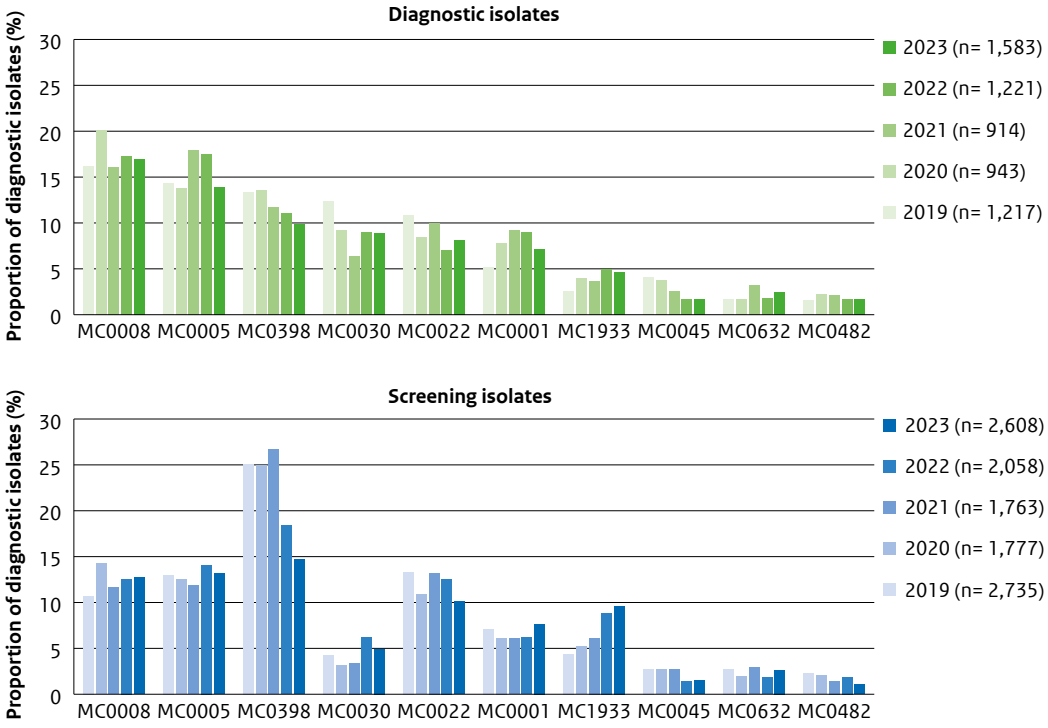


Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic S. aureus isolate per patient per year was selected.
The prevalence of MRSA isolates was based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2). If these tests were lacking, prevalence was based on laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin.
[1] *From GP patients only wound or pus isolates were selected.*

Molecular characteristics of MRSA based on Type-Ned

In 2023, the RIVM received 4,974 *S. aureus* isolates from human origin that were *mecA* or *mecC* gene positive and 4,204 isolates fulfilled the selection criteria specified in the methods section. The absolute number of MRSA isolates included in the analysis in 2023 is higher compared to previous four years (minimum: 2,694 isolates in 2021, maximum: 3,963 isolates in 2022). The majority of isolates were cultured from samples submitted to the MML by hospitals (n= 2,739; 65%), followed by GPs (n= 1,097; 26%), municipal health services (n= 169; 4%) and long-term care facilities (n= 81; 2%). Based on the sample material, 62% (n= 2,608) of the isolates were isolated from screening samples. A total of 1,583 isolates (38%) were diagnostic isolates, of which the majority were cultured from wound material or pus (1,223/1,583; 77%) and 43 isolates were cultured from blood (3%). The above-mentioned percentages are comparable to previous years. In 2023, 28 methicillin-resistant *Staphylococcus argenteus* (MRSArg) isolates were submitted, of which 21 (75%) were screening isolates. Fifteen MRSArg isolates were submitted in light of a nosocomial outbreak.
For 2023, the MRSA population consisted of 1,027 different MLVA-types. The majority (784 MLVA types; 3,708 isolates) could be grouped into 27 MLVA-complexes (MCs). For the remaining 243 MLVA-types (496 isolates) no MC could be assigned. The most frequently identified MCs were MC0008 (n= 604 isolates; 14%), MC0005 (n= 566; 13%) and MC0398 (n= 544; 13%, Like in the previous years, the proportion of MC008 was higher in diagnostic (17%) than in screening isolates (13%), whereas the proportion of MC0398 was higher in screening isolates (15%) than in diagnostic isolates (10%). During the 2019-2023 surveillance period, there has been a decreasing trend in the proportion of MC0398 and MC0045 and an increasing proportion of MC1933 in both diagnostic and screening isolates (Figure 4.8.5.2).

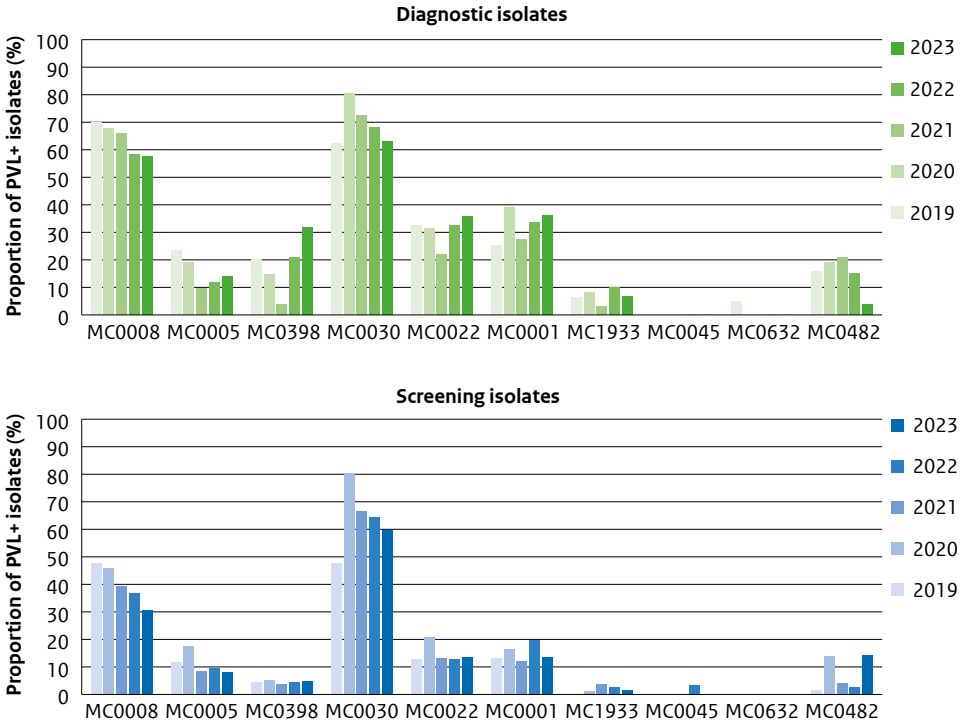
Figure 4.8.5.2 Trends in the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2019 to 2023) among diagnostic and screening isolates, based on enhanced MRSA surveillance data



The graph displays the proportions of MLVA-complexes per sampling year.
The first MRSA isolate per person sampled in the period 2019 to 2023 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 4,204 isolates submitted in 2023, 24 (0.6%) contained the *mecC* gene and no *mecA* gene, of which 11 were diagnostic isolates. Overall, 26% of the isolates (n= 1,114) were Panton-Valentine Leukocidin (PVL) positive. Except for 2021 (21%), the proportions of PVL-positive isolates were comparable in previous years. Of the 1,114 PVL-positive isolates, 478 (43%) were screening isolates and 632 (57%) were diagnostic isolates. Like in previous years, the proportion of PVL-positive isolates was higher in diagnostic isolates (632/1,583 isolates; 40%) than in screening isolates (478/2,608 isolates; 18%). The proportions of PVL-positive isolates were highest for MC0030 and MC0008 during the surveillance period 2019-2023 in both diagnostic and screening samples (Figure 4.8.5.3). However, for both of these complexes a decreasing trend in the proportion of PVL-positive isolates can be observed. In 2023, 32% of diagnostic LA-MRSA (MC0398) isolates (50/157) were PVL-positive, which is higher than in previous years. The majority of these isolates belonged to MLVA types MT0569 (22/50; 44%) and MT2306 (15/50; 30%). Only 5% of screening LA-MRSA isolates (18/383) were PVL-positive, which is comparable to previous years.

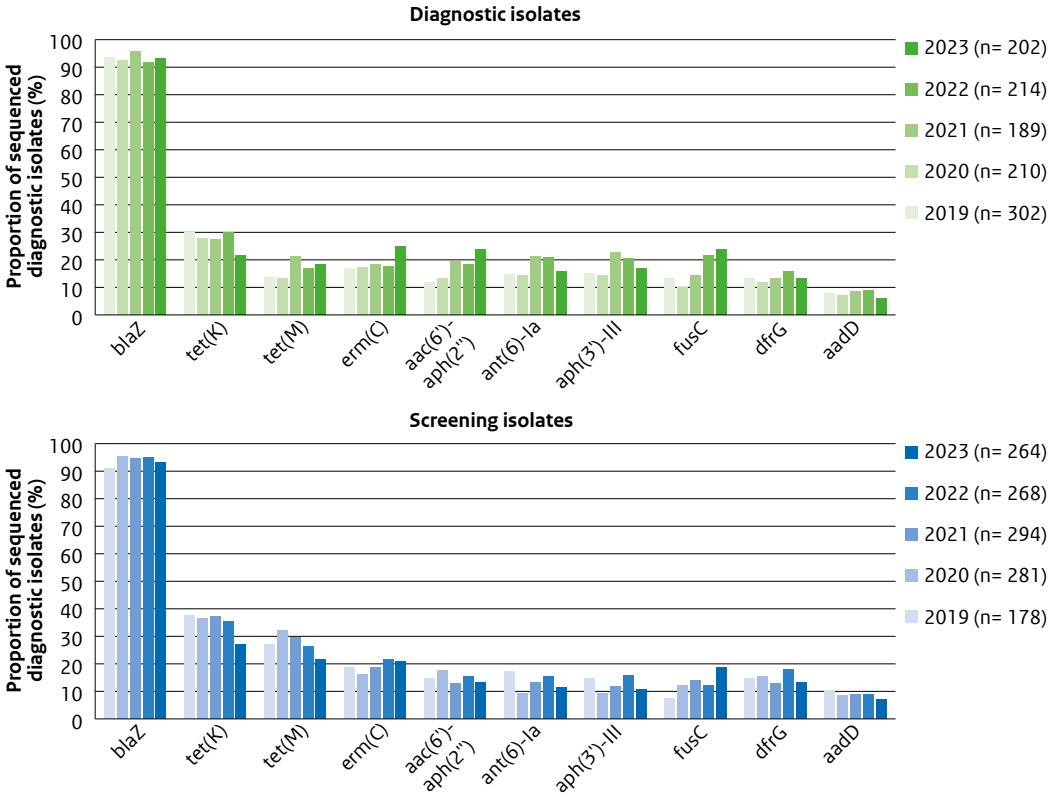
Figure 4.8.5.3 Trends in PVL positivity among the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2019 to 2023), based on enhanced MRSA surveillance data



The graph displays the proportion of PVL-positive isolates per MLVA-complex per sampling year.
The first MRSA isolate per person sampled in the period 2019 to 2023 was selected, 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.

In 2023, 468 of the MRSA isolates included for molecular analysis were also analysed through whole-genome sequencing. The isolates belonged to 55 different multi-locus sequence types (STs), of which ST5 was most frequently identified (n= 68; 15%), followed by ST22 (n= 51; 11%), ST398 (n= 47; 10%), ST6 (n= 47; 10%) and ST8 (n=44; 9%). Besides *mecA*, the antimicrobial resistance (AMR) genes *blaZ* (encoding beta-lactamase; 94%) and *tet(K)* (encoding tetracycline resistance ribosomal protection protein; 31%) were most frequently identified in the genomes of MRSA isolates obtained between 2019 and 2023. The other eight most frequently identified AMR genes in MRSA isolates putatively encode for resistance against tetracyclin (*tet(M)*), erythromycin (*erm(C)*), aminoglycosides (*aac(6')-aph(2'')*, *ant(6)-Ia*, *aph(3')-III*, *aadD*), fusidic acid (*fusC*) and trimethoprim (*dfrG*). Over the period 2019-2023 there is a trend of increasing proportion of isolates containing the *fusC* gene in both, diagnostic and screening isolates (Figure 4.8.5.4). In addition, the proportion of diagnostic isolates containing the *aac(6')-aph(2'')* gene is increasing and the proportion of screening isolates containing the *tet(M)* gene is decreasing (Figure 4.8.5.4).

Figure 4.8.5.4 Trends in the ten most frequently identified antimicrobial resistance genes in MRSA in the Netherlands (2019 to 2023), 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. MRSA isolates that were sequenced and fulfilled the inclusion criteria for molecular analysis are included.

MRSA clusters of special interest

In 2023, MRSA was the most frequently reported micro-organism reported to the Early warning and response meeting for Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR), both in hospitals and in long-term care facilities (see Chapter 4.8.7). In addition, MRSA was mentioned four times in the weekly report of the general Early warning and response meeting in 2023.

- May 2023. Expansion of an MRSA cluster, mainly in the community, possibly associated with men having sex with men and/or intravenous drug use. This concerns multiple MLVA-types, including MT2307 and MT6494, clustering by whole-genome multi-locus sequence typing.
- June 2023. A remarkable number of MRSA outbreaks on neonatology wards, reported to the SO-ZI/AMR. This concerns multiple MLVA-types.
- November 2023. A follow-up report on the increase of impetigo in the community caused by MRSA MT4627.
- December 2023. A cluster in the community with PVL-positive MRSA MT2306, associated with a massage parlour.

Clinical/epidemiological characteristics of patients with MRSA based on Type-Ned

In 2023, the persons from whom MRSA was cultured had a median age of 36 (range 0 - 99) years and 2,415 (57%) were male. Based on information provided at sample submission, diagnostic purposes were the reason for sampling in 48% of the persons (2,016/4,204), which is comparable to the 50% of last year and much higher than in the previous three years (increased from 37% in 2019 to 42% in 2021). Screening was the reason for sampling in 49% (2,056/4,204): in 39% (1,658/4,204) screening was performed because of presumed increased risk for MRSA carriage including active surveillance, and in 9% (398/4,204) because the person was part of a contact tracing investigation. For 3% (n=132) the reason of sampling was unknown.

For 51 of the 4,204 (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 1,217, including contacts in a contact tracing investigation, no additional data were available. Therefore, additional epidemiological questionnaire data for 2023 were available for 69% (n=2,885) of the persons.

In Table 4.8.5.2 a selection of the clinical/epidemiological data of included persons is summarised. Seventy-one percent (2,042/2,885) were sampled in the hospital, of which 44% in outpatient departments, 30% in inpatient departments and 5% during their stay in the Intensive care unit. In the group of persons that were sampled for screening/active surveillance, the large majority (95%) met the WIP risk category 1, 2, or 3[2], largely coinciding with the recently published SRI categories[3], whereas in diagnostic isolates this proportion was - as expected - much lower (44%). This percentage is comparable to 2021 (46%) and 2022 (45%) but was lower before (34% in 2019 and 39% in 2020). Work-related exposure to livestock animals was reported for 3% of persons with diagnostic samples and 17% of persons with samples that were taken for screening/active surveillance. The latter varied between 20 and 25% in the years before. The main group of livestock animals to which this group was exposed were pigs (77%), and from 93% of persons with a livestock-related profession an LA-MRSA was sampled. Out of all persons with LA-MRSA, 22% (n=24/108) of persons with diagnostic samples, and 77% (n=158/205) of persons that were sampled for screening/active surveillance, were persons with work related exposure to livestock animals. The proportion of persons for whom hospitalisation abroad for at least 24 hours during the previous two months was recorded was 134/1,606 (8%), comparable to 2022 (8%) and 2019 (9%), after a drop in

2020-2021 due to travel restrictions during the COVID-19 pandemic (5% in 2020, 6% in 2021)). The main geographic regions of previous hospitalisations in 2023 were Western Europe (22%), Western Asia, including Turkey (16%), and Southern Europe (12%). Turkey was the country most frequently reported (n=14/134, 10%), followed by Belgium (12/134, 9%). In 543/1,781 (30%) persons an underlying illness was reported (underlying illness unknown in 1,107 persons), with skin disorders (192/543, 35%) and diabetes (152/543, 28%) being the most mentioned underlying conditions.

Table 4.8.5.2 Epidemiological data of 2,885 MRSA positive persons (excluding employees of healthcare facilities) with an isolate in the enhanced MRSA surveillance system, with a sampling date in 2023

Characteristic	Diagnostic and screening combined[a]		Diagnostic		Screening/active surveillance	
	Data available (N)	n (%)	Data available (N)	n (%)	Data available (N)	n (%)
Sample location (hospital only)						
Outpatient departments	2,042	896 (44)	1,011	467 (46)	1,013	425 (42)
Inpatient departments (excluding intensive care units)	2,042	620 (30)	1,011	293 (29)	1,013	323 (32)
Intensive care units	2,042	95 (5)	1,011	44 (4)	1,013	51 (5)
Other/unknown	2,042	431 (21)	1,011	207 (20)	1,013	214 (21)
Risk factors						
Meeting WIP risk category 1,2, or 3[b,c]	2,419	1,698 (70)	1,178	522 (44)	1,212	1153 (95)
Work-related exposure to livestock animals	1,976	197 (10)	990	30 (3)	966	166 (17)
Pigs	197	145 (74)	30	17 (57)	166	127 (77)
Cattle	197	41 (21)	30	10 (33)	166	31 (19)
Other/unknown	197	11 (6)	30	3 (10)	166	8 (5)
Hospitalization abroad >24 hours during the previous two months	1,606	134 (8)	831	25 (3)	762	109 (14)
Western Europe	134	30 (22)	25	6 (24)	109	24 (22)
Western Asia (including Turkey)	134	22 (16)	25	5 (20)	109	17 (16)
Southern Europe	134	16 (12)	25	3 (12)	109	13 (12)
Other/unknown country	134	66 (49)	25	11 (44)	109	55 (50)
Living in asylum centre	2,670	693 (26)	1,379	149 (11)	1,246	530 (43)

WIP: Working Party in Infection Control.

[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] *WIP 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

An increase was found in the proportion of MRSA in ICUs from 2020 onwards. The explanation of this finding is currently unclear. No large clusters were found in ICUs based on next-generation sequencing data that was part of the enhanced MRSA surveillance. The number of MRSA outbreaks in hospitals, including ICUs, reported to SO-ZI/AMR was much higher in 2023 compared to the previous five years (n=20 compared to n<10 in each of the previous five years) (see also Chapter 4.8.7). In addition, there were some reports of community-acquired MRSA infections in 2023. Nevertheless, the observed MRSA percentage is still low compared to other countries[4]. In 2023, the first nosocomial outbreak with MRSArg in the Netherlands was reported, which resulted in an exceptionally higher number of MRSArg isolates submitted to Type-Ned MRSA. The outbreak was reported to SO-ZI/AMR. The high proportion of LA-MRSA MC0398 isolates is probably 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, which is a result of increasing numbers of non-LA-MRSA rather than of decreasing LA-MRSA. A higher proportion of PVL-positive LA-MRSA was observed in 2023. However, PVL-positive LA-MRSA types MT0569 and MT2306 were not associated with livestock, but could frequently be related to travel to and/or hospitalisation in south-eastern Asia.

With the interpretation of the surveillance results several factors should be taken into account. Within the ISIS-AR database, routine culture results from MMLs are collected. However, this can introduce overestimation of resistance percentages due to selective sampling by GPs 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*. Therefore, we only included diagnostic isolates to assess MRSA prevalence.

Secondly, 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.

Finally, although skin disorders are now the most reported underlying condition, this could be a result of wrongly recording impetigo as an underlying illness instead of a presentation of the MRSA infection.

Conclusions

- The overall proportion of routinely collected diagnostic *S. aureus* isolates that were MRSA positive in 2019-2023 was still 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.
- Percentages for MRSA obtained from diagnostic samples were quite stable over time, except in intensive care units in which the prevalence increased from 2% to 4% between 2019 and 2023.
- Gradual shifts occurred in the proportion of isolates belonging to the ten most frequently identified MLVA-complexes. Trends were mostly similar in screening and diagnostic samples.
- LA-MRSA MC0398 is no longer the predominant MRSA clade. With MC0008, MC0005 and MC0398 constituting 14%, 13% and 13% of the MRSA isolates in 2023, the most frequently identified MLVA-complexes are more equally distributed.
- In 2023, 40% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 18% of the screening isolates were PVL-positive. MC0008 and MC0030 isolates had the highest proportion of PVL-positive isolates, but the proportion is decreasing over the past years.

- In 2023, 32% of diagnostic LA-MRSA (MC0398) isolates were PVL-positive. Seventy-four percent of these isolates belonged to MLVA types MT0569 and MT2306, for which professional contact with livestock was not reported.
- The top 10 most occurring antimicrobial resistance genes among diagnostic and screening MRSA isolates encode for beta-lactam, tetracycline, erythromycin, aminoglycoside, fusidic acid and trimethoprim resistance.
- In 2023, the first nosocomial outbreak of methicillin-resistant *S. argenteus* in the Netherlands was reported.
- The majority of persons with samples that were taken for screening/active surveillance, met WIP-category 1,2 or 3[2] (95%), with the main risk factor being work-related exposure to livestock animals (17%).
- Hospitalisation abroad for at least 24 hours during the previous two months was recorded in 8% of the MRSA positive persons. The main geographic regions of recent hospitalisations abroad of MRSA positive persons were Western Europe (22%) and Western Asia (16%).

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4.8.6 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 4.8.7). 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 2023, 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 37 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 2023, 9 outbreaks with VRE_{fm} in the Netherlands were reported to the SO-ZI/AMR (see section 4.8.7), 7 of which were in hospitals. This number of outbreaks is comparable to the 9 outbreaks that were reported in 2022, but lower than the 19 outbreaks reported in 2019. In the years before 2019, 10 to 15 outbreaks per year were reported. In total, since the start of SO-ZI/AMR in April 2012, 137 outbreaks with VRE_{fm} have been reported in the Netherlands. 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 2023 in the Netherlands based on ISIS-AR is shown in table 4.8.6.1. Figure 4.8.6.1 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.

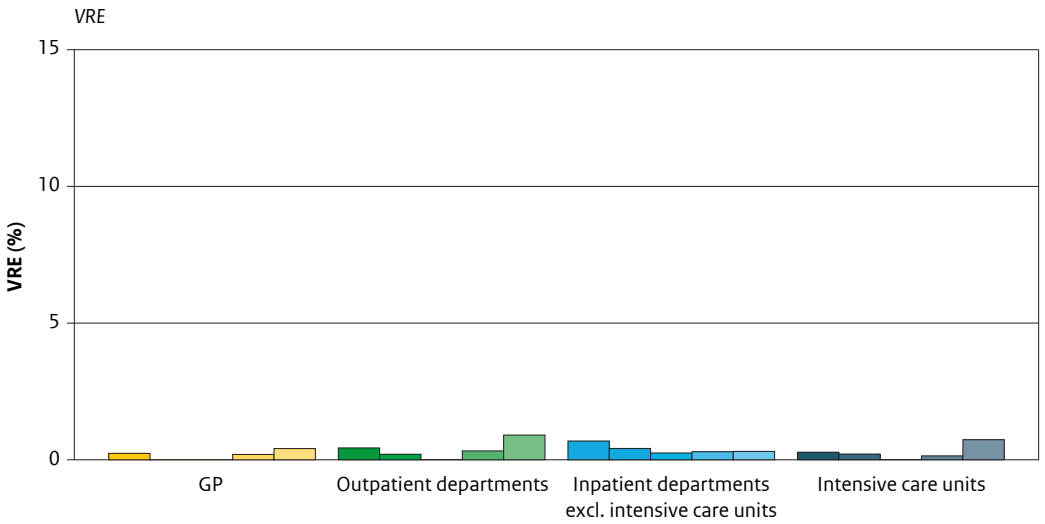
The absolute numbers of VRE_{fm} isolates from screening samples of inpatient hospital departments (including intensive care units), from 37 laboratories continuously reporting to ISIS-AR show a range of 55-155 positive isolates per year, with the lowest number in 2020 (Table 4.8.6.2).

Table 4.8.6.1 Vancomycin-resistant *E. faecium* (VRE_{fm}) in diagnostic isolates in the Netherlands in 2023, based on ISIS-AR data

Type of department	Tested isolates, N	VRE, N (%)
General practitioner	489	2 (0)
Outpatient departments	553	5 (1)
Inpatient departments excl. intensive care units	2,611	8 (0)
Intensive care units	546	4 (1)
Total	**4,199**	**19 (0)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first 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 2023, with (VRE_{fm}) being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 4.8.6.1 Percentage of diagnostic VRE_{fm} isolates of the total number of *E. faecium* isolates from diagnostic isolates in the Netherlands (from left to right 2019 to 2023), based on ISIS-AR data



Numbers are based on a selection of 37 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.

Table 4.8.6.2 Absolute numbers of vancomycin-resistant *E. faecium* (VRE_{fm}) isolates in the Netherlands, 2019-2023, based on ISIS-AR data

	General practitioner and outpatient departments			Inpatient departments including intensive care units			Total		
Year	Diagnostic	Screening	Total	Diagnostic	Screening	Total	Diagnostic	Screening	Total
2019	3	60	63	20	110	130	23	170	193
2020	1	39	40	13	62	75	14	101	115
2021	0	38	38	7	57	64	7	95	102
2022	3	42	45	9	133	142	12	175	187
2023	7	39	46	12	165	177	19	204	223

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first E. faecium isolate per patient was selected, with a preference for the first diagnostic isolate if both diagnostic and screening samples were taken.
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 2023, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

The number of reported VRE_{fm} outbreaks in 2023 was comparable to that of 2021 and 2022 and higher than in 2020, but still lower compared to 2019. This could be 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 above pre-COVID levels.
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.
In the coming period, the need for a national VRE_{fm} surveillance system will be discussed.

Conclusions

- The number of reported hospital outbreaks with VRE_{fm} in 2023 was comparable to 2021 and 2022, but still lower compared to 2019, 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 and varies marginally below 1%.
- The absolute number of positive screening VRE_{fm} isolates continued to increase to above the pre-COVID-19-period. The absolute number of diagnostic isolates does not show an increase.

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4.8.7 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 hospitals and to prevent spread to other health care facilities through early warning and reporting.[1] Since 2015 long-term care facilities (LTCFs) are also invited 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 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. Moreover in 2017, a financial compensation rule has been introduced 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.[4]

Methods

Notifications and monthly updates of outbreaks are submitted through a web-based application 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. In the monthly meetings, the SO-ZI/AMR reassesses the phase of each notified outbreak which has not yet been 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 infection control measures, which are thus incomplete or not sufficiently effective.

Results

Table 4.8.7.1 provides an overview of the 44 outbreaks reported in 2023. These were reported by 37 different healthcare institutions: 34 outbreaks in hospitals and 10 in LTCFs, including two in rehabilitation centres. Most outbreaks (n=33) ended in 2023, 10 ended before July 1 2024 and 1 continued after July 1 2024.
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 6. The maximum number of involved patients was much higher in hospitals compared to LTCF (111 vs 8).
In hospitals, a remarkable number of 20 methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks were reported, compared to <10 in each of the previous five years. Nine (45%) of these were MRSA outbreaks in a paediatric or neonatology ward. In three occasions, an MRSA outbreak resulted from transmission of an index patient who was transferred from another hospital with a known MRSA outbreak. The MRSA

positive findings from the first hospital were only announced to the second hospital, after the patient had already been transferred and admitted. The finding of a relatively high number of hospital outbreaks with MRSA related to a paediatric or neonatology ward was reported more extensively in the weekly report of the general Early warning and response meeting.
The number of reported outbreaks in hospitals with vancomycin-resistant *Enterococcus faecium* (VRE) (n=7) was comparable to 2022 (n=9). Three outbreaks were with carbapenemase-producing (CP) gram-negatives (2 outbreaks with CP Enterobacterales and 1 with CP *P. aeruginosa*). In 2023, the first hospital outbreak with methicillin-resistant *Staphylococcus argenteus* in the Netherlands was reported, which was additionally described more in detail in the weekly report of the general Early warning and response meeting.
In LTCFs MRSA was most often reported, as in previous years. Still, the number of reported MRSA outbreaks in LTCFs (n=6 in 2023) has been lower than before since 2021 (8 in 2022 and 3 in 2021 compared to 11 in 2020 and 17 in 2019).
One outbreak was classified as phase III. This concerned an extensive, long lasting VRE outbreak in a hospital which was reported to SO-ZI/AMR in September 2023. Retrospectively, the outbreak had already started in November 2021, and it was noted by the hospital staff at the end of 2022. The microbiological detection of the outbreak strain was complicated, because the VRE outbreak strain was not always vancomycin-resistant, and it only grew after long incubation periods at specific VRE culture plates. In the beginning of the outbreak, the routine laboratory work flow was based on standard VRE culture methods, without longer incubation time, and therefore, the strain was initially not detected. VRE detection through polymerase-chain reaction (pcr) was not routinely implemented in this laboratory. As a result of the initial difficulties in diagnostics, the outbreak was only recognized after it had been going on for a while, which has contributed to the long duration and a large number of patients involved. As of June 1 2024, the outbreak had not yet been contained and the total number of positive patients was 111. The spread of this outbreak strain and the diagnostic difficulties were described in the weekly report of the general Early warning and response meeting.

Discussion

The total number of 44 outbreaks in 2023 was higher than in 2020-2022 (n=34, 27 and 36 in 2020, 2021 and 2022 respectively), but still lower than in 2017-2019, when around 60 outbreaks were reported each year. Especially in those earlier years, the number of outbreak notifications by LTCFs were higher (n=20, 25 and 21 in 2017, 2018 and 2019 respectively) compared to 2023 (n=10), which could be a result of the increased attention to HRMO prevalence in the setting of Dutch LTCFs, leading to multiple publications in those years and a National Point Prevalence Study in LTCFs conducted in 2018.[5-8] This raised vigilance might have waned during the COVID-pandemic and onwards.
In 2023, a high number of MRSA outbreaks were reported by hospitals compared to previous years. Almost half of these involved transmission on paediatric or neonatology wards. In three occasions, an MRSA outbreak resulted from transmission of an index patient who was transferred from another hospital with a known MRSA outbreak. A delay in diagnostics and/or insufficient communication of MRSA positive results from one hospital to another could have played a role. It is important to stay aware of the possibility of HRMO transmission between hospitals through transferals of HRMO positive patients. Although it is to be expected that MRSA positive results of patients are communicated before and during patient transferals, this turns out not to be effective at every occasion. In the Netherlands, MRSA screening is not routinely performed in all patients in neonatal intensive care units. In the national guidelines for MRSA infection prevention[9], no specific advice on routine MRSA screening of patients on NICUs or their parents is included.

Table 4.8.7.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2023

	Hospitals (n=34) n	LTCFs[4] (n=10) n	Total 2023 (n=44) n
Microorganism (resistance mechanism)[1]			
Staphylococcus aureus (MRSA)	20	6	26
Enterococcus faecium (VRE)	7	2	9
Enterobacterales (CPE) (various species)	2	1	3
Klebsiella pneumoniae (ESBL)	1	1	2
Pseudomonas aeruginosa (CPPA)	1		1
Staphylococcus argenteus (MRSArg)	1		1
Clostridioides difficile	1		1
Serratia marcescens (non-HRMO)	1		1
Highest level phase[2]			
phase I	7	6	13
phase II	26	4	30
phase III	1		1
Median number of patients[3] (range)	5 (1-111)	6 (1-8)	5 (1-111)
Duration outbreak			
<1 month	2	1	3
1-6 months	26	7	33
>6 months	6	2	8
Median duration outbreak in days from start or reporting date until end of the outbreak (range)	97 (22-266)	70 (19-294)	89 (19-294)

n: number of outbreaks

1 *MRSA=methicillin-resistant Staphylococcus aureus; VRE=vancomycin-resistant Enterococcus faecium; CPE=carbapenemase-producing Enterobacterales; ESBL=extended-spectrum β-lactamase producing; CPPA=carbapenemase-producing Pseudomonas aeruginosa; MRSArg=methicillin-resistant Staphylococcus argenteus; HRMO=highly resistant microorganism*

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 four outbreaks, one patient and multiple health care workers were involved*

4 *Including two rehabilitation centres*

Conclusions

- On average, four outbreaks a month were reported to the SO-ZI/AMR in 2023, which is still lower compared to the pre-COVID-19 era, but higher compared to 2020-2022.
- Most outbreaks were classified as maximum as phase II and one hospital outbreak was classified as phase III, which concerned a long lasting VRE outbreak with a high number of patients involved.
- A high number of MRSA outbreaks in hospitals was reported in 2023. Almost half of them were related to paediatric or neonatal wards.
- The number of VRE outbreaks in hospitals was stable at n=7.
- In LTCFs, most outbreaks concerned MRSA.

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4.9 Resistance in specific pathogens

4.9.1 *Neisseria meningitidis*

Introduction

Neisseria meningitidis isolates cultured from cerebrospinal fluid (CSF) and/or blood in microbiological laboratories in the Netherlands are submitted for serogrouping and susceptibility testing to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) at the Amsterdam UMC.
For *N. meningitidis*, the EUCAST criteria for phenotypic penicillin susceptibility testing have a single breakpoint defining susceptible (≤ 0.25 mg/L) or resistant strains (> 0.25 mg/L).

Methods

From 2014-2023, 1,188 invasive meningococcal disease cases were reported to the NRLBM, including culture-negative PCR-positive cases. In total, 1,014 isolates were available for the study period; 712 isolates from blood, 275 isolates from CSF, and 27 blood isolates from patients with a PCR-positive, but culture-negative CSF-sample. The number of meningococcal isolates per year in this period ranged between 25 isolates (2021) and 186 isolates (2018). In 2023, 88 meningococcal isolates were available, 26 from CSF and 62 from blood. The MIC for penicillin was determined by Etest using Mueller-Hinton Fastidious Agar (MHF) plates and incubation at 36°C under 5% CO_2 for 18-24 h. EUCAST criteria for resistance were applied. In case of resistance to penicillin, susceptibility to ceftriaxone was also assessed by Etest using MHF plates and incubation at 36°C under 5% CO_2 for 18-24 h. Since January 2023, rifampicin susceptibility testing was discontinued for cost-saving, availability of resistance information through ISIS-AR, and generally low rifampicin resistance of *N. meningitidis* in the past 10 years (2 out of 1,013 strains, 0.2%).

Results

In 2023, all received meningococcal isolates obtained from CSF (n=26) were susceptible to penicillin, which is similar to data from 2021 and 2022 (table 4.9.1.1). For meningococcal isolates cultured from blood (n=62), 97% (n=60) were susceptible to penicillin (table 4.9.1.2). The two penicillin resistant isolates were both sensitive to ceftriaxone. Overall, only 7 out of 1,014 (0.7%) submitted isolates displayed phenotypic penicillin resistance in the last 10 years.

Discussion

The breakpoint for phenotypic penicillin susceptibility was altered by EUCAST in 2021, whereby only 2 categories remained; isolates are either susceptible or resistant to penicillin. Penicillin resistance among *N. meningitidis* isolates in the Netherlands is rare, whether applying the former or current EUCAST criteria for penicillin susceptibility.

Conclusions

- The number of invasive meningococcal disease isolates is still 35% lower in 2023 compared to 2019 (pre-COVID-19) but has more than tripled compared to the lowest recorded number in 2021.
- Penicillin resistance in *N. meningitidis* isolates is rare in the Netherlands.

Table 4.9.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2014-2023

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2014	33	**100**	0	**0**	33
2015	32	**100**	0	**0**	32
2016	36	**100**	0	**0**	36
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

* *MIC values in mg/L.*

Table 4.9.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2014-2023

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2014	40	**100**	0	**0**	40
2015	52	**100**	0	**0**	52
2016	101	**100**	0	**0**	101
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

* *MIC values in mg/L.*

4.9.2 *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 from Sexual Health Centres (SHC) across the Netherlands. In 2023, 15 out of the 24 SHC participated in GRAS, which together accounted for 82% 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 number of gonorrhoea diagnoses reported by SHC was around 6,000 from 2015 to 2020, and increased since then to 13,853 diagnoses in 2023. The percentage of diagnoses including a susceptibility test result remained stable around 33% since 2016 (33.5% in 2023, Figure 4.9.2.1).

Gonococcal resistance to ciprofloxacin was around 30% between 2014 and 2018 but increased since then and was 63.1% in 2023. Resistance to cefotaxime has been slowly decreasing since 2014 and was 0.3% in 2023. For azithromycin, resistance increased from 2.2% in 2014 to 30.6% in 2023. No resistance was reported to ceftriaxone (Figure 4.9.2.2). 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 4.9.2.3a). This continued until 2023. For azithromycin a shift towards higher MICs is also observed over time (Figure 4.9.2.3b).

Figure 4.9.2.1 Number of reported gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test result at Sexual Health Centres, 2014-2023

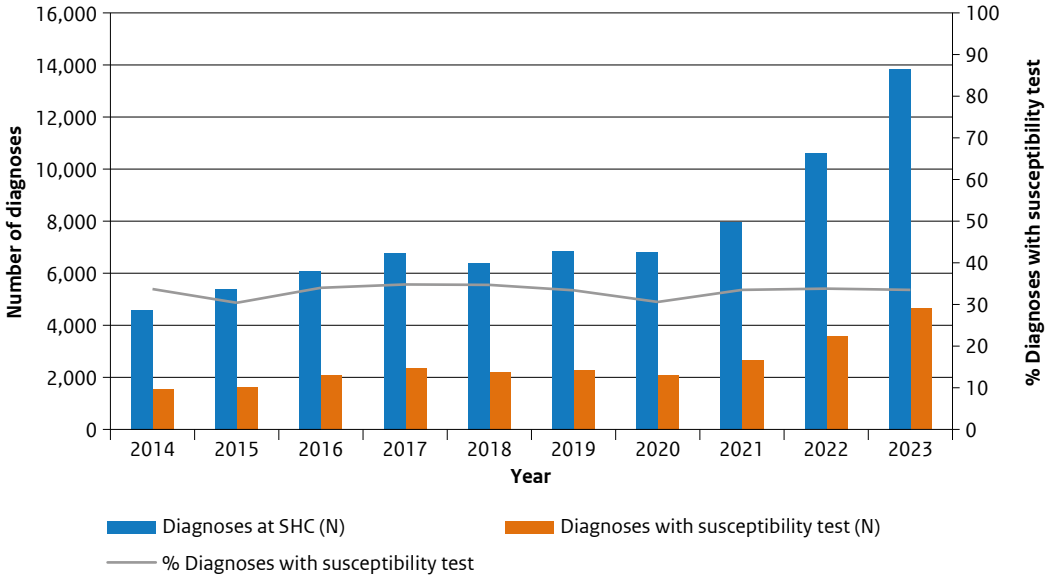
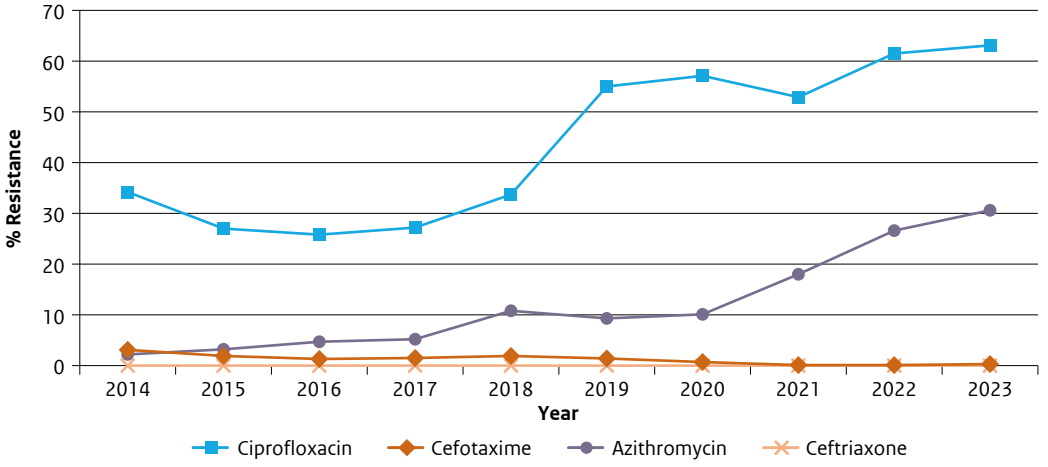


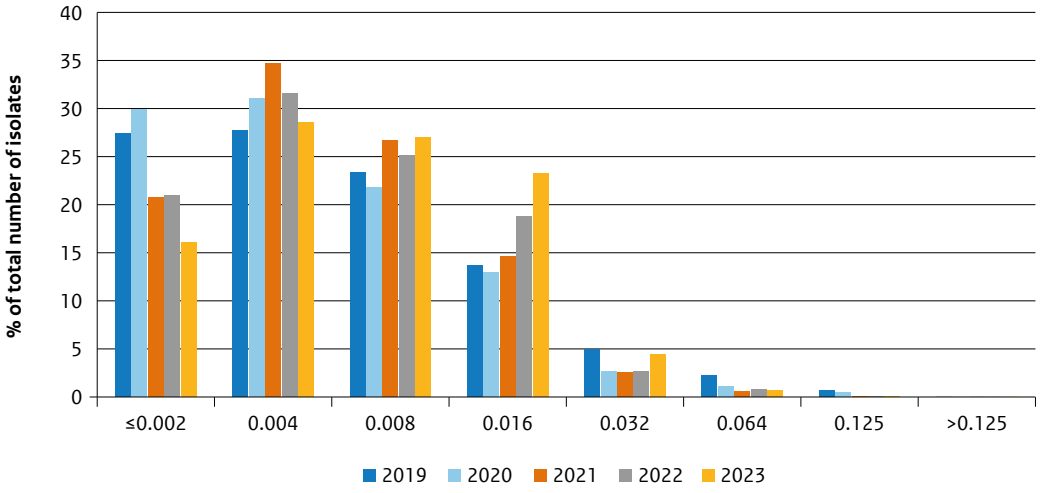
Figure 4.9.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2014-2023



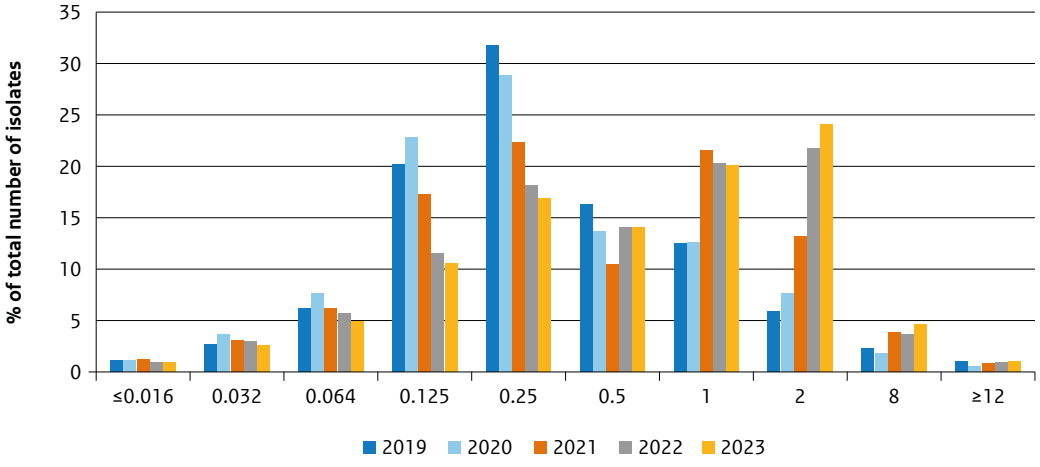
No resistance to ceftriaxone has been reported.

Figure 4.9.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2019-2023

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 2023 in one-third (33.5%) of all gonorrhoea diagnoses at the SHC susceptibility testing 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 is cultured, of which ~50% is culture negative, making susceptibility testing impossible. The number of gonorrhoea diagnoses almost doubled since 2021. This increase in diagnoses was mainly due to increased gonorrhoea positivity rates among heterosexuals, and especially among women and heterosexual men. This is also reflected in the distribution of isolates between sexes: in 2021, 85% of isolates were from men who have sex with men (MSM), 7% from heterosexual men and 7% from women. In 2023, 71% of isolates were from MSM, 11% from heterosexual men and 17% from women. 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. Yet, trends of decreasing susceptibility have been observed for multiple antimicrobial agents monitored in GRAS. Resistance to ciprofloxacin yearly increases and more than doubled since 2016, to 63.1% in 2023. MIC values for azithromycin also continue to increase. These findings are remarkable, because both ciprofloxacin and azithromycin are not first-choice treatments for gonorrhoea, according to guidelines. However, both are antibiotics that are also prescribed for a range of other infectious diseases, which might contribute to the decreasing gonococcal susceptibility.

These trends call for a continued effort to monitor the prevalence and emergence of antimicrobial resistance in gonococci, especially since the number of gonorrhoea infections is rising.

Conclusions

- Thanks to the GRAS programme, with stable inclusion rates over time, around 33% of 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 2019.
- Resistance to ciprofloxacin and azithromycin continues to increase.

References

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2 The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. Available from http://www.eucast.org/clinical_breakpoints/.

4.9.3 *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 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, resistance testing and the whole genome sequencing (WGS) typing of *M. tuberculosis* complex isolates in the Netherlands. These results are used to guide tuberculosis therapy of individual patients, as well as investigations of transmission and are used for the purpose of national TB surveillance. 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 laboratories in the Netherlands are involved in the diagnosis of TB and all send any *M. tuberculosis* complex isolates 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. 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 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, for which the combination of the results of WGS and phenotypic testing (a composite reference standard) yield more reliable predictions than phenotypic testing alone. Comparisons of molecular and phenotypic resistance testing have been described by Jajou *et al*[1] and Walker *et al*[2]. These studies form the basis of the current testing algorithm.[4]

Results

After a fall in cases between 2019 and 2022, in 2023 the number of notified TB cases increased by 11.5%; from 635 patients in 2022 to 709 patients in 2023.

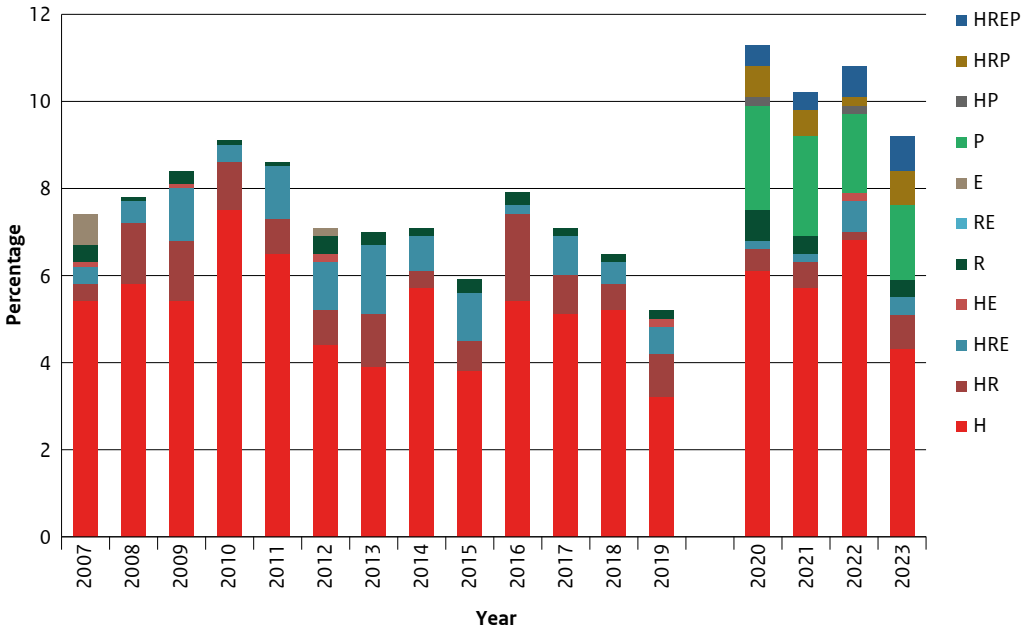
The decrease in TB cases in the Netherlands observed in the previous 3 years is considered to be at least partially related to the COVID-19 pandemic, reduced immigration and delayed diagnosis.

In 2023, of the 709 notified cases, 517 (73%) were culture confirmed. Isolates from these confirmed cases were received at the RIVM and subjected to WGS.

Approximately 90% of these isolates were predicted to be sensitive for first line antimycobacterial drugs on the basis of no relevant resistance mutations detected by WGS. In 9.1% (47/517) of the sequenced isolates resistance to one or more antimycobacterial drugs was detected. This mainly concerned isoniazid

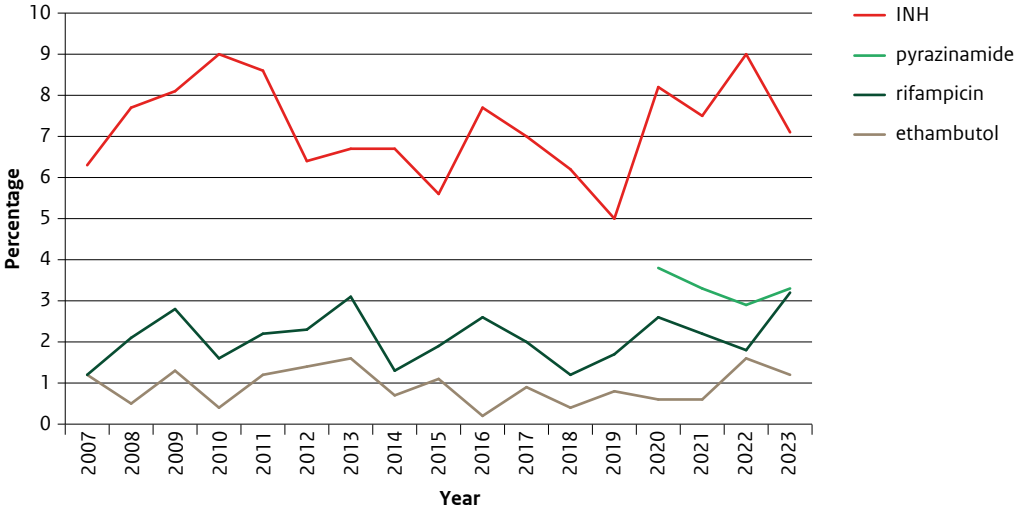
resistance (see figure 4.9.3.1). In total 14 multi-drug resistant (MDR)-TB cases (defined as resistant to at least both isoniazid (INH) and rifampicin) were detected and 2 TB cases resistant to at least rifampicin (RR). These observations were initially based on the detection of resistance mutations in WGS data from isolates for which resistance to at least one agent was predicted. In those isolates, subsequently phenotypic susceptibility testing was performed to confirm the genotypic findings and to screen for additional resistance according to our protocols.[4]
MDR and RR-TB cases represented 3.1% (16/517) of the culture positive cases diagnosed in 2023, which is an increase compared to the combined MDR and RR resistance in 2022 (1.8%).

Figure 4.9.3.1 Percentage combined antibiotic resistance for *M. tuberculosis* complex isolates 2007-2023



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 4.9.3.2 Percentage antibiotic resistance for *M. tuberculosis* complex isolates 2007-2023



Discussion

In 2023, 9.1% (47/517) of the isolates tested in the Netherlands revealed some form of resistance, compared to 10.9% (48/441) in 2022. From 2023 we have monitored 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 number of MDR isolates, in the Netherlands, increased slightly to 3.1% of all isolates, a total of 16 MDR/RR-TB cases. The extended hospitalization, complicated MDR-TB treatment, and contagious nature of this disease continues to justify special attention for these 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.

Conclusions

- Resistance to the first line antimycobacterial drugs remained almost stable over the last years.
- Although the number of MDR-TB cases was stable in recent years (average of 10 cases per year) in 2023 there was a slight increase in MDR cases to 16 (3.1% of culture confirmed cases). This trend is carefully monitored.
- The increase of 11.5% of the number of TB cases in the Netherlands in 2023 is remarkable. This may be a regression to the mean after the sharp decline in TB notification in 2020, which was presumably related to the COVID-19 pandemic. In 2021 there was also an increase of 10% compared to 2020.

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3 WHO, *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance: 2nd edition* 2023, World Health Organization: Geneva.

4 Anthony R, Groenheit R, Mansjö M, de Zwaan R, Werngren J. The Relative Positioning of Genotyping and Phenotyping for Tuberculosis Resistance Screening in Two EU National Reference Laboratories in 2023. Microorganisms. 2023; 11(7):1809. https://doi.org/10.3390/microorganisms11071809

4.9.4 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 with 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), Polio/Enterovirus, Mumps virus, Measles virus, Rubella virus, 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.

References

[1] HIV Monitoring Rapport 2022 | Stichting HIV Monitoring (hiv-monitoring.nl)

4.9.4.1 Influenza virus

Introduction

When vaccination against influenza is not available or fails due to antigenic mismatch with circulating viruses, influenza antiviral drugs can be used for (post exposure) prophylaxis as well as treatment of influenza cases with (expected) severe course of disease. In the Netherlands, the following agents are approved: the M2 ion channel blockers (M2B) amantadine and rimantadine, which act against type A viruses only, the neuraminidase enzyme inhibitors (NAI) oseltamivir and zanamivir and the acidic endonuclease inhibitor baloxavir marboxil (BXM), both classes of agents with potency against type A and B viruses. M2B prevents uncoating of the virus in the cell and BXM inhibits replication of the viral genome, both thereby inhibiting viral replication. In contrast, NAI prevent the release of progeny virus from the cell, which limits the infection rate of other cells. Seasonal influenza type A viruses have become fully resistant against M2B by 2010. Monitoring of NAI susceptibility of seasonal human influenza viruses is performed since the 2005/2006 winter season and of BXM susceptibility since 2019/2020.[1,2]

Methods

Monitoring of influenza antiviral susceptibility is embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinels, which is carried out by the Nivel Netherlands Institute for Health Services Research and the National Institute for Public Health and the Environment (RIVM) location of the National Influenza Centre (NIC). A subset of viruses detected in hospital laboratories and peripheral laboratories are submitted to the Erasmus Medical Centre or the RIVM locations of the NIC. For viruses of these both sources patient information on antiviral treatment, travel history and immune competence status in the 14 days preceding the time of specimen collection are collected. Furthermore, influenza viruses collected from COVID-19 testing sites organized by the municipal health service during 2021/2022, as well as those obtained through the community-based respiratory infection surveillance system (Infectieradar)[3] since 2022/2023, are included in the assessment. For viruses of these both sources patient information on antiviral treatment, travel history and immune competence status in the 14 days preceding the time of specimen collection are not collected.
Antiviral susceptibility is monitored using whole genome Nanopore sequencing for known reduced antiviral susceptibility markers for both NAIs and BXM. For a subset of influenza viruses, the susceptibility to NAIs is determined using an enzyme inhibition assay, which generates a 50% inhibitory concentration of the drug (IC_{50}) to confirm the impact of known markers for reduced antiviral susceptibility and to discover new markers. The use of antiviral drugs is monitored using data available from the Foundation for Pharmaceutical Statistics (SFK) collecting data from more than 97% of the community pharmacies in the Netherlands serving 15.8 million people (93%) of the Dutch population.

Results

Findings for the influenza seasons 2005/2006 through 2019/2020 are presented in NethMap 2016 and NethMap 2023.[1,2] Table 4.9.4.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2020/2021 influenza season. Up to 2022/2023, each season, none or only very few viruses with reduced antiviral susceptibility to NAI and recently BXM have been detected, and, if status reported to RIVM, were frequently associated with antiviral drug use. However, information on preceding antiviral treatment is often lacking. During the 2023/2024 season, A(H1N1)pdm09 viruses with the double amino acid substitution NA-I223V;S247N emerged, which appeared to be mildly reduced inhibited by oseltamivir.

The percentage double mutant viruses of sequenced A(H1N1)pdm09 viruses reached just over 10% in the peak of virus detections; overall for the season it accounted for 3.7% of all A(H1N1)pdm09 sequenced. For none of the patients with the double mutant virus with data on antiviral treatment, oseltamivir treatment was reported. Additionally, during the 2023/2024 season, among A(H1N1)pdm09 viruses two with NA-H275Y associated with highly reduced inhibition by oseltamivir were detected, next to one with NA-I223T associated with reduced inhibition by oseltamivir. Only for one NA-H275Y case the antiviral treatment status was known; the virus was retrieved during oseltamivir treatment.

Figure 4.9.4.1 shows the utilization of oseltamivir since 2020, as reported by the SFK. Oseltamivir prescriptions peak when the circulation of influenza viruses peaks. Zanamivir has not been prescribed since 2020 and BXM has still not been prescribed since its EU authorization early 2021, as reported by the SFK.

Discussion

In the Netherlands, and globally, the proportion of NAI reduced susceptible influenza viruses remained very low up to 2022/2023.[4] However, during the 2023/2024 season oseltamivir mildly reduced inhibited double mutant A(H1N1)pdm09 viruses emerged in The Netherlands. Preliminary analyses of all sequences in the influenza database of GISAID for the 2023/2024 season by April 2024, showed that the highest number of detected double mutant viruses are found in Europe, with reports still coming in from across the world. The clinical impact of these viruses is considered low because of low level reduced inhibition by oseltamivir, but clinical studies are needed to provide more conclusive results. According to the WHO, NAIs are still deemed appropriate for prophylaxis and treatment. Very few natural BXM reduced susceptible viruses were detected in the Netherlands, similar to the very low prevalence globally.[4] The findings together illustrate that it is important to continue monitoring the susceptibility of influenza viruses for NAIs and BXM.

Conclusions

- Mildy reduced inhibited A(H1N1)pdm09 viruses by oseltamivir with NA-I223V and NA-S247N double amino acid substitutions emerged during the 2023/2024 season, accounting for 3.7% of all A(H1N1) pdm09 sequenced. This was not associated with the use of antivirals prior to specimen collection, for cases for which antiviral treatment status was known.
- Sporadically, baloxavir marboxil reduced susceptible virus and neuraminidase inhibitor (highly) reduced inhibited virus with other amino acid changes in the neuraminidase protein have been detected. The latter was frequently associated with the use of antivirals prior to specimen collection if antiviral treatment status was known.
- Prescriptions of oseltamivir remain low with sharp increases during every influenza epidemic, even during the 2020/2021 season when hardly any influenza viruses were detected due to the COVID-19 pandemic measures.
- Zanamivir has not been prescribed since late 2020 and baloxavir marboxil has not been prescribed since its registration in 2021.

Table 4.9.4.1 (Highly) reduced inhibition/susceptibility of influenza viruses by NAIs and BXM in the Netherlands, 2020/2021 - 2023/2024[1]

	A(H1N1)pdm09		A(H3N2)		B	
Season	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[8]	34/834 (4.1%)[9]	0/644 (<1%)	0/402	0/382 (<1%)	0/26	0/19

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 inibition by zanamivir and normal inhibition by oseltamivir were detected; by phenotypic testing two were indeed reduced inhibited by zanamvir and with fold-change around the reduced inhibited threshold 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 1 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.

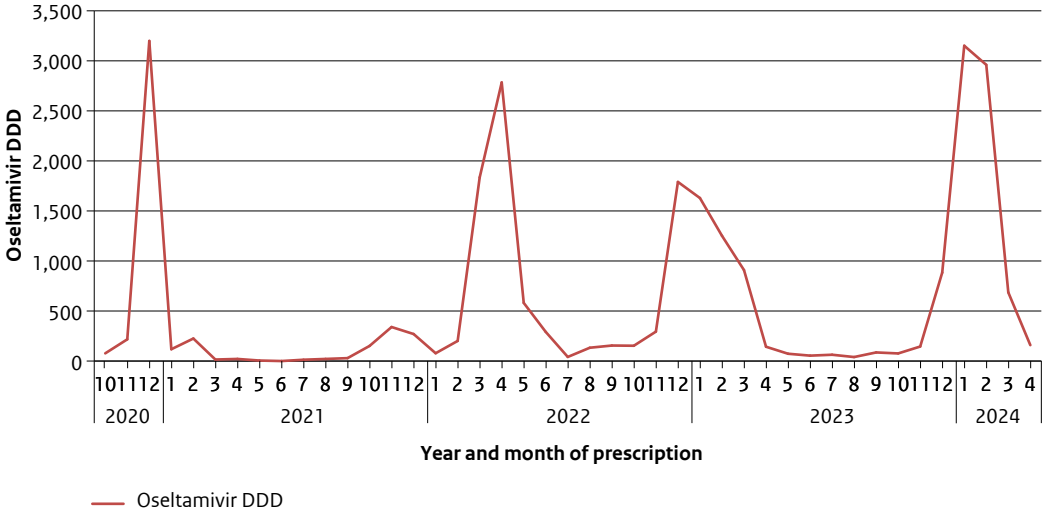
7 *A cluster of B/Victoria viruses emerged almost exclusively in The Netherlands with NA-K360E previously associated with highly reduced inhibition by peramivir. However, by phenotypic testing of 9 of the 89 viruses detected they appeared normal inhibited by peramivir, likely due to compensating additional amino acid substitutions A395V and L396F/S in the close vicinity of the 360 position in the 3D structure of the neuraminidase.*

8 *Preliminary data up to week 20/2024.*

9 *Thirty-one viruses with NA-I223V and NA-S247N (double mutant) were detected. Independently each single amino acid substitution has been associated previously with a slight shift in fold-change but still normal inhibited by oseltamivir and zanamivir. Fourteen of the double mutants were tested phenotypically and 13 were found to be reduced inhibited by oseltamivir (median fold-change 12.3; range 10.4-16.1) and normal inhibited by zanamivir. One double mutant virus had a fold-change of 9.5 just below the reduced inhibited threshold of 10. For none of the patients for which the treatment status was known oseltamivir treatment was reported.*
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 know; 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.

Figure 4.9.4.1 Prescriptions of oseltamivir in the Netherlands, 2020/2021 - 2023/2024

Shown are the Defined Daily Doses (DDD) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands.



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2 NethMap 2024. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2023. National Institute for Public Health and the Environment, June 2019.

3 Infectieradar (https://www.infectieradar.nl/welcome)

4 Govorkova EA, Takashita E, Daniels RS, Fujisaki S, Presser LD, Patel MC, Huang W, Lackenby A, Nguyen HT, Pereyaslov D, Rattigan A, Brown SK, Samaan M, Subbarao K, Wong S, Wang D, Webby RJ, Yen HL, Zhang W, Meijer A, Gubareva LV. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2018-2020. Antiviral Res. 2022 Apr;200:105281. doi: 10.1016/j.antiviral.2022.105281. Epub 2022 Mar 12. PMID: 35292289.

4.9.4.2 Human Immunodeficiency virus

Antiviral Drug Resistance data - HIV Summary as part of the national repository with reference to the HIV monitoring rapport 2023, Stichting HIV Monitoring[1]

Introduction

Human Immunodeficiency virus (HIV) is characterized as HIV-1 and HIV-2 and 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 small 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 on 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 which in turn affect drug's efficacy. A full characterization of circulating HIV strains with drug resistance is crucial to prevent HIV transmission and HIV-associated morbidity and mortality.[3]

HIV resistance can be categorized as pre-treatment drug resistance resulting from the transmission of HIV strains harbouring resistance associated mutations (RAM) and 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.

In the Netherlands, as in other countries, viral resistance tests are recommended at the start of the treatment (or established baseline viral loads) since 2003, though not performed in all HIV treatment centers. These tests help to determine the most appropriate therapeutic scheme while attempting to minimize the emergence/spread of HIV drug resistance.

Methods

HIV genotypic resistance analyses are performed in the Netherlands at entry into care and later on if antiretroviral treatment fails. By obtaining resistance analyses at baseline individual patient treatment can be optimized in both adults and infants, 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 HIVdb which infers antiretroviral drug susceptibility and resistance scores.[4] The 2022 International Antiviral Society-USA (IAS-USA) HIV drug resistance mutation list was used to score major resistance-associated mutations.[5]

Results

By the end of December 2022, 21,074 people with HIV were in care and on ART in the Netherlands. Antiretroviral resistance 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. HIV-1 subtype B (73.8%), followed by non-B subtypes (26.2%), including circulating recombinant forms (i.e. CRF_02AG (6.8%), subtype C (5.0%), and CRF_01AE (3.7%)) were the main identified HIV-1 subtypes.

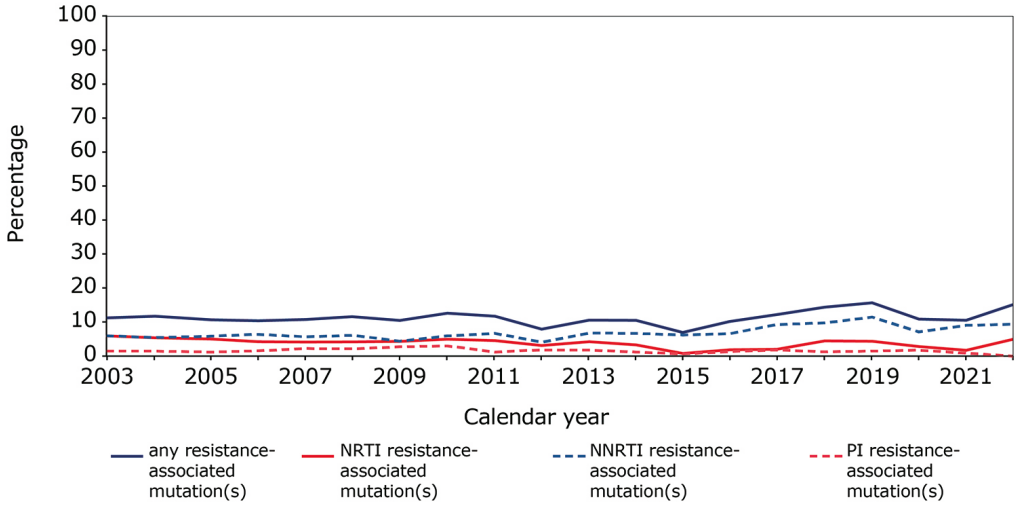
Transmitted drug resistance

Transmitted drug resistance is considered when RAM can be detected prior to the initiation of ART. From 2003-2022, 9,125 HIV-1 sequences were obtained from 8,806 ART-naïve individuals. Analysis was performed on 9,111 reverse transcriptase sequences available from 8,795 individuals; 8,572 protease sequences were available from 8,268 individuals; 412 integrase sequences were available from 411 individuals.

Overall, 282 (3.2%) individuals screened for transmitted drug resistance harboured high-level resistance to at least one antiretroviral drug, 73 (0.8%) to at least one nucleotide/nucleoside reverse transcriptase inhibitor (NRTI); 209 (2.4%) to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI); and 36 (0.4%) to at least one protease inhibitor (PI), as depicted in Figure 4.9.4.2. Noteworthy, from 411 people that had an integrase sequence available prior the time of entry into care (ART), only one major integrase resistance-associated mutation was detected (Y134Y/C).

Overall, more than 97% of individual infection isolates seemed fully susceptible while 2.8% (n=244) harboured high-level resistance in one drug class; 0.3% (n=27) in two drug classes; and less than 0.1% (n=5) to three drug classes (i.e. NRTIs, NNRTIs and PIs).

Figure 4.9.4.2 Annual percentage of patients with evidence of transmitted HIV drug resistance over time
Note: RAS, resistance associate substitutions. ***In*** HIV Monitoring report 2023 (p. 135)



Acquired drug resistance

From 2000 to 2022, 4,905 HIV-1 sequences from 2,933 individuals who received ART for at least four months when the HIV RNA was above 500 copies/ml, were analyzed (Figure 4.9.4.3). From these, 748 received mono or dual therapy in the past, representing 25.5% (748/2,933) of the individuals with a sequence result available. In summary, analysis was performed on 3,518 sequences from 2,185 individuals who had been ART-naïve before initiating ART; 4,589 reverse transcriptase sequences from 2,903 individuals; 4,582 protease sequences from 2,754 individuals, and 563 integrase sequences were available from 437 individuals.

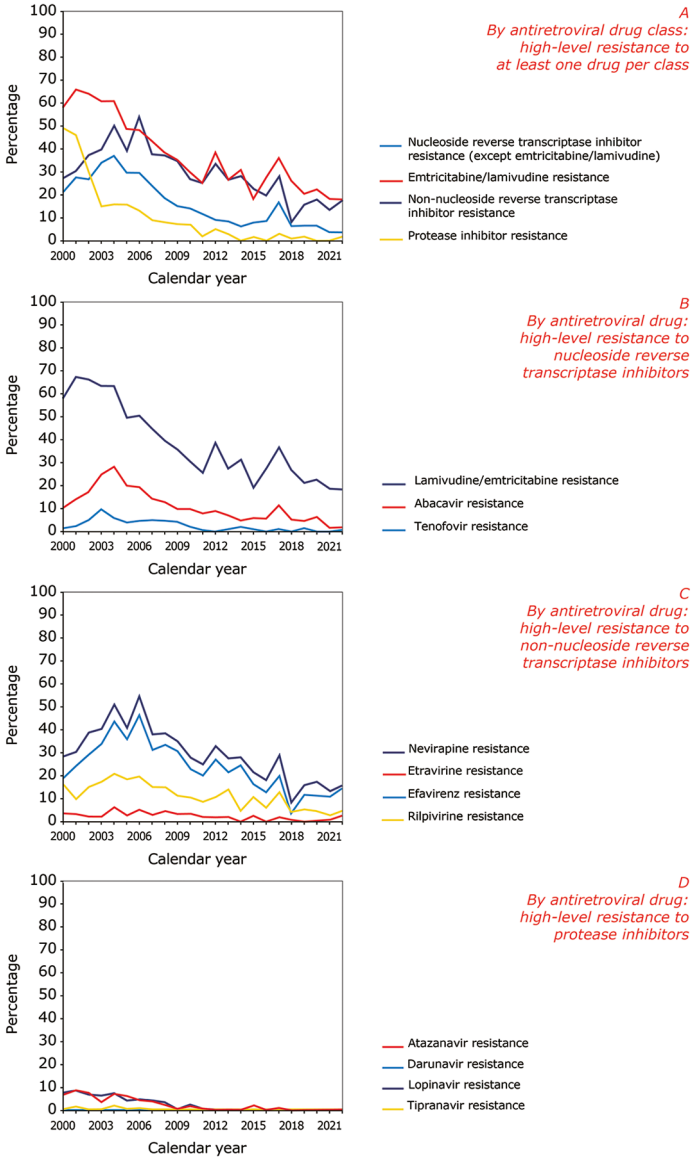
A total of 2,939 (59.9%) harboured high-level resistance to at least one antiretroviral drug. High-level NRTI resistance was detected in 2,966 (60.5%) sequences; of those, 2,531 (85.3%) harboured high-level resistance to emtricitabine or lamivudine. Notably, of the 1,869 individuals ever identified as harbouring the M184V or M184I mutation who were still in care in 2022, 1,205 (64.5%) were still on ART containing lamivudine or emtricitabine, of whom 925 (76.8%) had undetectable HIV-RNA at their last visit. In addition, 1,764 (36.6%) harboured high-level resistance to at least one NNRTI, and 1,036 (22.6%) to at least one PI.

The available 563 integrase sequences originated from 437 people who received integrase-containing ART for at least four months; 43 were pre-treated with monotherapy or dual NRTI therapy before initiating ART, and 394 were ARV-naïve before initiating ART. Most people had initiated ART years before; the median time between ART initiation and testing for integrase inhibitor resistance was 10.4 years (IQR 4.8-15.8). From the 473 individuals, 52 displayed at least one acquired major mutation associated with integrase inhibitor resistance. The following RAMs could be detected: N155H/N, R263R/K, E92E/Q, Y143R and Y143Y/C, T66T/I, Q148H/R, S147S/G (major INSTI RAM); T97 (T97A/T), T66T/A/K/I, L74I/L/M, G140G/S, E138K/A (minor RAM).

PrEP related drug resistance

Genotypic resistance test results were performed for 65 (73.0%) of the 89 individuals who reported having used PrEP prior to first entering HIV care. In 13 (20.0%) reverse transcriptase (RT) RAM (M184V/I) were demonstrated indicating ongoing 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).

Figure 4.9.4.3 The percentage of sequences with evidence of high-level resistance by drug class per year, obtained at the time of virological failure when receiving combination ART, among previously antiretroviral drug-naïve people. Graphs depicting high level resistance to antiretroviral drug (class): A) at least one drug within class, B) nucleoside reverse transcriptase inhibitors, C) non-nucleoside reverse transcriptase inhibitors, D) protease inhibitors. Note: additional information on high-level resistance to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors, available in original report. ***In*** HIV Monitoring report 2023 (p. 140/141)



Discussion

In the Netherlands, the proportion of antiretroviral resistance shows decreasing trends among transmitted and acquired drug resistance groups (Figure 4.9.4.2). While transmitted HIV drug resistance results from transmission of an HIV strain containing RAM, acquired resistance mostly results from intentional or unintentional decreased treatment adherence or decreased exposure to ART resulting from drug-drug interactions. Data shows high rates of NRTI resistance among MSM that had used PrEP.
Noteworthy, incomplete suppression of viral replication by ART, may enable HIV mutations, henceforth a possible ART failure since all antiretroviral drugs, including newer classes, have an associated risk of ineffectiveness due to drug-resistant HIV virus emergence. Continuous monitoring in both newly diagnosed infections 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 suppress viral loads, 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 the SHM.

Conclusions

- Globally, viral suppression rates in patients with HIV receiving ART is high and has been improving. Among those with ART failure, the annual percentage with acquired antiviral resistance remained low; similarly to other high-income settings.
- Overall, 3.2% of individuals screened for transmitted drug resistance harboured high-level resistance to at least one antiretroviral drug and 59.9% of individuals screened for acquired drug resistance harboured high-level resistance to at least one antiretroviral drug.
- Only one major integrase resistance-associated mutation was detected (Y134Y/C) among 411 people that had an integrase sequence available prior the time of entry into care (ART).

References

1 Stichting HIV Monitoring (hiv-monitoring.nl)
2 Fact Sheet: HIV Drug Resistance (who.int) (data from 17.November.2022; accessed on July, 2023)
3 Global action plan on HIV drug resistance 2017–2021. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
4 HIV Drug Resistance Database (stanford.edu)
5 Wensing AM et al. 2019 update of the drug resistance mutations in HIV-1. Top. Antivir. Med. 27, 111–121 (2019).

4.9.5 The antibiotic susceptibility profile of anaerobic bacteria

Introduction

As for aerobic bacteria, resistance among anaerobic bacteria is also increasing. The human commensal microbiome consists for more than 95% out of anaerobic bacteria, and infections caused by this group of bacteria are often caused by these commensals. Here, trends in antibiotic resistance of anaerobic bacteria isolated from human clinical specimens at the University Medical Center Groningen in 2023 is reported.

Methods

As in previous years, about 1000 anaerobic isolates, isolated in 2023, were included in this overview. Isolates were retrieved from a variety of human clinical specimens, e.g. abdominal samples, orthopedic materials, pus, drain fluids, blood cultures, etc. All cultures were performed for diagnostic purposes. Isolates were identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany), complemented with an in-house made database for the identification of anaerobic bacteria which were not represented in the manufacturer's database. Presence of the *cfiA* gene, encoding a metallo-beta-lactamase, in *Bacteroides fragilis* isolates was assessed using the MALDI-TOF MS subtyping module. MIC-values for the different antibiotics were determined using gradient strips (bioMerieux, Marcy-l' Étoile, France), according to the manufacturer's instructions. The MIC-value for clindamycin was determined for all anaerobes isolated, as was metronidazole with the exception of *Actinomyces* spp., *Cutibacterium* spp. and bifidobacteria. *Bacteroides*, *Parabacteroides* and *Prevotella* were only tested for amoxicillin when they were not producing beta-lactamase. Amoxicillin-clavulanic acid (fixed concentration of clavulanic acid) was tested for all Gram-negative anaerobic bacteria. The MIC-value for meropenem was determined for *Bacteroides*, *Parabacteroides* and *Prevotella* isolates.
Resistance was assessed using the most current EUCAST breakpoints (v14.0). These guidelines provide species/genus specific breakpoints for *Bacteroides* (including *Parabacteroides* and *Phocaeicola*), *Prevotella*, *Fusobacterium necrophorum*, *Clostridium perfringens* and *Cutibacterium acnes*. No breakpoints for *Prevotella* spp., *C. perfringens* and *C. acnes* are available for amoxicillin-clavulanic acid, since growth of these bacteria is already inhibited by low concentrations of clavulanic acid. For all other anaerobic bacteria the MIC-values given in the guidance document "when no breakpoints are available" by EUCAST were used. Use of an antibiotic with an MIC above this value is discouraged.

Results

An overview of all measured MIC_{50} and MIC_{90} values and the resistance percentage is shown in Table 4.9.5.1. As in previous years metronidazole resistance among *Bacteroides* and *Prevotella* isolates was observed. One *Prevotella bivia* isolate had an MIC of 128 mg/L and a *Bacteroides thetaiotaomicron* had an MIC of 12 mg/L. Both isolates were susceptible for meropenem. One *Clostridium tertium* isolate was resistant to metronidazole. Furthermore, 9 (6%) of the *Bacteroides* isolates were resistant to meropenem: one *B. thetaiotaomicron* isolate (MIC 2 mg/L), one *Bacteroides ovatus* isolate (>32 mg/L) and 7 *B. fragilis* isolates (MICs ranging from 1.5 to >32 mg/L). Two of those seven meropenem-resistant *B. fragilis* isolates (MIC 1.5 and 6 mg/L) did not harbor the *cfiA* gene in their genome, while the other five isolates did harbor the gene (MIC 12 - >32 mg/L). One *Prevotella bergensis* isolate was also resistant to meropenem, with an MIC of 0.75 mg/L. Part of the *Parabacteroides* isolates (7/11) were tested for meropenem and all were shown to be susceptible, with MICs ranging from 0.094 mg/L to 0.38 mg/L.
Resistance for amoxicillin was observed among *Fusobacterium* and *Peptostreptococcus* spp., 15.4% and 17.6% respectively.

Discussion

The breakpoints of antibiotics for anaerobic bacteria have been extensively modified by EUCAST and are currently more adapted to the different anaerobic genera, each having its own characteristics. In most cases the breakpoints have been lowered, resulting in different resistance rates. Since these modifications are still in progress, we chose to present the rate of resistance using the current breakpoints. When resistance rates were compared with those in previous years, the resistance percentage was determined using the breakpoints from previous years. The breakpoints for metronidazole remained similar, with the exception of the breakpoint for *F. necrophorum*, which was changed to 0.5 mg/L. In the last decade metronidazole resistance among *Bacteroides* and *Prevotella* spp. varied from 0 till 2%. In 2023 resistance rates for this antibiotic were 0.6% and 0.9%, respectively. The breakpoint for meropenem was lowered which resulted in a higher proportion of *Bacteroides* isolates determined to be resistant, and one resistant *Prevotella* isolate with an MIC slightly higher than the established breakpoint. Meropenem-resistant *B. fragilis* isolates with an MIC for meropenem >1 mg/L but <8 mg/L did not harbor the *cfiA* gene, which encodes a metallo-beta-lactamase believed to be responsible for carbapenem resistance. Presence of antibiotic resistance genes in the meropenem resistant *B. thetaiotaomicron* and *B. ovatus* isolate was not determined. The resistance rate of 6% for meropenem in *Bacteroides* spp. does not correlate with the resistance rate for amoxicillin-clavulanic acid of 14.1%. In order to assess whether meropenem resistance remained stable among this group of bacteria, the resistance percentage of the 2023 isolates was also reinterpreted using the previous breakpoints and by using those breakpoints, the resistance percentage was determined to be 4.7%, which was higher than in earlier years. The highest rate observed so far was 4% in 2022.

No breakpoints for amoxicillin for *Bacteroides* and *Parabacteroides* species have been established, since they are assumed to be intrinsic resistant. For all other genera the breakpoint is significantly lower than previously, resulting in resistance among the gram-positive anaerobic cocci genera, which was previously not observed. Especially among *Peptostreptococcus* species and clostridia, a high resistance rate for amoxicillin was determined, with resistance rates of 17.6% and 33.3%, respectively, although the absolute numbers of tested isolates are low.

Clindamycin resistance differs between the genera, but is highest among the *Parabacteroides* isolates, with a resistance rate of 90.9%.

Conclusions

- Metronidazole resistance among clinical *Bacteroides* and *Prevotella* isolates remained stable.
- The modified breakpoints for meropenem lead to an increased resistance rate among *Bacteroides* isolates.
- Not all meropenem resistant *B. fragilis* isolates harbor a *cfiA* gene in their genome.
- Amoxicillin resistance is observed among gram-positive anaerobic bacteria.
- High rates of clindamycin resistance are observed among *Parabacteroides* isolates, which should be taken into account in case of empiric therapy.

Table 4.9.5.1 MIC_{50}, MIC_{90} and percentage resistance of clinically anaerobic isolates for the different antibiotics

	amoxicillin			amoxi-clav			clindamycin			metronidazole			meropenem		
Genus	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R
Gram-negative anaerobes															
Bacteroides spp. (148-149)[a,b]	nd[c]	nd	nd	0.25	8	14.1	3	>256	41.2	0.38	0.75	0.6	0.19	0.75	6
Dialister spp. (10)	0.047	0.19	0	<0.016	0.094	0	0.38	0.75	30	0.38	4	10	nd	nd	nd
Fusobacterium spp. (26-27)	0.023	1	15.4	<0.016	0.016	3.7	0.064	0.19	7.4	<0.016	0.064	0	nd	nd	nd
Parabacteroides spp. (11)	nd	nd	nd	1.5	2	9.1	4	>256	90.9	0.19	0.75	0	nd	nd	nd
Porphyromonas spp. (18-22)	<0.016	0.032	5.6	<0.016	0.023	4.5	<0.016	>256	18.2	0.047	1.5	0	nd	nd	nd
Prevotella spp. (113-119)	nd	nd	nd	0.016	0.38	nd	0.023	>256	21.1	0.25	1.5	0.9	0.047	0.125	0.9
Veillonella spp. (37-38)	0.5	1.5	48.6	0.38	1	23.7	0.19	0.38	0	1.5	3	2.7	nd	nd	nd
Gram-positive anaerobes															
Actinomyces spp. (96)	0.125	0.38	5.2	nd	nd	nd	0.38	>256	31.3	nd	nd	nd	nd	nd	nd
Anaerococcus spp. (40)	0.032	0.094	0	nd	nd	nd	0.094	>256	27.5	0.19	0.75	0	nd	nd	nd
Bifidobacterium spp. (10)	0.25	1.5	30	nd	nd	nd	0.032	0.094	0	nd	nd	nd	nd	nd	nd
Clostridium spp. (33)	0.5	2	33.3	nd	nd	nd	0.75	48	57.6	0.5	1.5	3	nd	nd	nd
Clostridium perfringens (11)[d]	0.032	0.064	0	nd	nd	nd	3	128	90.9	0.5	0.75	0	nd	nd	nd
Cutibacterium acnes (200-201)	0.064	0.19	4.5	nd	nd	nd	0.047	0.19	4.5	nd	nd	nd	nd	nd	nd
Cutibacterium spp. (31)[e]	0.19	0.38	3.2	nd	nd	nd	0.047	>256	25.8	nd	nd	nd	nd	nd	nd
Finegoldia magna (77)	0.25	0.38	1.3	nd	nd	nd	1	>256	58.4	0.25	0.5	0	nd	nd	nd
Parvimonas micra (43)	0.023	0.125	2.3	nd	nd	nd	0.25	3	20.9	0.094	0.25	0	nd	nd	nd
Peptoniphilus spp. (56)	0.016	0.19	0	nd	nd	nd	0.38	128	46.4	0.38	1.5	0	nd	nd	nd
Peptostreptococcus spp. (16-17)	0.125	0.75	17.6	nd	nd	nd	0.19	1	17.6	0.032	0.19	0	nd	nd	nd

[a] *Number of isolates. For some genera, not all isolates were tested for all antibiotics.*

[b] *Bacteroides spp.: includes Phocaeicola spp.*

[c] *Not determined.*

[d] *Clostridium spp.: excluding C. perfringens and C. difficile, but including genera which were previously named Clostridium, e.g. Hungatella.*

[e] *Cutibacterium spp.: excluding C. acnes.*

4.9.6 *Clostridioides difficile*

Introduction

Clostridioides difficile is an intestinal bacterium that can be found in humans and animals. In both, asymptomatic colonization with toxin-producing *C. difficile* 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). Currently, the surveillance is coordinated by the national CDI Expert Centre, a collaboration between the Center for Infectious Disease Control (CIb) of RIVM and Leiden University Medical Centre (LUMC). Whole genome sequencing (WGS) offers the opportunity to better understand and map pathogen transmission and epidemiology. For *C. difficile*, historically ribotyping was used for this purpose. In 2022, we validated core genome multi-locus sequence typing (cgMLST) for routine bacterial typing.[1] Current first-line therapeutics recommended in national and international guidelines are fidaxomicin and vancomycin, and metronidazole in conditional cases.[2] Notably, fidaxomicin resistance has recently been observed in Europe in recurrent CDI after treatment with fidaxomicin, reports on vancomycin resistance have caused concern in the United States, and a new molecular mechanism for metronidazole resistance has been described.[3,4,5] Therefore, the surveillance report of 2023 includes more detailed information on *C. difficile* resistance in the Netherlands. At the end of the year 2023, Public Health England reported on fifty cases with a new RT955, that has caused two large clusters in UK hospitals and is resistant to metronidazole, fluoroquinolones, and erythromycin. RT955 is genetically similar to the epidemic RT027, and both belong to sequence type (ST) 1. We queried our database for this RT that has caused international concern.

Methods

As of 2022, the 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 Centre 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. 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 continued to be offered for all microbiology laboratories in the Netherlands for typing *C. difficile* isolates of patients with severe disease or isolates from a suspected outbreak. We here present circulating ribotypes over the years, and for 2023 also the cgMLST typing results.[1] In addition, 100 isolates were randomly selected to assess Minimum Inhibitory Concentrations (MICs) of metronidazole, vancomycin and fidaxomicin using the agar dilution method, using EUCAST breakpoints version 14.0 (2024).

Results

In 2023, 378 fecal samples/isolates were sent to the Expertise Centre for the sentinel surveillance and *ad hoc* typing. In the CDI sentinel surveillance, 249 cases (median age 68, IQR 57-76) met inclusion criteria, of which 202 *C. difficile* isolates could be cultured and typed. We received 28 samples for *ad hoc* typing of severe cases or suspected outbreaks. One center noted an increased CDI incidence and submitted 12 isolates. Here, cgMLST analysis confirmed clonal relationship of two RT027/ST1 isolates with one allele difference; two RT078/ST11 strains had 4 alleles difference, not suspicious for transmission. No CDI outbreaks (>2 related cases) were confirmed in 2023.

CDI incidence was relatively stable with 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 increased from 1.0% (95%CI 0.0-2.1) in 2022 to 4.2% (95%CI 1.7-6.7, n=10) in 2023; the range of the previous 10 years was 1-4%. The percentage of patients with severe disease continued to increase from 16.1% (95%CI 13.7-18.5) in 2018-2019 to 30.3% (95%CI 25.0-35.6) in 2022 and 39.9% (95%CI 33.8-46.0) in 2023. The age of severe CDI cases remained stable with a median age of 69.5 years. The proportion of cases with a complicated course, i.e. with 30-day mortality, needing ICU admission or surgery, did not increase and was 13% in 2023. The proportion of cases reported to be community onset cases also appeared to increase from 47% (41.3-52.7) in 2022 to 56% (49.7-62.1) in 2023. Key epidemiological characteristics over time are shown in Table 4.9.6.1.

In 2023, RT014/020 continued to be the most prevalent RT accounting for 16.7% of isolates. The increased proportion of RT106 in 2022 (5.3%) was not sustained and decreased to 1.5% in 2023. See the Figure 4.9.6.1 for the proportions of the 5 most common ribotypes in *C. difficile* sentinel surveillance samples in the current and past year(s). The most common STs in 2023 were ST8 (n=24, 13.2%, includes RT002), ST2 (n=22, 12.1%, includes RT014/020), ST11 (n=22, 12.1% includes RT078/126) and ST6 (n=16, 8.8%, includes RT005). ST1, which includes RT027 and the newly identified RT955, was not detected in the five centers participating in the sentinel surveillance. RT955 was not found in *ad hoc* typing samples either or in our historic database with Dutch isolates. However, RT955 was found in strains cultured in Poland and Serbia which were sent for characterization to our laboratory in 2020 and 2023. These strains have been sequenced for comparison with the UK strains in an international effort to map origin and spread of RT955.

No resistance to metronidazole, vancomycin and fidaxomicin was detected. One isolate (RT002/ST8) had a vancomycin MIC of 2 mg/L. MIC_{50} and MIC_{90} values (mg/L) and range of MICs per sequence type and per ribotype can be found in Table 4.9.6.2. Notably, overall MIC90 of vancomycin was 1.0 mg/L in 2023, while the MIC_{90} reported in NethMap 2021 per ribotype did not exceed 0.125 mg/L.

Discussion

The trend of more severe CDI cases first noted during the second COVID-19 wave in the Netherlands[6] has continued. 2023 data suggest an increase in CDI-attributable mortality, however, confidence intervals of attributable mortality in 2022 and 2023 are overlapping. Vancomycin MIC_{90} has increased, but there was still no vancomycin resistance detected using EUCAST cut-off levels. Revised national (2023) and international treatment (2021) guidelines are anticipated to result in increased prescription of vancomycin and fidaxomicin.[2] It has recently been suggested that reduced vancomycin susceptibility is associated with treatment failure, however, this association has not been confirmed.[7] Altogether, this emphasizes the importance of monitoring resistance in *C. difficile*. A new RT955 causing outbreaks in the UK is currently not found in the Netherlands. The CDI Expertise Center is supporting international efforts to map the

distribution and characterization of this new RT. To investigate the occurrence of *C. difficile* in cattle, the National Institute for Public Health and the Environment (RIVM) collaborated with the CDI Expert Center on a study at dairy farms. A predominance of RT695 and RT078/126 was found[8], the latter being highly prevalent in clinical isolates from humans. Monitoring of circulating ribo- and sequence types, investigations into *C. difficile* occurrence outside human health (One Health perspective), and *ad hoc* typing are relevant for preparedness by early recognition of new circulating strains and control of potential outbreaks.

Table 4.9.6.1 Key epidemiological characteristics of *C. difficile* surveillance over the years 2009-2023

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
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
Location of onset													
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%	55%	53%	44%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%	45%	47%	56%
Course and outcome[1]													
Severe CDI[2]	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%	21%	30%	40%
Uncomplicated course[3]	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%	89%	83%	87%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%	2%	1%	4%
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%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	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

[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] *2022 data is based on 5 laboratories.*

Figure 4.9.6.1 Proportions of the 7 most common ribotypes in time in *C. difficile* sentinel surveillance samples

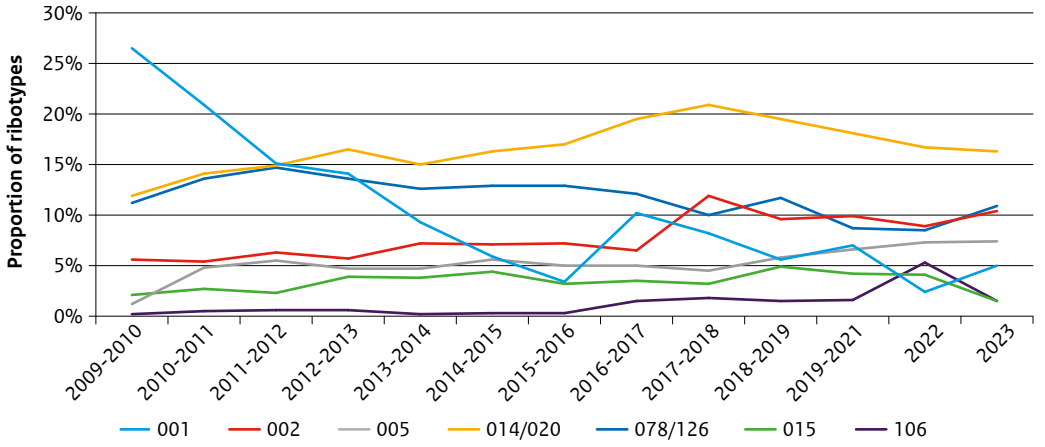


Table 4.9.6.2 MIC_{50}, MIC_{90}, and range (mg/L) per sequence type (ST) and ribotype (RT) of 100 randomly selected *C. difficile* surveillance isolates sampled in 2023

	MIC_{50}	MIC_{90}	Range
Total (n=100)			
Metronidazole	0.25	0.25	0.125-0.5
Vancomycin	0.5	1	0.5-2
Fidaxomicin	0.125	0.125	0.03-0.25
ST2 (n=13)			
Metronidazole	0.25	0.4	0.125-0.5
Vancomycin	0.5	1	0.5-1
Fidaxomicin	0.125	0.2	0.06-0.25
ST6 (n=9)			
Metronidazole	0.25	.	0.125-0.25
Vancomycin	0.5	.	0.5-1
Fidaxomicin	0.125	.	0.06-0.25
ST8 (n=12)			
Metronidazole	0.25	0.25	0.125-0.25
Vancomycin	1	1.7	0.5-2
Fidaxomicin	0.125	0.25	0.06-0.25
ST11 (n=11)			
Metronidazole	0.25	0.5	0.25-0.5
Vancomycin	0.5	1	0.5-1
Fidaxomicin	0.125	0.125	0.06-0.125
ST14 (n=8)			
Metronidazole	0.25	.	0.125-0.25
Vancomycin	0.75	.	0.5-1
Fidaxomicin	0.125	.	0.06-0.125
Other sequence types (n=47)			
Metronidazole	0.25	0.25	0.125-0.5
Vancomycin	0.5	1	0.5-1
Fidaxomicin	0.125	0.15	0.03-0.25

	MIC_{50}	MIC_{90}	Range
Total (n=100)			
Metronidazole	0.25	0.25	0.125-0.5
Vancomycin	0.5	1	0.5-2
Fidaxomicin	0.125	0.125	0.03-0.25
RT002 (n=11)			
Metronidazole	0.25	0.25	0.125-0.5
Vancomycin	1	1.8	0.5-2
Fidaxomicin	0.125	0.25	0.06-0.25
RT005 (n=8)			
Metronidazole	0.25	.	0.125-0.25
Vancomycin	0.5	.	0.5-1
Fidaxomicin	0.125	.	0.125-0.25
RT014 (n=11)			
Metronidazole	0.25	0.25	0.125-0.25
Vancomycin	0.5	1	0.5-1
Fidaxomicin	0.06	0.125	0.06-0.125
RT020 (n=7)			
Metronidazole	0.25	0.25	0.25-0.25
Vancomycin	1	.	0.5-1
Fidaxomicin	0.125	.	0.125-0.25
RT078 (n=7)			
Metronidazole	0.25	.	0.25-0.5
Vancomycin	0.5	.	0.5-1
Fidaxomicin	0.125	.	0.06-0.125
Other ribotypes (n=56)			
Metronidazole	0.25	0.25	0.125-0.5
Vancomycin	0.5	1	0.5-1
Fidaxomicin	0.125	0.125	0.03-0.25

Conclusions

- No CDI outbreaks were detected in 2023.
- There is a continued increase in severe CDI cases and community-onset CDI.
- Vancomycin MIC_{90} has increased, but no vancomycin resistance was detected.
- The newly reported metronidazole resistant RT955 was not found in the Netherlands, but was present in our database amongst isolates from Serbia and Poland.

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4.9.7 *Aspergillus fumigatus*

Introduction

Aspergillus fumigatus is a saprobic mold that thrives on decaying plant material and may cause respiratory diseases in humans, including chronic and acute pulmonary aspergillosis. *Aspergillus* diseases are treated with medical triazoles, including itraconazole, voriconazole, posaconazole and isavuconazole which can be administered both orally and intravenously. Azole resistance has emerged primarily driven by resistance selection through the use of azole fungicides in our 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. Azole monotherapy was shown to be less effective in azole-resistant invasive aspergillosis and alternative treatment options are limited. Surveillance is performed to determine resistance levels in hospitals and trends in the distribution of resistance mutations.

Methods

In five University Medical Centers (UMCs) and five teaching hospitals clinical *A. fumigatus* isolates were 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 reference laboratory for MIC-testing and sequence-analysis of the *Cyp51A* gene. MIC-testing is performed using the EUCAST microbroth dilution method and using recommended clinical breakpoints. Underlying disease information was collected for patients harboring a triazole-resistant isolate. The resistance frequency based on the number of patients screened was determined for all participating centers and compared with previous years.

Results

In 2023, *A. fumigatus* isolates from 1,354 culture-positive patients were screened for triazole resistance, including 649 (range 65 to 188 per center) patients from UMCs and 705 (range 92 to 204 per center) patients from teaching hospitals. Overall, 124 azole-resistant *A. fumigatus* isolates were recovered from 102 patients. The overall resistance frequency was 7.5%, with a resistance frequency of 11.6% (75 of 649 patients) in UMCs and 3.8% (27 of 705 patients) in teaching hospitals (Table 4.9.7.1). The resistance frequency in three UMCs was above 10%, which is the recommended threshold to consider changing empirical antifungal treatment regimen. In all teaching hospitals the resistance frequency was below 10%, ranging from 0.9% to 7.1%.

Of the 124 *A. fumigatus* isolates that were analyzed for resistance mutations in the *Cyp51A* gene, a tandem repeat (TR_{34} or TR_{46}) was detected in 101 isolates (81.5%), of which 71 TR_{34} and 30 TR_{46}. The presence of TR_{34} or TR_{46} is indicative of environmental resistance selection rather than in-host resistance development during antifungal therapy. Isolates harboring TR_{34} included 67 with TR_{34}/L98H only, while four isolates (4 of 71; 5.6%) harbored additional short nucleotide polymorphisms (SNPs). Additional SNPs were found in 21 of 30 isolates (70%) with a TR_{46}, recovered from patients from six different hospitals. Ten azole-resistant *A. fumigatus* isolates harbored no mutations in the *Cyp51A* gene, indicating that other (unknown) resistance mechanisms are present.

Of the 102 patients with triazole-resistant *A. fumigatus*, data regarding underlying condition was available for 75 patients. Of these, 34 (45.3%) suffered from a chronic lung disease, 16 had a solid organ transplant and four had a hematological malignancy as underlying disease. The presence of *Aspergillus* disease was not classified in these patients.

Discussion

The azole resistance rates in 2023 are similar to previous years, with resistance rates exceeding 10% in three of ten surveillance centers. Resistance rates were lower in teaching hospitals compared to UMCs. Similar to previous years genotype variations were observed in azole-resistant isolates harboring TR_{34} and TR_{46}. Although TR_{46}/Y121F/T289A is the default resistance genotype, the majority of isolates with TR_{46} (21 of 30) showed variations consisting of additional SNPs in the *Cyp51A* gene or variations in the number of TRs in the gene promoter. These variations might alter the azole susceptibility phenotype and will not be detected by current *Aspergillus* resistance PCR tests. TR_{34} and TR_{46} mutations are associated with environmental resistance selection, and continued azole fungicide pressure might drive increasing resistance genotype variation. Resistant isolates with no mutations in the *Cyp51A* gene might also indicate increasing variation in resistance mechanisms.

An acknowledged drawback of our pathogen surveillance is the lack of clinical data, especially the classification of *Aspergillus* disease. This would require collection of clinical and microbiological data. Furthermore, patients with hematological malignancy are underrepresented as these are commonly diagnosed with biomarkers, rather than positive cultures. Research is ongoing to investigate if enhanced *Aspergillus* disease surveillance is feasible, meaning that the resistance burden and implications can be assessed in specific *Aspergillus* diseases, e.g. invasive aspergillosis and chronic pulmonary aspergillosis.

Conclusions

- Triazole resistance frequency in 2023 was 11.6% in UMCs and 3.8% in teaching hospitals, which is stable in comparison with previous years.
- In two of five UMCs and all teaching hospitals the azole resistance frequency was below the 10% threshold.
- Overall, 81.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism, with TR-genotype variants especially frequent in TR_{46} isolates.

Table 4.9.7.1 Triazole resistance proportion in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers and 5 teaching hospitals, 2018-2023

	2018		2019		2020		2021		2022		2023	
	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)
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)
Radboudumc	196	23 (11.7)	230	23 (10)	193	20 (10.4)	205	25 (12.2)	175	18 (10.3)	172	13 (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)
AmsterdamUMC	81	13 (16)	51	6 (11.8)	172[a]	16 (9.3)	173	20 (11.6)	175	16 (9.1)	110	18 (16)
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)**
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)
St. Antonius 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)
PAMM*	81	4 (4.9)	147	8 (5.4)	150	3 (2)	129	6 (4.7)	141	4 (2.8)	114	1 (0.9)
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)
Isala	195	13 (6.7)	222	18 (8.1)	183	10 (5.5)	222	20 (9)	237	11 (4.6)	204	6 (3.0)
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)**

[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*

5 Antimicrobial stewardship monitor in hospitals

5.1 Organization of antimicrobial stewardship teams in the Netherlands

Methods and results

A web-based survey was sent to all 69 acute care hospitals in the Netherlands to assess the composition and the type of monitoring performed by antimicrobial stewardship teams (A-teams) in the Netherlands in 2023. The results of the hospitals that completed the questionnaire (n=48; 70%) are presented as percentages of the responding hospitals in Table 5.1. The A-team characteristics and activities are described comparing data with the previous five years. This year the presence of a 'specialist antimicrobial stewardship', 'specialist in training' or 'other' were added to the question on A-team characteristics. Furthermore, three potential A-team monitoring activities were added to the questionnaire: 'antibiotic allergy evaluation and delabeling' (n=21; 44%), 'contributing data to the AMSM (Antimicrobial Stewardship Monitor) (or are planning to do so)' (n=32; 67%), and 'other monitoring activities' (n=10; 21%).

Table 5.1 Trends in A-team characteristics and activities between 2019-2023

	2019	2020	2021	2022	2023
Survey response rate, N (%)*	39 (51%)	37 (51%)	60 (85%)	39 (54%)	48 (70%)
A-team characteristics					
Presence of an A-team in responding hospitals	97%	100%	100%	100%	100%
A-team consists of at least:					
≥1 clinical microbiologist	100%	97%	95%	100%	100%
≥1 hospital pharmacist	97%	100%	95%	100%	100%
≥1 infectious disease specialist	71%	76%	68%	77%	83%
≥1 nurse/nurse specialist	21%	32%	18%	33%	29%
≥1 infection prevention specialist	16%	14%	15%	13%	10%
≥1 specialist antimicrobial stewardship**	N.A.	N.A.	N.A.	N.A.	15%
≥1 specialist in training**	N.A.	N.A.	N.A.	N.A.	25%
≥1 other**	N.A.	N.A.	N.A.	N.A.	40%
Time spent on stewardship per team, median [hours per week], (range)***	21.0 (2-144)	N.A.	15.0 (0-98)	16.0 (3-73)	16.0 (0-100)
Budget provided by hospital board of directors	55%	54%	67%	77%	61%
Financial support, median [FTE], (range)****	0.6 (0.05-3.30)	0.9 (0.1-2.6)	N.A.	0.9 (0.1-2.9)	0.7 (0-2.8)
Occasional and continuous monitoring of					
Antibiotic allergy evaluation and delabeling**	N.A.	N.A.	N.A.	N.A.	44%
Restricted antimicrobials	95%	N.A.	N.A.	100%	85%
Guideline adherence for empirical antimicrobial use	39%	N.A.	N.A.	49%	54%
IV-oral switch	58%	N.A.	N.A.	77%	77%
De-escalation after 48-72 hrs of empirical therapy	37%	N.A.	N.A.	49%	56%
Bedside consultation *S. aureus* bacteremia	77%	N.A.	N.A.	94%	83%
Therapeutic drug monitoring	44%	N.A.	N.A.	72%	71%
Correct diagnostics	13%	N.A.	N.A.	18%	N.A.
Surgical prophylaxis	3%	N.A.	N.A.	15%	23%
Other [a]**	N.A.	N.A.	N.A.	N.A.	21%
Contributing data to AMSM (or planning to do so)**	N.A.	N.A.	N.A.	N.A.	67%

N.A. = Data Not Available
AMSM = Antimicrobial Stewardship Monitor
* *Total number of hospitals in the Netherlands has changed. Total number of hospitals in 2019: 76, in 2020: 73, in 2021: 71, in 2022: 72, in 2023: 69.*
** *This year newly added parameter.*
*** *Data available for 46 hospitals.*
**** *Data available for 29 hospitals.*
[a] *Includes OPAT, annual point prevalence studies, weekly patient-antibiotic use discussions, de-escalation after 7 days IV, course duration evaluation, dose optimalisation.*

5.2 Quality of antimicrobial use

Methods

Participating hospitals and Data acquisition

The Antimicrobial Stewardship Monitor (AMSM) supports hospitals in obtaining information on the quality of antibiotic use. Currently, 30 hospitals have joined the AMSM with an additional ten hospitals that are in the process of joining.

Data reported here were extracted from the interactive dashboard of the AMSM. This dashboard provides benchmarked feedback information to A-teams and uses structured data already recorded in the electronic medical records (EMR). Benchmarking against the national benchmark (NL benchmark) or the academic benchmark is possible, where the first consists of data submitted by all participating hospitals and the latter consists of only data from academic hospitals.

As part of the 'basic data set', participating hospitals were asked to provide individual patient data, in contrast to Chapter 3, where purchase or dispensing data was used, for all patients admitted to the hospital and for whom antimicrobials were prescribed during admission. These data are submitted as two datasets:

- antimicrobial prescriptions (both clinical and those started at discharge with ATC codes starting with J01, J02, or J04) irrespective of administration route. 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. For example:
  - 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)

Moreover, if possible and available, indications for prescriptions are also submitted. Only a limited number of hospitals submitted this information. As part of the 'basic data set', hospitals also submit four values, so called 'volume indicators', separate from the above mentioned datasets each year. The 'volume indicators' are: number of unique patients with a hospital admission, number of clinical admissions, number of one-day admissions and number of patient days.

Hospitals could provide more data in addition to the basic data set ('extensive data set'). This included data on when antimicrobial drug concentrations were determined (therapeutic drug monitoring) and, if recorded as structured data in the EMR, the judgment by the A-team on whether the indications of prescriptions of (restricted) antimicrobials were according to the local antibiotic guidelines and on whether the intravenous-oral switch was performed according to local guidelines. Participating hospitals upload their data to the interactive dashboard annually, biannually or quarterly.

Here, data are shown from the 'basic data set' from hospitals that provided complete data (i.e., data from an entire year containing antimicrobial prescriptions including patient information from all patients hospitalized >1 day) for 2022 and 2023. Data for 2022 were not available in the previous NethMap report and are therefore included in this report. For this analysis, complete data submitted by 20 and 18 participating hospitals was retrieved from the dashboard for 2022 and 2023, respectively. Overall, 18 hospitals could simultaneously be compared between 2022 and 2023.

Indicators

We derived so-called 'proxy indicators' from the data in the AMSM. These metrics are a combination of quantity metrics and quality indicators providing an indication of the appropriateness of several aspects of antibiotic use in the treatment of infections. The following 'proxy indicators' were included: empiric treatment, IV-oral switch, streamlining and surgical prophylaxis. In addition to previous years, we included 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. Compared to DDD's, DOTs are not influenced by dosage variations.

Definitions

Individual antimicrobial prescriptions included all individual oral and IV prescriptions of antimicrobial therapy. The following definitions were 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.
- **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. Patient days are extracted from the above mentioned 'volume data'.
- **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:** unique patient; an individual with a clinical admission lasting at least 24 hours.
- **Proxy indicator 'surgical prophylaxis':** Antimicrobials or combinations of antimicrobials, 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.
- **Proxy indicator 'empiric treatment':** IV antimicrobial course/combination of courses given on the day of admission, which was not considered as surgical prophylaxis. A switch from an empiric IV course to an oral or other IV antimicrobial course was defined as a switch in therapy occurring between 24 hours before and 24 hours after stopping the empiric treatment.

Importantly, while the dashboard contained complete data from participating hospitals, some data may still be missing. Missing data affects the proportion of useable information in the interactive dashboard; therefore, hospitals with many missing data of a parameter needed to calculate the specific 'proxy indicator' were excluded from the results of that indicator. Additionally the type of hospital can influence the results of certain 'proxy indicators', which also justified the exclusion of those hospitals.

Results

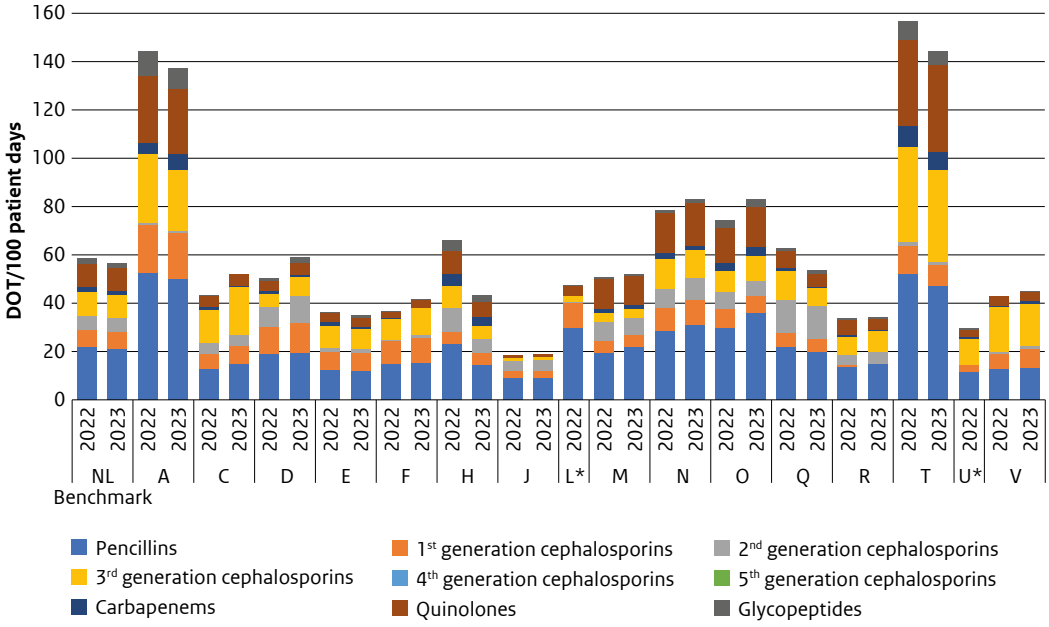
5.2.1 Participating hospitals

Twenty hospitals in 2022, (4 academic and 16 non-academic) and 18 hospitals in 2023, (5 academic and 13 non-academic) participated and provided complete data for the basic data set. Only data from the basic data set are shown.

5.2.2 Days of Therapy

Figure 5.2.1 shows DOT per 100 patient days for both 2022 and 2023 compared to the NL benchmark for frequently used antimicrobial groups (penicillins, cephalosporins, carbapenems, quinolones and glycopeptides). Sixteen hospitals provided data in 2022 and 14 in 2023. Antibiotic use (both in terms of amount and preferred choice) clearly varies between hospitals, with hospitals A and T notably well above the NL benchmark and hospital J well below it.

Figure 5.2.1 Days of therapy (DOT) per 100 patient days for penicillins, cephalosporins, carbapenems, quinolones and glycopeptides compared to the NL benchmark in 2022 for 16 hospitals and in 2023 for 14 hospitals



** No data available for 2023.*

5.2.3 Restricted antimicrobials

Overall, the percentages of restricted antimicrobial courses used in non-academic and academic hospitals in 2022 and 2023 are similar for carbapenems, quinolones, glycopeptides and piperacillin-tazobactam (Table 5.2.1). In non-academic hospitals, the percentage of carbapenem courses as part of total prescribed courses ranged from 0.2-2.7%, quinolone courses from 2.2-8.8%, glycopeptide courses from 0.1-1.9% and piperacillin-tazobactam courses from 0.01-6.4% in 2022 and 2023. In academic hospitals, these percentages varied from 1.7-3.5% for carbapenem courses, 5.2-9.3% for quinolone courses, 2.1-4.3% for glycopeptide courses and 0.03-5.3% for piperacillin-tazobactam courses.

The percentage of amoxicillin-clavulanic acid courses as part of total prescribed courses varied between 2022 and 2023. In 2022, non-academic hospitals reported 0.4-16.2%, while academic hospitals reported 6.6-8.2%. In 2023, these percentages ranged from 6.5-14.9% for non-academic hospitals and 6.3-8.2% for academic hospitals.

The use of three additional antimicrobials, that recently became available through a dedicated centralized procedure at the RIVM, being aztreonam, cefiderocol and ampicillin-sulbactam were also analyzed. These antimicrobials were used infrequently in 2022 and 2023: a total of 12 courses of aztreonam were used in non-academic hospitals in 2022 and 23 in 2023, while academic hospitals reported eight courses in 2022 and 20 in 2023 (data not shown). Cefiderocol was used only in academic hospitals, with two courses in 2022 and six in 2023. Additionally, one course of ampicillin-sulbactam was administered in one academic hospital in 2023.

Table 5.2.1 Use of restricted antimicrobials in non-academic and academic hospitals in 2022 and 2023 for 19 and 17 hospitals, respectively

Antibiotic group/ Antibiotic	Non-academic hospitals		Academic hospitals	
	2022 (n=14)	**2023** (n=12)	**2022** (n=5)	**2023** (n=5)
Carbapenems	**0.2 - 2.5%**	**0.3 - 2.7%**	**1.8 - 3.5%**	**1.7 - 3.4%**
	12/6,977 - 543/22,106	52/15,782 - 637/23,553	475/26,776 - 1,364/39,317	437/25,726 - 1,264/37,541
Quinolones	**2.2 - 8.8%**	**2.8 - 7.4%**	**5.2 - 8.6%**	**5.2 - 9.3%**
	148/6,666 - 467/5,330	575/20,313 - 1,736/23,553	1,392/26,776 - 1,626/19,028	1,400/27,011 - 1,574/17,017
Glycopeptides	**0.1 - 1.9%**	**0.2 - 1.5%**	**2.3 - 4.3%**	**2.1 - 4.0%**
	36/25,479 - 123/6,666	50/25,538 - 185/12,651	580/24,969 - 1,147/26,776	543/25,726 - 1,084/27,011
Amoxicillin-Clavulanic acid	**0.4 - 16.2%**	**6.5 - 14.9%**	**6.6 - 8.2%**	**6.3 - 8.2%**
	23/6,666 - 1,127/6,977	1,208/18,620 - 3,800/25,538	2,593/39,317 - 2,435/29,615	1,691/27,011 - 2,114/25,726
Piperacillin-Tazobactam	**0.04 - 5.4%**	**0.01 - 6.4%**	**0.03 - 5.3%**	**0.06 - 5.1%**
	11/28,757 - 1,198/22,106	1/6,734 - 1,507/23,553	8/26,776 - 1,318/24,969	16/27,011 - 1,301/25,726
Aztreonam	**0.004 - 0.04%**	**0.01 - 0.09%**	**0.004 - 0.02%**	**0.003 - 0.05%**
	1/25,479 - 8/17,811	2/25,538 - 17/18,620	1/24,969 - 7/29,615	1/37,541 - 14/29,487
Cefiderocol	**N.A.**	**N.A.**	**0,01%**	**0.004 - 0.070%**
			2/39,317	1/25,726 - 3/17,017
Ampicillin-Sulbactam	**N.A.**	**N.A.**	**N.A.**	**0.003%**
				1/29,487

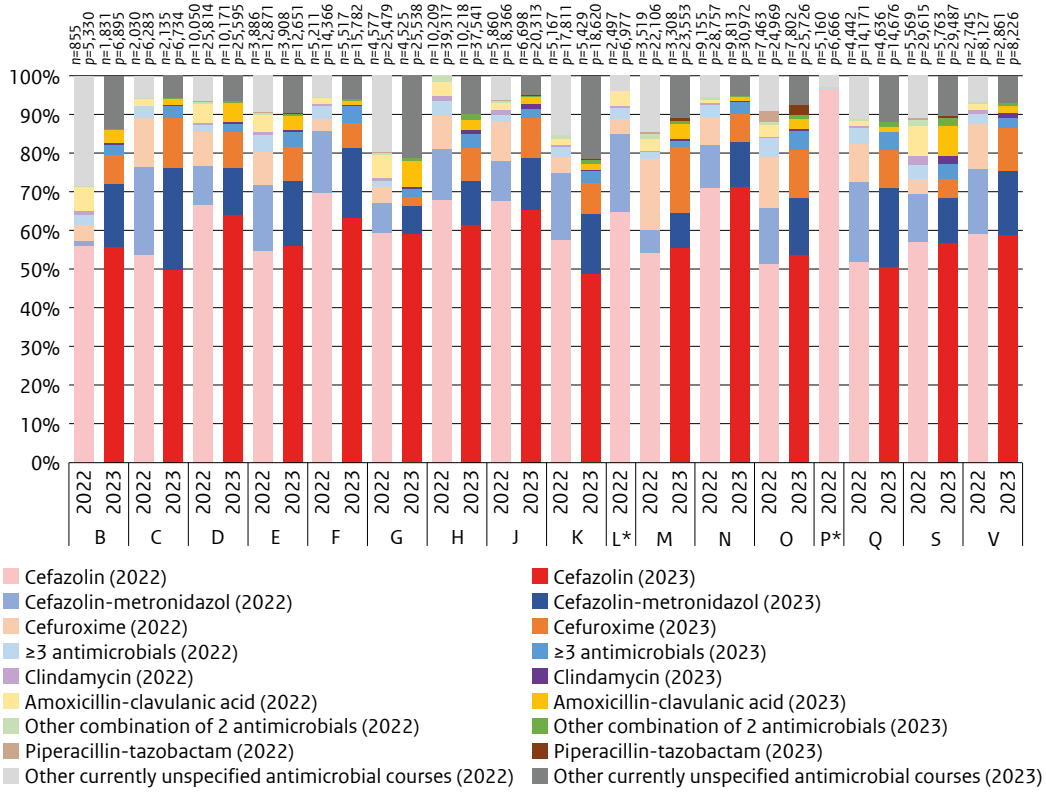
The total number of hospitals (n) providing data are displayed in the table above next to the years.
The percentages in bold show the use of restricted antimicrobial courses in hospitals, ranging from lowest to highest use in a year.
The light grey values indicate the number of restricted antimicrobial courses used (from lowest to highest) as proportions of the total number of antimicrobial courses prescribed in a year.
N.A. = Data Not Available.

5.2.4 Surgical prophylaxis

The most commonly prescribed agents as preoperative prophylaxis, according to our proxy definition, are summarized in Figure 5.2.2 for 2022 and 2023. 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. Two additional hospitals were excluded because perioperative prescriptions were missing. Data for the remaining 17 and 15 hospitals are shown for 2022 and 2023, respectively. All hospitals used cefazolin as backbone for surgical antimicrobial prophylaxis in both 2022 and 2023. Perioperative cefazolin use in 2022 was on average 59% (range 51-71%) and in 2023 cefazolin use was on average 58% (range 45-71%).

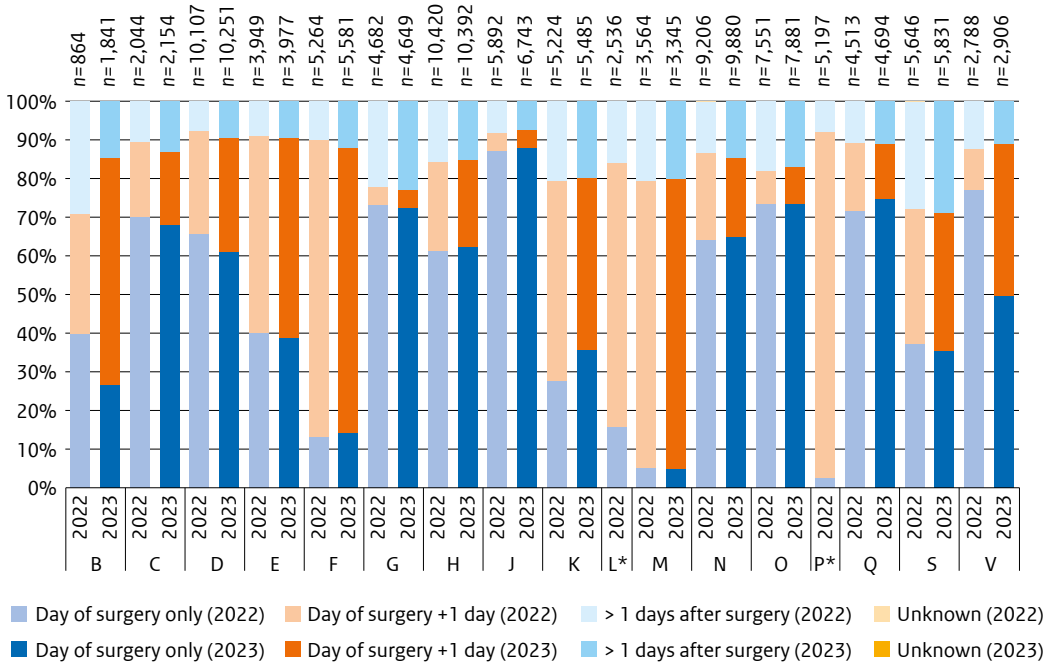
Figure 5.2.3 shows the duration of antimicrobial prophylaxis after surgery. Perioperative antimicrobial prophylaxis should generally be discontinued within 24 hours after surgery. In 2022 on average, 85% (range 71-92%) of surgical antimicrobial prophylaxis courses were discontinued on the day of surgery or the day after based on data from 17 hospitals. In 2023 on average, 85% (range 71-93%) of surgical antimicrobial prophylaxis courses were discontinued on the day of surgery or the day after based on data from 15 hospitals.

Figure 5.2.2 Antibiotics used for surgical antimicrobial prophylaxis in 17 and 15 hospitals in 2022 and 2023, respectively



Total number of courses used (p) and total number of courses used for surgical prophylaxis (n) are displayed above the columns.
** No data available for year 2023.*

Figure 5.2.3 Distribution of the duration of surgical antimicrobial prophylaxis in 17 and 15 hospitals in 2022 and 2023, respectively



Total number of courses used for surgical prophylaxis (n) is displayed above the columns.
Unknown: indicates courses with duration > 365 days.
** No data available for year 2023.*

5.2.5 Intravenous to oral switch and escalation

Most hospitals in the Netherlands use either cefuroxime or ceftriaxone as empirical treatment for most infections, including sepsis of unknown origin. Only in 2022 one hospital used amoxicillin-clavulanic acid as the backbone. Figure 5.2.4 shows the duration of these antibiotic courses. Empiric therapy courses containing prescriptions without stop dates were excluded from this analysis (Figures 5.2.4 to 5.2.7). In 2022 and 2023 data from 19 and 18 hospitals were used, respectively.

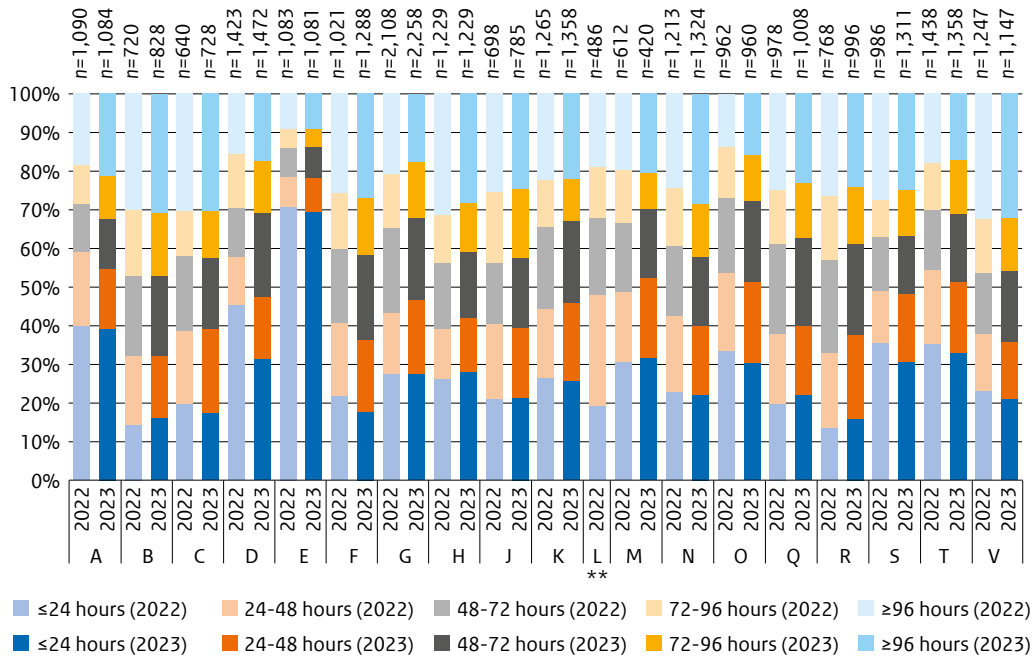
Each participating hospital had a similar trend in the duration of empiric therapy for both 2022 and 2023 (Figure 5.2.4). For all hospitals, 67% (mean, range 53-86%) and 64% (mean, range 54-86%) of the courses given as empiric therapy had a duration of less than 72 hours in 2022 and 2023, respectively.

For all courses given as empiric treatment, regardless of the empiric treatment duration, 58% (mean, range 39-71%) of empiric treatments were discontinued without starting another course, 27% (mean, range 14-44%) was switched to oral treatment and 16% (mean, range 9-26%) were switched to other IV antibiotics in 2022 (Figure 5.2.5). In 2023, 56% (mean, range 36-66%) of empiric treatments were discontinued without starting another course, 28% (mean, range 13-49%) were switched to oral treatment and 16% (mean, range 11-26%) were switched to other IV antibiotics.

Figure 5.2.6 shows the follow-up after empiric treatment upon admission, depending on the duration of the initial empiric therapy. In 2022, after 24 hours or less of empiric treatment, 46% (mean, range 20-67%) was discontinued without starting another course, 16% (range 8-22%) was switched to oral treatment and 38% (range 21-59%) was switched to other IV antibiotics. In 2023, these values were 45% (mean, range 29-71%), 16% (range 8-25%) and 40% (range 21-57%), respectively. After 48-96 hours of empiric treatment, 55% (mean, range 30-74%) of courses in 2022 were discontinued without starting another course, 40% (mean, range 22-65%) was switched to oral treatment, and 6% (mean, range 3-10%) were switched to other IV antibiotics. In 2023, after 48-96 hours of empiric treatment, 52% (mean, range 27-75%) were discontinued, 42% (range 22-69%) were switched to oral treatment and 6% (range 2-11%) were switched to other IV antibiotics. In both years, antibiotic courses were switched to other IV courses more frequently when empiric therapy lasted 24 hours or less compared to 48-96 hours. Conversely, courses were discontinued more often when empiric therapy lasted 48-96 hours than when it lasted 24 hours or less.

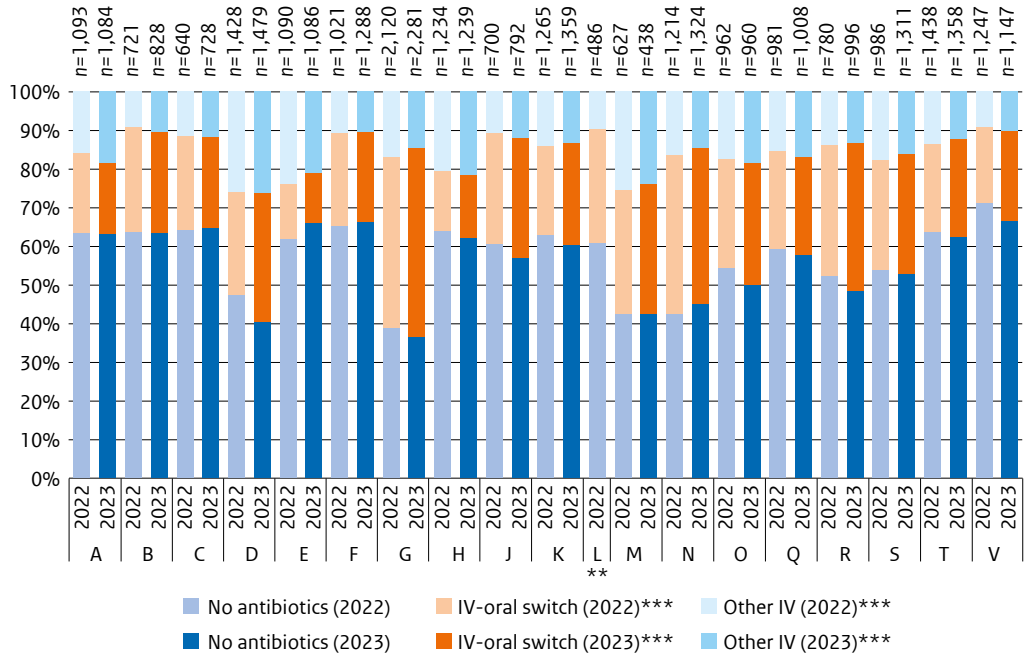
Lastly, for 2022 and 2023, empiric treatment was escalated to an aminoglycoside-containing regimen, piperacillin-tazobactam or carbapenem, in only a very small fraction of the patients (Figure 5.2.7). There was little variation within hospitals over the two years as well as between the hospitals.

Figure 5.2.4 Duration of cefuroxime, ceftriaxone or amoxicillin-clavulanic acid* courses started on the day of admission ('empiric treatment') in 19 and 18 hospitals in 2022 and 2023, respectively



* *cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*
** *No data available for year 2023.*
Total number of courses used as empirical treatment (n) is displayed above the columns.
Hospital G, M and R had noteworthy proportions (range 10-57%) of prescriptions missing a stop date (2022).
Hospital G and M had noteworthy proportions (18% and 69%, respectively) of prescriptions missing a stop date (2023).
Hospital P is excluded because of its hospital type; only 7 courses used as empirical treatment (2022). In 2023 no data was available.

Figure 5.2.5 Discontinuation or change to oral or other intravenous antibiotic treatment of all cefuroxime, ceftriaxone or amoxicillin-clavulanic acid* courses started on the day of admission ('empiric treatment') in 19 and 18 hospitals in 2022 and 2023, respectively



* *cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*

** *No data available for year 2023.*

****Switch occurred between 24 hours before and 24 hours after stop.*

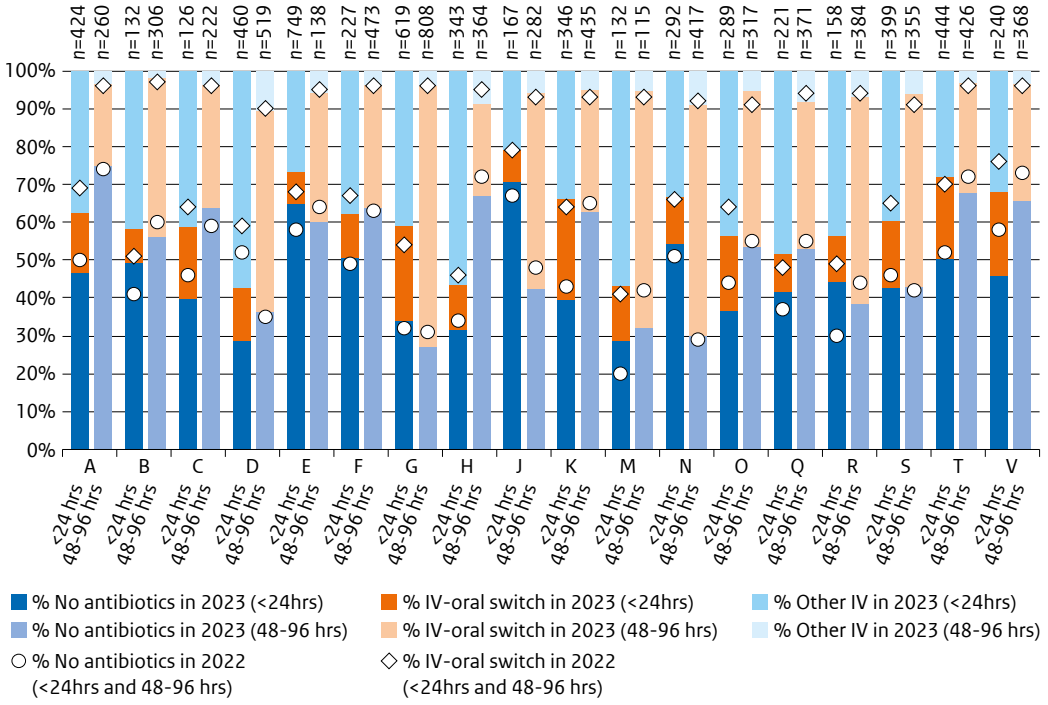
Total number of courses used as empirical treatment (n) is displayed above the columns.

Hospital G, M and R had noteworthy proportions (range 10-56%) of prescriptions missing a stop date (2022).

Hospital G and M had noteworthy proportions (17% and 68%, respectively) of prescriptions missing a stop date (2023).

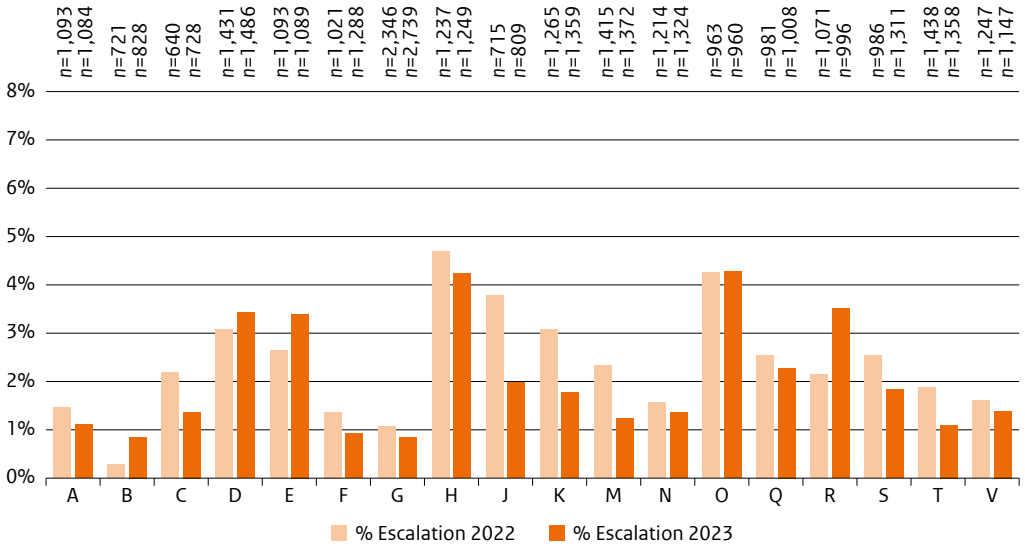
Hospital P is excluded because of its hospital type; only 7 courses used as empirical treatment (2022). In 2023 no data was available.

Figure 5.2.6 Discontinuation or change to oral or other intravenous antibiotic treatment of all cefuroxime/ ceftriaxone * courses started on the day of admission ('empiric treatment') in 18** hospitals depending on duration of initial IV therapy in 2022 and 2023



* *cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*
** *Hospital L is not shown in the figure due to missing data for 2023 but is included in the calculations for year 2022.*
Total number of courses used as empirical treatment (n) in 2023 is displayed above the columns.
Hospital P is excluded because of its hospital type; only 7 courses used as empirical treatment (2022). In 2023 no data was available.

Figure 5.2.7 Percentage of empiric antibiotic cefuroxime or ceftriaxone courses*, started on the day of admission ('empiric treatment'), where a new course with aminoglycosides, piperacillin-tazobactam or meropenem was started between 24 and 96 hours after initiation of cefuroxime/ceftriaxone in 18 hospitals in 2022 and 2023



** cefuroxime or ceftriaxone, depending on what the preferred empiric treatment for sepsis of unknown origin*
Total number of courses (n) is displayed above the columns.
Hospital L had no data available since amoxicillin-clavulanic acid is used as empiric treatment for sepsis of unknown origin and escalation is only known for cefuroxime/ceftriaxone.

Discussion

Antimicrobial stewardship programs (ASP) are well embedded in Dutch hospitals for several years now. The A-team's composition in 2022 and 2023 is similar to previous years. The response rate for the evaluation questionnaire in 2023 was 70% whereas as in 2022 it was 54%. Only 61% of responding hospitals indicate that structural financial support for A-team activities is present. The amount of available full-time equivalent (fte) ranges from 0 to 2.8 fte.

After publication of the SWAB guideline for the 'Approach to suspected Antibiotic Allergy' in 2022, 44% of hospitals have initiated activities to assess antibiotic allergies and/or have started programs to reduce unnecessary allergy labels within their A-teams. This while successfully maintaining other activities such as monitoring of restricted antimicrobials and IV-Oral switch. Of the responding hospitals, 67% indicate that they contribute data to the AMSM or are making preparations to do so.

This is the third year that we have extracted data from the interactive dashboard of the SWAB AMSM and the first year we publish data on DOT/100 patient days. The AMSM provides benchmarked feedback to A-teams and uses structured data already recorded in the EMR. For 2022 and 2023 the dashboard contained complete data (i.e. data from an entire year containing antimicrobial prescriptions and linked patient information from all patients hospitalized >1 day) from 20 and 18 hospitals, respectively. In 2021, 19 hospitals provided data. Five new hospitals provided data in 2022 and 2023 compared to 2021, however 4 hospitals that provided data in 2021 were not able to provide data the following two years (or the data was incomplete). This explains the minor increase and decrease in number of participating hospitals in 2022 and 2023, respectively. For this analysis only the data from the years 2022 and 2023 were compared.

A few important points need to be mentioned with regard to the data that are uploaded by participating hospitals, to the AMSM, that can influence the reported results of the proxy indicators and DOTs/100 patient days:

- As previously mentioned, 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' and 'duration of empiric therapy' for a certain hospital can indicate missing stop dates. Additionally, low numbers of surgery days may indicate missing surgery day data. As a result not all hospitals submitting data could contribute to all the 'proxy indicators'.
- The results of the 'proxy indicators' is also influenced by the type of hospital. For example, a highly specialized hospital focusing on a particular type of surgery may exhibit relatively higher preoperative prophylactic antimicrobial use but relatively lower empirical intravenous antimicrobial use.
- Three (~16%), hospitals were missing preoperative prescriptions: For one hospital the prescriptions were documented in a separate system parallel to the main EMR. For two hospitals prescriptions were missing because they were not extracted correctly.
- Clinical prescriptions and prescriptions started at discharge (discharge prescriptions) are included in the data. However, for at least two hospitals the discharge prescriptions were initially missing from the data set because these prescriptions were not 'labeled' as clinical prescriptions (which was the assumption) but as outpatient prescriptions. Missing prescriptions can influence the calculation of course duration.

The coordinator of the AMSM assists hospitals and monitors data quality, collaborating with individual A-teams to enhance the completeness and validity of the data provided by hospitals. Each hospital extracts their data from the EMR using a script that occasionally requires updates/improvements and uploads the data to the interactive dashboard. With every improvement, hospitals are requested not only to use the updated version of the script for future data but also to update previously uploaded data in the dashboard using the new script. In summary, data validation is an ongoing collaborative effort between the coordinator and participating hospitals.

In 2022, two non-academic hospitals (an orthopedic center rarely using empirical therapy and a hospital using amoxicillin-clavulanic acid as standard empirical therapy for sepsis of unknown origin) submitted data. In 2023 however, there was no data submitted by both hospitals potentially explaining the difference in the use of the restricted antimicrobial amoxicillin-clavulanic acid between the two years (0.4%-16.2% and 6.5%-14.9% for 2022 and 2023, respectively).

The addition of DOT/100 patients to the AMSM provides a quantity metric for evaluating antimicrobial use across hospitals. We observed substantial variation between hospitals. The DOT/100 patient days (Figure 5.2.1), in comparison to the NL benchmark, indicates that two hospitals use markedly high numbers of antimicrobials while one hospital uses notably low numbers. Ideally, this should be a reason for the A-teams of the concerned hospitals to do further/in-depth analysis on the quantity of antibiotic use per specialty or department, facilitated by the AMSM.

The data required to calculate the DOT/100 patient days consists of two parts. While individual patient data, including prescription and admission data, are often uploaded, the second component, volume data, is however submitted less frequently. As a result, the number of hospitals reporting DOT/100 patient days is lower than the number of hospitals represented in figures 5.2.2-5.2.7 and table 5.2.1.

We have derived 'proxy indicators' from the volume data, reflecting the appropriateness of different aspects of antibiotic use. For example, we used an indicator to reflect the extent to which surgical prophylaxis was given after the operation, whereas postoperative continuation is never indicated. For both 2022 and 2023, 85% (mean) of surgical antimicrobial prophylaxis courses were discontinued on the day of surgery or the day after, ranging from 71-93%, similar to 2021 (82%, range 76-96%). We chose the day of surgery and the day after as adequate surgical prophylaxis because in some hospitals prophylaxis prescription is continued until 0.00 AM and therefore it counts for the day after. There was variation between hospitals in duration of surgical prophylaxis and the data suggest that especially those hospitals being below the mean percentage of discontinued prophylactic courses on the day of surgery or the day after may choose this a topic for an improvement intervention.

The percentage of cefazolin as surgical prophylaxis in 2022 is 59% (51-71%) and in 2023 it was 58% (49-71%), which is lower than expected. Because we used a 'proxy indicator' to calculate the percentage of cefazolin used for surgical prophylaxis, we may have misclassified therapeutic antimicrobial prescriptions as prophylactic prescriptions. This misclassification could result in an overestimation of the total number of courses considered as surgical prophylaxis. Data validation is important to overcome this uncertainty.

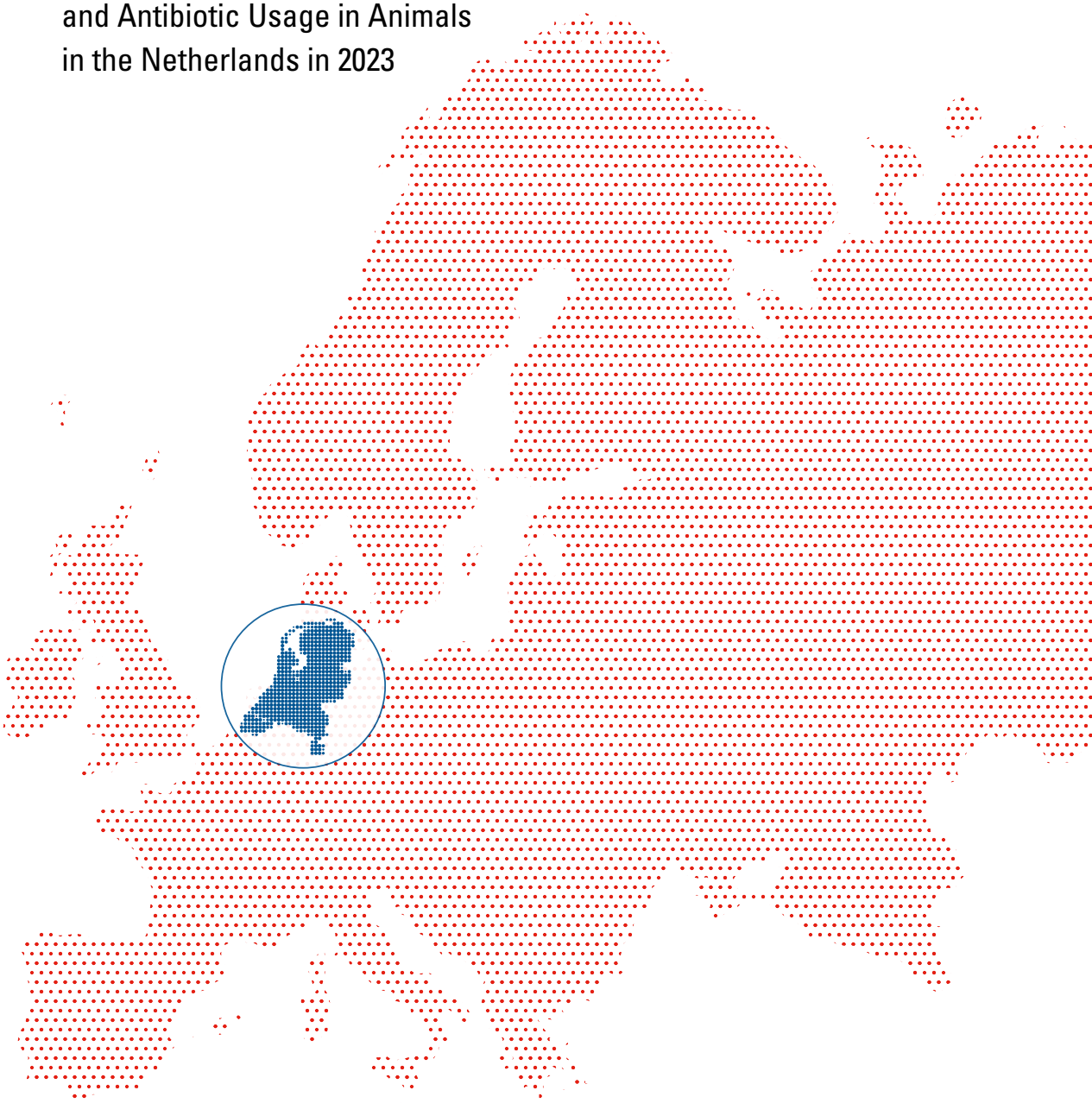
We used the antibiotic course(s) started on the day of admission and not classified as surgical prophylaxis as a proxy for empiric treatment, highlighting in this analysis specifically the treatment of sepsis with an unknown origin (i.e., intravenously administered courses of cefuroxime, ceftriaxone and amoxicillin-clavulanic acid). By focusing on the cornerstone of local antibiotic policy, we described how long this therapy was used. Future validation steps and linking indications to antibiotic use will provide more insight into how to interpret these data, but the large variation in the timing of the switch to oral therapy suggests that some A-teams should perform more in-depth audits on this topic.

Conclusions

- Of the hospitals participating in the yearly A-team survey (70% of all hospitals), 100% had a formal A-team, but only 61% indicated that structural financial support for A-team activities is present. This year one of the newly added activities to the A-team survey was 'antibiotic allergy evaluation and delabeling'; 44% (n=22) of the hospitals indicate including this in their A-team activities.
- Twenty and 18 (~25%) acute care hospitals (2022, total n=72 and 2023, total n=69) extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor for 2022 and 2023, respectively.
- On average, in 59% and 58% of surgical interventions, cefazolin was used as backbone of surgical antimicrobial prophylaxis in 2022 and 2023, respectively.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 82% (range 76-96%) of surgical antimicrobial prophylaxis courses were discontinued on the day of surgery or the day after in both 2022 and 2023.
- Of all patients receiving empirical therapy upon admission, 16% (mean, range 8-25%) was switched to oral treatment within 24 hours in both 2022 and 2023, while 40% (mean, range 22-65%) and 42% (mean, range 22-69%) were switched to oral treatment between 48-96 hours in 2022 and 2023, respectively.
- Data validation will enhance and further develop the AMSM. Benchmarking against hospitals of the same type is essential for the improvement of antibiotic stewardship activities within hospitals and therefore relevant for the clinic.

MARAN 2024

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2023



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

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

WAGENINGEN UNIVERSITY & RESEARCH

Universiteit Utrecht

SDa Autoriteit Diergeneesmiddelen

MARAN 2024

Monitoring of Antimicrobial Resistance
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in 2023

November 2024

Contents

Colophon

This report is published under the acronym MARAN-2024 by Wageningen Bioveterinary Research (WBVR) in collaboration with the Food and Consumer Product Safety Authority (NVWA), Wageningen Food Safety Research (WFSR), the National Institute for Public Health and the Environment (RIVM), the Netherlands Veterinary Medicines Institute (SDa) and the Faculty of Veterinary Medicine, Utrecht University (FD).
The information presented in MARAN-2024 is based on total sales data and animal specific usage of antimicrobial agents in livestock and the occurrence of antimicrobial resistance and specific resistance genes in bacteria of animal origin and of relevance to public health in 2023.

MARAN-2024 is published in a combined back-to-back report with NETHMAP-2024. The combined report is available on the website of WBVR at www.wur.nl/maran. More detailed information on the usage of antibiotics per animal species is available on the website of the Netherlands Veterinary Medicines Institute (www.autoriteitdiergeneesmiddelen.nl).

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NVWA, Utrecht: Petra Dop, Rianne Hagen-Lenselink, Jack van Lier, Joke Remijnse-Poffers, Ben Wit

Acknowledgements

This study on antimicrobial resistance in food-animals was primarily financed by the Ministry of Agriculture, Nature and Food Quality through the project 'Antimicrobial Resistance Research in Animals', grant number WOT-01-002-038.

The work of the Food and Consumer Product Safety Authority within the domain Microbiology is financed by the Ministry of Health, Welfare and Sport.

The work of the National Institute for Public Health and the Environment (RIVM) is financed by the Ministry of Health, Welfare and Sport.

The work of Wageningen Food Safety Research (WFSR) is financed by the Ministry of Health, Welfare and Sport.

The work of University of Utrecht is financed by the Ministry of Agriculture, Nature and Food Quality.

The work of the Netherlands Veterinary Medicines Institute is financed in equal amounts by public (Ministry of Agriculture, Nature and Food Quality) and private funds (animals production sectors for cattle, pigs, poultry and veal calves and the Royal Dutch Veterinary Association).

The authors thank Mr. Drs. J.F. Schutte, E. van't Ende and A. de Goeij from FIDIN for providing detailed insight into the national sales data of antimicrobials in the veterinary sector.

1 Summary

Antibiotic Usage

In 2023, a total of 117 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is an increase of 4.5% compared to 2022. A decrease in sales by 76.4 % over the years 2009-2023 is attained (with 2009 considered a reference year by the Dutch Government). A small increase in sales of AVMPs in the Netherlands in 2023 is reflected by indeed 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, with turkeys as the big exception with a substantial use reduction in 2023. 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 in 2023, overall decrease since 2011 is 83.4% in sales. Of the fluoroquinolones, 49% is applied is sectors not yet monitored; an overall decrease of 92.1% since 2011 is observed.

Antimicrobial resistance

In 2023, 1413 human *Salmonella* isolates and 726 isolates from non-human sources were tested for antimicrobial susceptibility. Over all isolates, the highest resistance proportions were observed for ciprofloxacin (21%), nalidixic acid (21%), ampicillin (18%), sulfamethoxazole (19%), tetracycline (17%), trimethoprim (9%) and chloramphenicol (6%). Over all sources, the highest levels of resistance were observed for monophasic *S.* Typhimurium, *S.* Infantis, *S.* Chester, *S.* Paratyphi B var. Java, and *S.* Typhimurium. One carbapenemase-producing *Salmonella* (OXA-48) was found in a human isolate (monophasic *S.* Typhimurium). Among human *S.* Typhimurium isolates, decreasing trends in resistance against ampicillin (34%) and tetracycline (18%) were observed since 2010. Among human *S.* Enteritidis isolates, a decrease in resistance against ampicillin (10%), ciprofloxacin and nalidixic acid (27%) was observed. However, in general there is an increasing trend in resistance against fluoroquinolones in human isolates since 2010. In total, 20 (1.4%) ESBL-producing human clinical isolates were detected, which is comparable to previous years (1.8% in 2022 and 1.4% in 2021). Levels of resistance among isolates from broiler meat were high for ciprofloxacin (52%), nalidixic acid (52%), tetracycline (33%) and sulfamethoxazole (68%).

In 2023, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof remained at a high level for fluoroquinolones (FQ) and tetracycline. Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, but was observed at low levels in *C. jejuni* from veal calves and *C. coli* from broilers and poultry meat. A notably higher level of erythromycin resistance was observed in *C. coli* from veal calves again. In humans, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates. Resistance levels increased in 2022 and 2023 compared with 2020 and 2021, when resistance most likely dropped due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 travel restrictions, which is associated with higher resistance proportions than domestically acquired campylobacteriosis. However, resistance levels for *C. jejuni* were generally still lower than before the COVID-19 pandemic, but back to pre-pandemic levels for *C. coli*. Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2023, which is a concern for public health. It was, however, lower compared to 2019, before the COVID-19 pandemic. Resistance to erythromycin, as representative for the first choice antibiotic class in human medicine, remained low.

In Shiga-toxin producing and Enteropathogenic *E. coli* serotype O157 (STEC/EPEC O157), a decrease in proportions of resistance against ampicillin, tetracycline, trimethoprim and sulfamethoxazole compared to 2022 was observed. The proportion of resistance of ciprofloxacin was higher in human STEC/EPEC non-O157 *E. coli* than in human STEC O157. No ESBL-producing isolates were detected in STEC O157, but resistance to third generation cephalosporins was detected in three STEC/EPEC non-O157 *E. coli* isolates through presence of ESBL genes in two isolates (CTX-M-1 and CTX-M-15). In a third isolate, a mutation in the AmpC promotor region was detected.

Amongst indicator *E. coli* obtained from caecal samples of broilers, pigs and veal calves, the levels of resistance stabilised for most antibiotics in the last five years. Resistance in *E. coli* from faecal samples of dairy cattle remained traditionally low. Proportions of resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim remained relatively high in broilers, pigs, and (white) veal calves. Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers (25.6%) in contrast to the low prevalence observed in pigs (1,7%) and veal calves (4.3%) and the complete absence in dairy cattle. In addition, resistance to third generation cephalosporins was low or absent amongst (randomly isolated) indicator *E. coli* in 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 *E. coli* isolates from chicken meat, decreasing levels of resistance were observed with a tendency to flatten in the more recent years. Except for the lower FQ resistance in retail pork, resistance patterns in *E. coli* obtained from pork were comparable to broiler meat with the highest resistances monitored for ampicillin, trimethoprim, sulfamethoxazole and tetracycline. Over time, no major changes occurred in *E. coli* from pork. In bovine meat, levels of resistance are traditionally low with fluctuating percentages below 5% for most antimicrobials tested. In addition, *E. coli* obtained from imported beef showed similar low levels of resistance.

The prevalence of resistance to extended-spectrum cephalosporins (ESC) in randomly selected *E. coli* has been steadily low for several years in all livestock species. In contrast, selective culturing revealed an increase of ESC resistance in dairy cattle and broilers in recent years. In dairy cattle, the prevalence of selectively isolated ESC-resistant *E. coli* significantly increased over the past 5 years from 10.3% in 2019 to 18.0% in 2023. A significant increase in the prevalence of selectively isolated ESC-resistant *E. coli* was also observed in broilers over the past 4 years from 10.2% in 2020 to 20.3% in 2023. However, the increase in

broilers is partially attributed to a change in the sampling method from 2022 onwards in accordance with the updated European legislation (Commission Implementing Decision (EU) 2020/1729). As in former years, Whole Genome Sequencing of ESC-resistant *E. coli* shows that genetic clustering is mostly seen within production sectors, although some spill-over appears to occur.

In 2023, the prevalence of the colistin resistance gene *mcr* was low in livestock and meat. No carbapenemase-producing *Enterobacteriaceae* were detected in samples from livestock, companion animals and meat. In 2023, 4.5% of the investigated sheep farms was tested positive for MRSA.

The level of resistance of indicator *E. coli* from livestock and meat further stabilised in 2023. An unexpected increase of ESC resistance was observed in dairy cattle. In spite of the long-term AMU reduction, continuous high levels of resistance are observed for fluoroquinolones and tetracycline in *Campylobacter* isolates from humans and poultry. Colistin-resistance remains present at low levels, while no CPE was detected in samples from livestock, companion animals or meat.

2
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 (https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports).

2.1 Total sales of veterinary antibiotics in the Netherlands 2023

2.1.1 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 2023 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. 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 94.2% of total sales in 2023. 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 paragraph 2.2.

2.1.2 Trends in total sales

Table 1 shows the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. In 2023 in total 117 tonnes of AVMPs were sold, representing an increase of 4.5% in comparison with 2022. A decrease in sales by 76.4 % over the years 2009-2023 is attained (with 2009 considered the reference year by the Dutch Government).

Figure 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 2023. Antimicrobial use (in kg) in livestock sectors is presented as bars in which the use in different animal species can be distinguished. Figure 1 shows a slightly decreasing trend in liveweight of Dutch livestock. Compared to 2009 the liveweight of Dutch livestock has decreased by 12%. 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 almost 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 2023 between sales and usage in monitored sectors was 5.8% as illustrated in Figure 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 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 3.5% compared to 2022. The fraction of doxycycline (not specified in Figure 2) was in 2023 63.7% of the total sales of tetracyclines (55.9% in 2022, fluctuations between 31% and 69% in the years 2011-2021).

Penicillins
Second place in mass, also sales of penicillins (including aminopenicillins) increased in 2023 compared to 2022, with 4.9%. The distribution of broad and narrow spectrum penicillins (in mass sold) is comparable to previous years with 71.9% aminopenicillins.

(Fluoro)quinolones

The sales of fluoroquinolones increased with the 16kg (+15.9% compared to 2022) that were reduced in 2022. An overall reduction of 92.1% was realized since 2011. In 2023, 51% 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 3.4% in 2023 when compared to 2022 after the sharp rise in 2022; 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 will be evaluated in the near future for the Dutch situation.

Cephalosporins 3rd/4th generation

Sales of these AVMPs were relatively stable at a low level since 2016, fluctuating in kg range. In 2023 only four products were sold, representing less than 1 kg active substance.
A reduction of 99.97% of cephalosporins 3rd/4th generation sales has been achieved since 2011.

Polymyxins

Colistin sales decreased in 2023 again, with 5.0%. The reduction since 2011 is 83.4%. 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 DDDAF. 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, but for some sectors (laying hens) specific use data show differently and are reason for concern. On the other hand, many farms have zero colistin usage, this proportion of zero-use is increasing over years.

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

In Figure 3, 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 3) since monitoring of AMU was established.

Figure 4 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 also illustrates that use of fluoroquinolones (red bar) is low in all livestock sectors, use is the highest in turkeys, although an important overall use reduction of 34% and a limited fluoroquinolones use of 0.15 DDDANAT has been realized in 2023. In veal calves, a large sector using 86% first choice AVMP's, the steady decrease in total use was halted in 2020 (55%), and in 2023 the remaining reduction was 51%.
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 in 2023.
Expressing antibiotic use in number of Defined-Daily Dosage Animal like in Figure 3 and 4 shows that AMU in broilers, turkeys and in pigs in 2023 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 (https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports).

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 antibacterial substances to antimicrobial 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.

Conclusion
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 2023 is reflected by indeed 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, with turkeys as the big exception with a substantial use reduction in 2023.
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 in 2023, overall decrease since 2011 is 83.4% in sales. Of the fluoroquinolones, 49% is applied is sectors not yet monitored; an overall decrease of 92.1% since 2011 is observed.

Table 1 Antimicrobial veterinary medicinal product sales from 1999-2023 in kg (thousands) (FIDIN, 2024)

Year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20	'21	'22	'23
Betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43	36	40	34	29	30
Tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51	49	47	31	32
Macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25	23	24	21	19	21
Aminoglycosides	13	12	11	10	9	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
(Fluoro) quinolones	7	7	6	6	5	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
Trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33	29	30	32	22	24
Other antibacterials	11	12	11	11	7	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
Total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179	150	154	145	112	117

Figure 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 2009-2023

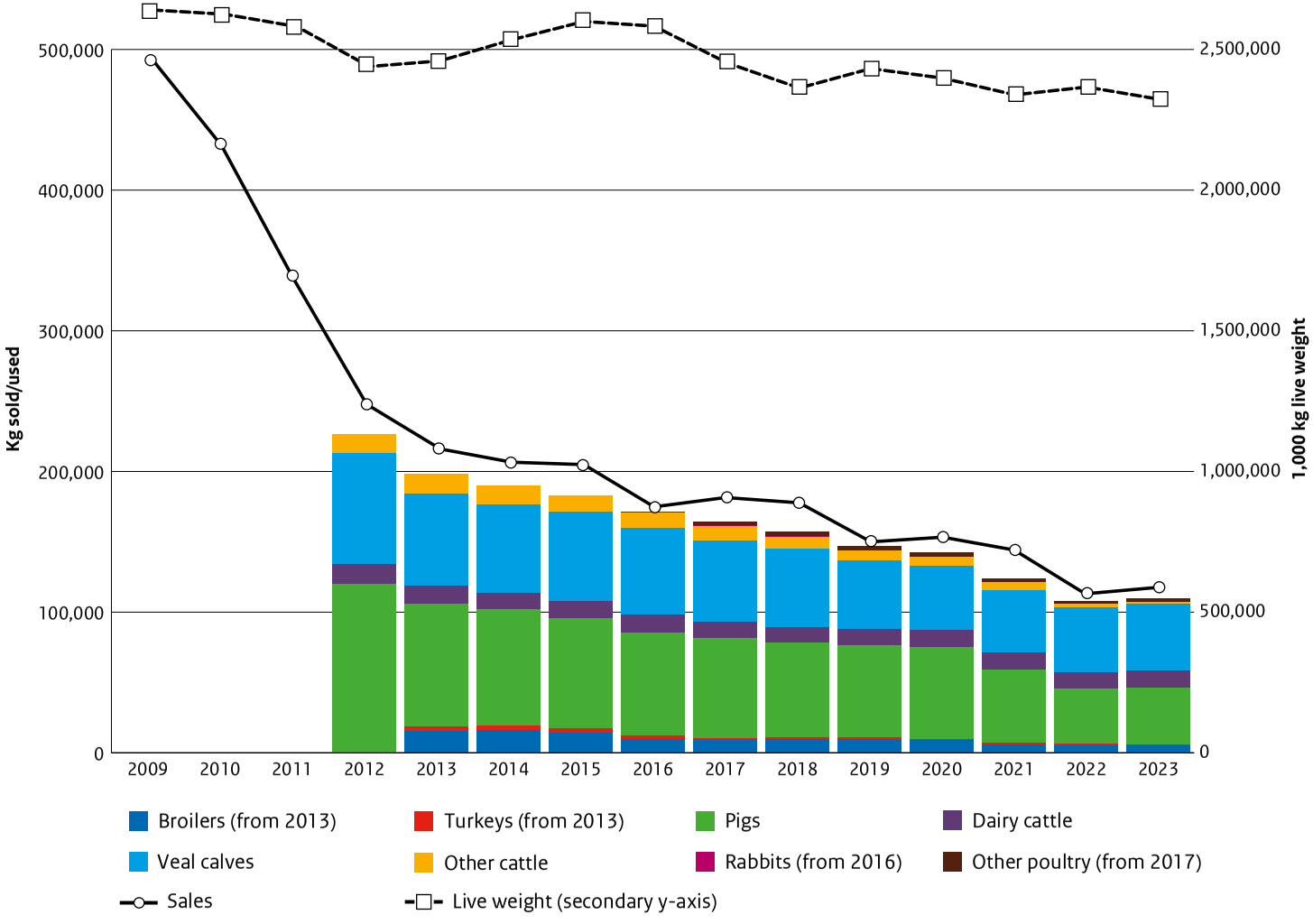


Figure 2 Antimicrobial Veterinary Medicinal Product sales by antibiotic class from 2011-2023 in kg (thousands); antibiotic class "other" comprises bacitracin, fusidic acid and nitro-imidazole's

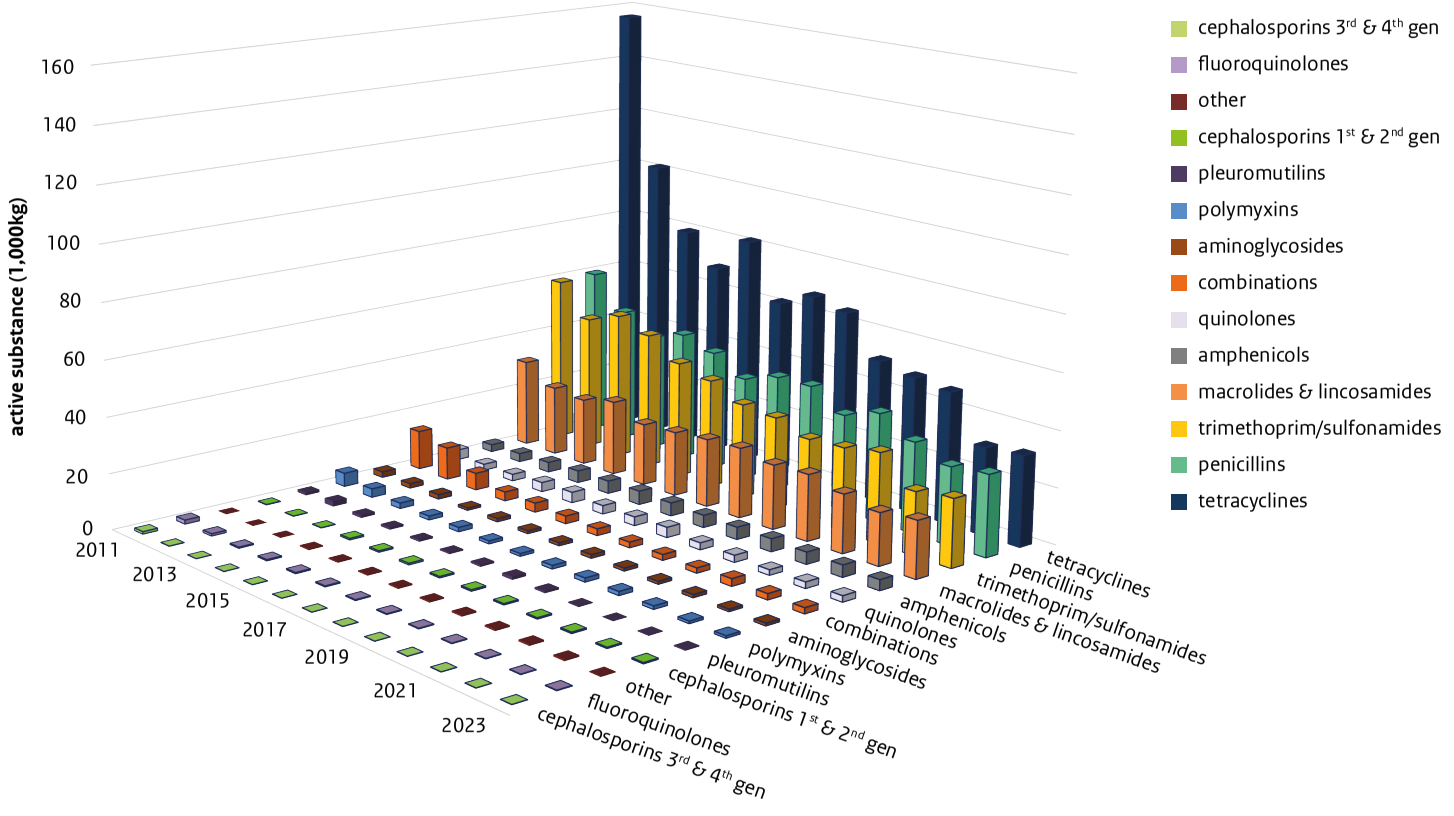


Figure 3 Number of animal-defined daily dosages per animal-year for rabbits (grey), turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2023 as DDDANAT) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area)

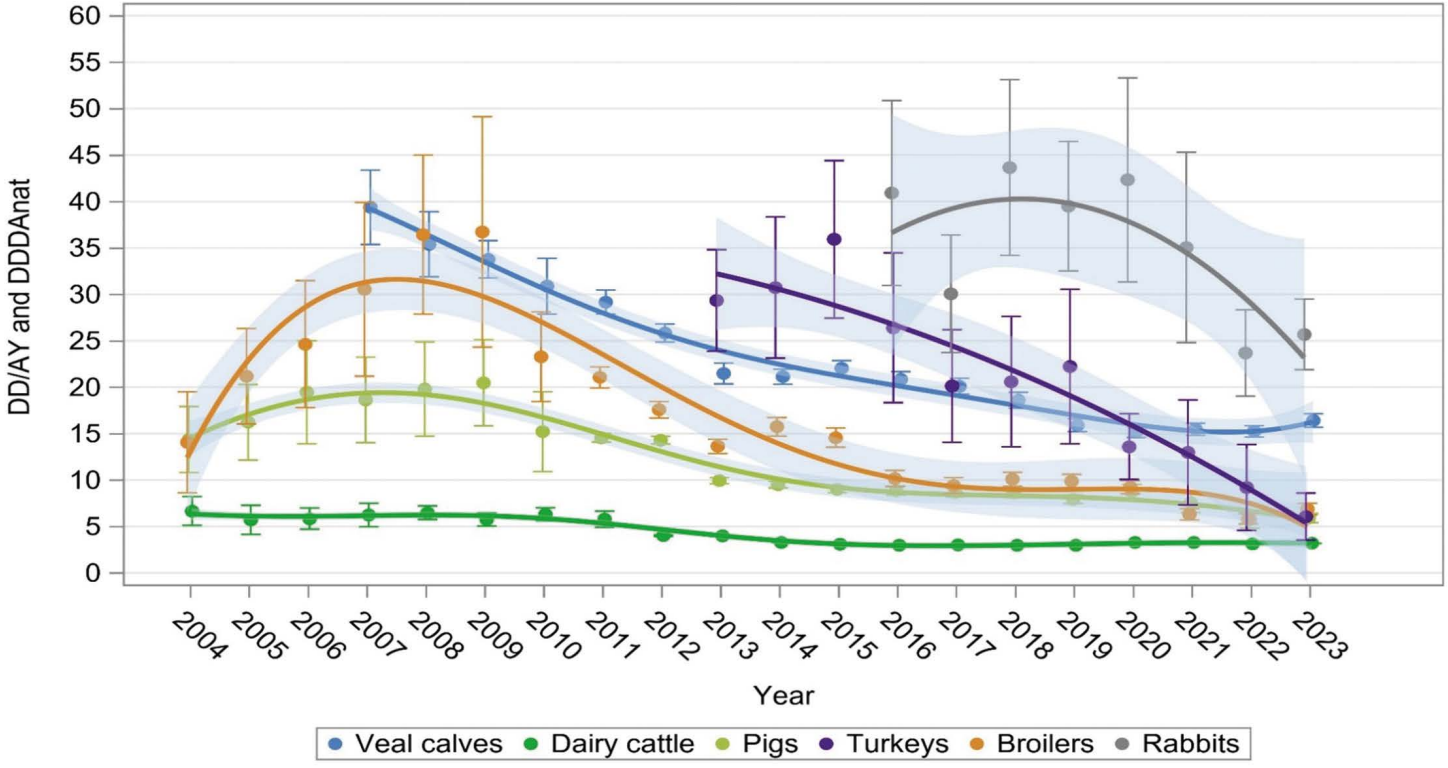
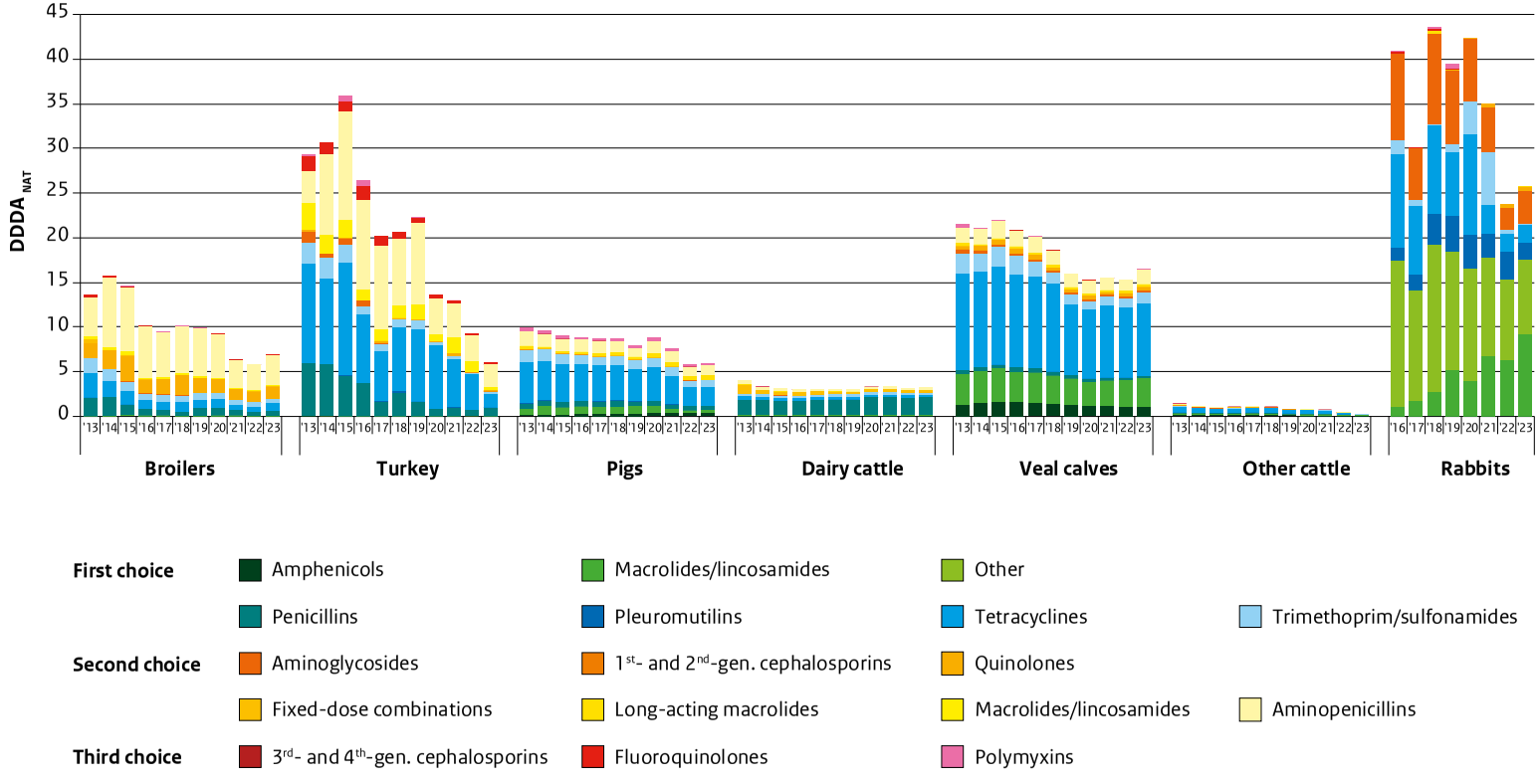


Figure 4 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by antibiotic class per animal sector over the years 2013-2023



3
Resistance data

This chapter describes susceptibility test results as determined in 2023 for the food-borne pathogens *Salmonella enterica* subsp. *enterica*, *Campylobacter* spp., *Escherichia coli* O157 and the commensal organism *E. coli*. 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.

3.1 Food-borne pathogens

3.1.1 *Salmonella*

This chapter presents antimicrobial resistance percentages of *Salmonella* isolates. These isolates were obtained from human salmonellosis patients, food-producing animals, food products of animal origin and other food products as potential sources of infection for humans via the food chain, and animal feed as potential source of infection for food-producing animals.

Highlights

1. In 2023, 1413 human *Salmonella* isolates and 726 isolates from non-human sources were tested for antimicrobial susceptibility.
2. Over all isolates, the highest resistance proportions were observed for ciprofloxacin (21%), nalidixic acid (21%), ampicillin (18%), sulfamethoxazole (19%), tetracycline (17%), trimethoprim (9%) and chloramphenicol (6%).
3. Over all sources, the highest levels of resistance were observed for monophasic *S.* Typhimurium, *S.* Infantis, *S.* Chester, *S.* Paratyphi B var. Java, and *S.* Typhimurium.
4. One carbapenemase-producing *Salmonella* (OXA-48) was found in a human isolate (monophasic *S.* Typhimurium).
5. Among human *S.* Typhimurium isolates, decreasing trends in resistance against ampicillin (34%) and tetracycline (18%) were observed since 2010.
6. Among human *S.* Enteritidis isolates, a decrease in resistance against ampicillin (10%), ciprofloxacin and nalidixic acid (27%) was observed. However, in general there is an increasing trend in resistance against fluoroquinolones in human isolates since 2010.
7. In total, 20 (1.4%) ESBL-producing human clinical isolates were detected, which is comparable to previous years (1.8% in 2022 and 1.4% in 2021).
8. Levels of resistance among isolates from broiler meat were high for ciprofloxacin (52%), nalidixic acid (52%), tetracycline (33%) and sulfamethoxazole (68%).

Resistance proportions overall

Human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories were sent to WBVR for antimicrobial susceptibility testing. Only the first isolate from a given patient tested in 2023 was selected for analysis (n = 1413). Moreover, 726 isolates from non-human sources were tested. These included isolates from broilers (n = 252), cattle (n = 72), pigs (n = 100), layers (n = 177), and sheep (n=81), as well as isolates from a diversity of other sources, including vegetables, herbs and other animals (e.g. goats, horses, turkey, etc., n = 44). The non-human isolates included 174 isolates from food products (e.g. meat and products thereof) analysed for antibiotic susceptibility by WFSR, the official food safety laboratory of the NVWA. Non-human isolates were mainly sent to the RIVM by the Animal Health Services in Deventer from a diversity of surveillance programs and diagnostic activities for clinical infections in animals, or they were obtained from WFSR (mainly non-clinical isolates) through its routine *Salmonella*-control activities on farms, slaughterhouses (e.g. EC/2073.2005 verification projects broiler neck skin) and food products sampled at different stage of the food chain. *Salmonella* isolates from primary poultry farms (n=254) were tested by WBVR, in line with Decision (EU) 2020/1729.

MIC distributions and resistance percentages of all 2139 *Salmonella* isolates from different sources tested for susceptibility in 2023 are presented in Table S01. The highest resistance proportions were observed for ciprofloxacin (21%), nalidixic acid (21%), sulfamethoxazole (19%), ampicillin (18%), tetracycline (17%), trimethoprim (9%), and chloramphenicol (6%). Low proportions of resistance (<5%) were found for amikacin, azithromycin, cefotaxime, ceftazidime, gentamicin, and tigecycline. For the first time a carbapenemase-producing *Salmonella* isolate resistant to meropenem was detected (monophasic *S.* Typhimurium carrying OXA-48), originating from faecal material of a patient without a known travel history. A second meropenem resistant clinical isolate (S. Enteritidis) was found, but could not be genotypically confirmed.

The class of fluoroquinolones is regarded as the treatment of choice for severe salmonellosis in adults. Currently, EUCAST recommends a clinical breakpoint of 0.06 mg/L for *Salmonella enterica*, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L) (www.eucast.org). Using the EUCAST recommended epidemiological cut-off value of 0.06 mg/L as breakpoint, 21% of *Salmonella* isolates from 2023 demonstrated an acquired resistance phenotype for ciprofloxacin (Table S01). Overall a very high level of ciprofloxacin resistance was observed for *S.* Chester (81%) (Table S02). High levels were also detected for *S.* Infantis (57%) and *S.* Paratyphi B var. Java (24%), and *S.* Enteritidis (22%). Similar patterns were observed for the quinolone nalidixic acid.

Table S01 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* isolates (N=2,139) tested for antibiotic susceptibility during 2023

Salmonella N = 2,139	MIC (%) distribution mg/L																		R%	95% CI
	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							29.4	49.5	3.4	0.1		0.1	17.6						17.8	16.2 - 19.5
Cefotaxime					97.5	1.4	0.1	0.05	0.05	0.9									1.1	0.8 - 1.7
Ceftazidime					57.5	38.1	3.2	0.2	0.2	0.2	0.6								1.0	0.6 - 1.5
Gentamicin						92.9	4.7	0.1	0.05	0.1	0.3	1.8							2.2	1.7 - 3.0
Tetracycline								80.7	2.4	0.3	0.05	0.1	16.5						16.6	15.1 - 18.2
Sulfamethoxazole										2.4	11.5	49.5	16.5	1.0			19.2		19.2	17.6 - 20.9
Trimethoprim					62.9	26.4	1.3	0.1	0.05			9.3							9.3	8.1 - 10.6
Ciprofloxacin	28.8	48.5	1.4	1.5	9.6	7.4	1.2	0.4	0.1	0.5	0.6								21.3	19.6 - 23.1
Nalidixic acid									73.2	6.3	2.1	3.7	0.1	14.7					20.5	18.9 - 22.3
Chloramphenicol										89.5	4.8	0.2	0.1	5.4					5.7	4.8 - 6.8
Azithromycin*								0.6	55.2	41.7	1.9	0.1	0.2	0.2					0.6	0.4 - 1.0
Colistin**							73.4	14.5	11.5	0.5		0.05							-	-
Meropenem		88.5	11.2	0.1	0.1														0.1	0.0-0.4
Tigecycline***					64.5	29.7	4.8	0.7	0.1	0.05									0.9	0.6 - 1.5
Amikacin									99.9	0.1									0.1	0.0 - 0.4

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 S02 Resistance (%) of the top 10 most prevalent *Salmonella* serovars in the Netherlands in 2023 (N tested)

	Enteritidis (732)	Typhimurium (190)	Typhimurium monophasic (167)	Infantis (102)	Diarizonae-61:k:1,5,(7) (79)	Paratyphi B vr. Java (79)	Chester (58)	Virchow (48)	Derby (43)	Dublin (36)
Ampicillin	15.5	88.8	44.9	10.2	4.2	4.5	0.0	30.3	18.2	95.5
Cefotaxime	0.0	3.1	1.1	4.5	2.8	0.0	0.0	0.0	0.0	81.8
Ceftazidime	0.0	1.0	0.0	4.5	2.8	0.0	0.0	0.0	0.0	81.8
Gentamicin	0.0	11.2	4.5	3.4	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline	7.4	81.6	33.7	53.4	0.0	4.5	0.0	3.0	22.7	95.5
Sulfamethoxazole	6.6	80.6	32.6	58.0	2.8	0.0	3.7	60.6	4.5	86.4
Trimethoprim	1.1	16.3	5.6	39.8	2.8	0.0	0.0	75.8	13.6	13.6
Ciprofloxacin	33.9	21.4	6.7	58.0	11.1	2.3	14.8	39.4	27.3	95.5
Nalidixic acid	34.3	21.4	5.6	59.1	11.1	2.3	14.8	45.5	22.7	95.5
Chloramphenicol	1.1	22.4	15.7	4.5	2.8	0.0	0.0	0.0	18.2	9.1
Azithromycin	0.7	6.1	1.1	1.1	0.0	0.0	0.0	0.0	4.5	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	10.2	1.1	8.0	0.0	0.0	0.0	0.0	0.0	13.6
Amikacin	0.0	0.0	1.1	0.0	0.0	0.0	3.7	0.0	0.0	0.0

Resistance proportions of the most prevalent serovars

The resistance patterns of *S.* Typhimurium and monophasic *S.* Typhimurium, which are both primarily associated with pigs and cattle as a reservoir, among human and non-human isolates are shown in Table S03. Resistance remained high for ampicillin, sulfamethoxazole, and tetracycline, especially among monophasic *S.* Typhimurium. Resistance to the clinically important drugs cefotaxime and ceftazidime was only detected among human isolates. Trends in resistance in human *S.* Typhimurium isolates are shown in Fig. S01.The percentage of resistance to tetracycline, ampicillin and sulfamethoxazole declined considerably; which is in line with the general downward trend since 2010. A decreasing trend was also observed for resistance to chloramphenicol. An increase in resistance to trimethoprim was observed, from 3% in 2022 to 11% in 2023.

In the Netherlands, *S.* Enteritidis is mainly associated with layers and their produced eggs. Fractions of resistance in *S.* Enteritidis are generally lower compared to *S.* Typhimurium. After an increase in 2022, a considerable decrease was observed in 2023 among human isolates in resistance against ampicillin (7% in 2021, 18% in 2022, 10% in 2023), ciprofloxacin and nalidixic acid (both 21% in 2021, 38% in 2022, 27% in 2023) (Table S04 and Fig S02). However, in general there is an increasing trend in resistance against fluoroquinolones in human isolates since 2010 (Fig S02). For layers, resistance against ciprofloxacin and nalidixic acid was 4% in 2023, compared to 0% in 2022 and 11% in 2021. In broilers resistance against ciprofloxacin and nalidixic acid was 16% 2023, while it was 44% in 2022 and 4% in 2021. However, the numbers of non-human isolates are relatively small and changes over time in the fraction of resistance should be interpreted with caution.

Table S03 Resistance percentages of *S.* Typhimurium and *S.* Typhimurium monophasic (N tested) isolated from humans and non-human sources in 2023

	S. Typhimurium (190)				S. Typhimurium monophasic (167)		
	Humans (131)	Cattle (21)	Pigs (14)	Other non-human sources (24)[a]	Humans (117)	Pigs (30)	Other non-human sources (20)[b]
Ampicillin	34.4	71.4	42.9	37.5	82.1	83.3	95.0
Cefotaxime	1.5	0.0	0.0	0.0	1.7	0.0	0.0
Ceftazidime	1.5	0.0	0.0	0.0	0.9	0.0	0.0
Gentamicin	2.3	57.1	7.1	20.8	4.3	0.0	15.0
Tetracycline	18.3	57.1	35.7	25.0	85.5	86.7	80.0
Sulfamethoxazole	23.7	66.7	64.3	37.5	81.2	76.7	95.0
Trimethoprim	11.5	4.8	42.9	12.5	11.1	16.7	15.0
Ciprofloxacin	8.4	4.8	7.1	0.0	13.7	0.0	0.0
Nalidixic acid	8.4	4.8	0.0	0.0	12.0	0.0	5.0
Chloramphenicol	7.6	66.7	42.9	20.8	15.4	20.0	20.0
Azithromycin	0.0	0.0	0.0	0.0	0.9	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.9	0.0	0.0
Tigecycline	1.5	0.0	0.0	0.0	0.9	0.0	0.0
Amikacin	0.0	0.0	0.0	0.0	0.9	3.3	0.0

a Non-human sources include broilers (9), horses (6), layers (6), pigeons (1), sheep (1), and shellfish (1)
b Non-human sources include broilers (9), cattle (5), horses (5), goats (1)

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans in 2000-2023

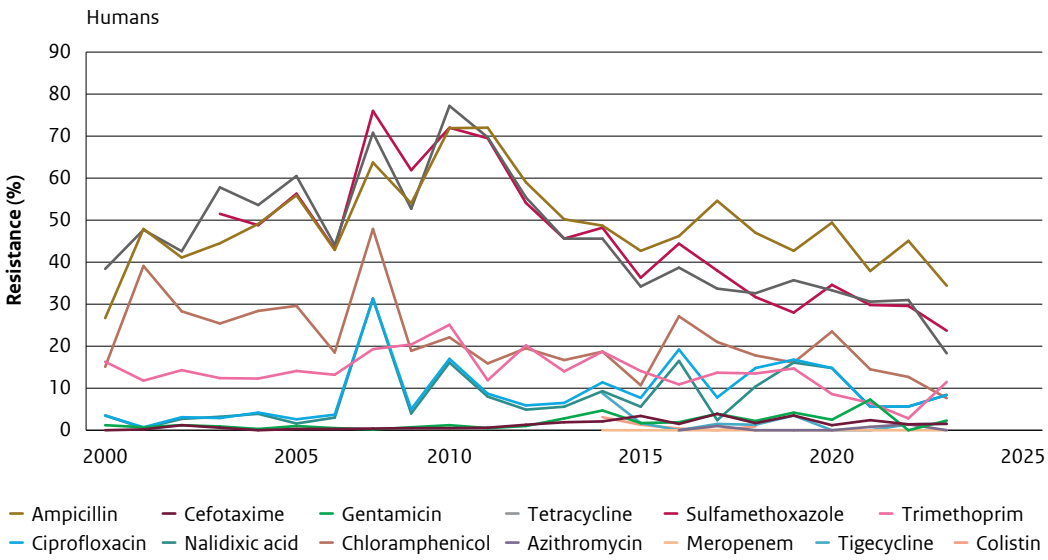
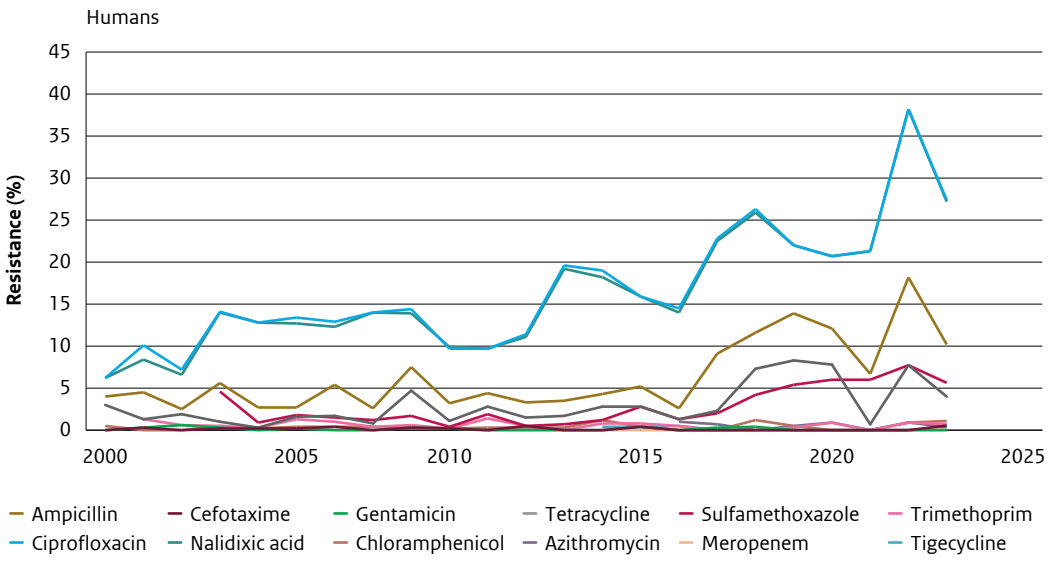


Table S04 Resistance percentages of *S.* Enteritidis (N tested) isolated from humans, layers and broilers in 2023

	S. Enteritidis (732)			
	Humans (220)	Layers (24)	Broilers (16)	Other non-human sources (19)[a]
Ampicillin	10.2	0.7	0.0	0.0
Cefotaxime	0.5	0.0	0.0	0.0
Ceftazidime	0.4	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0
Tetracycline	4.0	0.0	0.0	0.0
Sulfamethoxazole	5.6	0.7	0.0	0.0
Trimethoprim	0.7	0.0	0.0	0.0
Ciprofloxacin	27.4	3.7	16.0	5.3
Nalidixic acid	27.2	4.4	16.0	5.3
Chloramphenicol	1.1	0.0	0.0	0.0
Azithromycin	0.4	0.0	0.0	0.0
Meropenem	0.2	0.0	0.0	0.0
Tigecycline	0.4	0.0	0.0	0.0
Amikacin	0.0	0.0	0.0	0.0

a Other non-human sources include horses (3), cattle (2), sheep (1), vegetables (1), and unspecified non-human sources (12)

Figure S02 Trends in resistance (%) of *S.* Enteritidis isolated from humans from 2000-2023



ESBLs in *Salmonella*

The emergence of multidrug resistant *Salmonella* strains with resistance to fluoroquinolones and extended-spectrum cephalosporins is a serious development, which results in severe limitations for effective treatment of human infections. In 2023, the total number of genotypic confirmed ESBL *Salmonella* isolates was 20/2139 (0.9%) (compared to 14/1156 (1.2%) in 2022, 10/1264 (0.8%) in 2021, 6/1170 (0.5%) in 2020). All 20 isolates were obtained from humans and included *S.* Enteritidis (n=3), *S.* Kentucky (n=3), *S.* Infantis (n=2), *S.* Schwarzengrund (n=2), *S.* Typhimurium (n=2), *S.* Agona (n=1), *S.* Anatum (n=1), *S.* Apeyeme (n=1), *S.* Braenderup (n=1), *S.* Give (n=1), *S.* Heidelberg (n=1), *S.* Muenster (n=1), and monophasic *S.* Typhimurium (n=1).

Table S05 Resistance (%) of *Salmonella enterica* isolated from different types of raw meat in the Netherlands in 2023

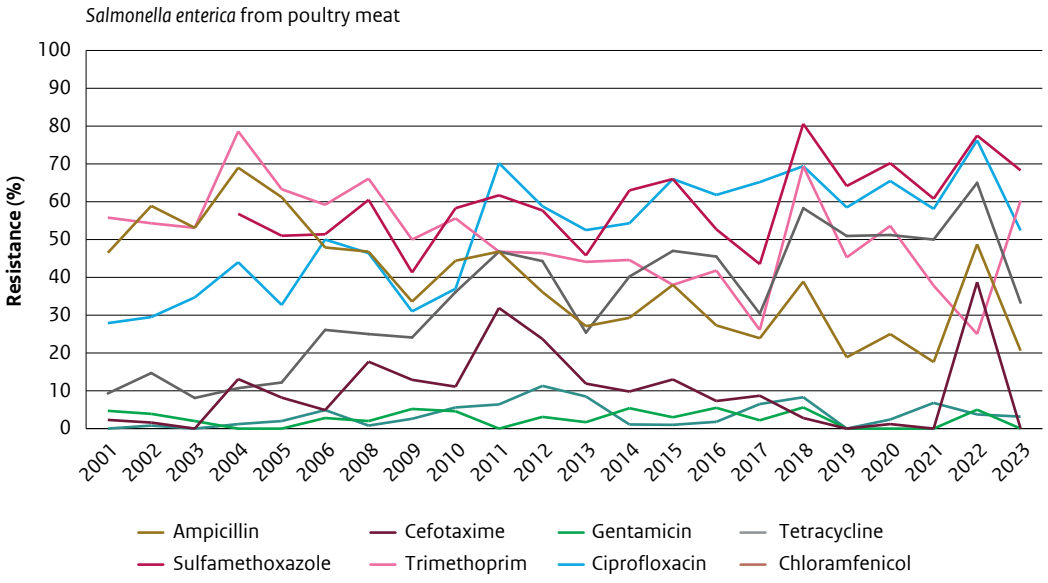
	Broiler meat[a]	Other meat[b]
	N = 63	N = 27
Ampicillin	20.6	25.9
Cefotaxime	0.0	0.0
Ceftazidime	0.0	0.0
Gentamicin	0.0	3.7
Tetracycline	33.3	18.5
Sulfamethoxazole	68.3	14.8
Trimethoprim	60.3	18.5
Ciprofloxacin	52.4	11.1
Nalidixic acid	52.4	11.1
Chloramphenicol	3.2	7.4
Azithromycin	1.6	0.0
Meropenem	0.0	0.0
Tigecycline	12.7	0.0
Amikacin	0.0	3.7

a Fresh chicken meat sampled at retail and chicken neck skin from verification projects
b Other meat includes pork (10), sheep (8), beef (7) and goats (2)

Salmonella from chicken meat, other meat sources and spices

Table S05 shows resistance data of *Salmonella* isolates from broiler meat and other meat sources, and in Fig. S03 the trends in resistance of *Salmonella* isolates from broiler meat are presented. In general, levels of resistance among isolates from broiler meat decreased compared to 2022, but were still high for resistance to sulfamethoxazole (68%), ciprofloxacin (52%), nalidixic acid (52%) and tetracycline (33%). Resistance to trimethoprim increased from 25% in 2022 to 60% in 2023. It should be noted that the level of resistance is also depending on the serotypes that are found. Furthermore, in 2022 almost 50% of *Salmonella* isolates originating from broiler meat were imported from outside of the EU, these isolates had considerably higher levels of resistance compared to Dutch/EU meat. No imported broiler meat was tested in 2023.

Figure S03 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meats in the Netherlands from 2001-2023



3.1.2 *Campylobacter*

In this chapter, the occurrence and trends in antimicrobial resistance in *Campylobacter jejuni* and *C. coli* are described. Isolates were obtained from samples collected from food animals, meat and humans.

Highlights

1. In 2023, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof remained at a high level for fluoroquinolones and tetracycline.
2. Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, but was observed at low levels in *C. jejuni* from veal calves and *C. coli* from broilers and poultry meat. A notably higher level of erythromycin resistance was again observed in *C. coli* from veal calves.
3. In humans, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates. Resistance levels increased in 2022 and 2023 compared with 2020 and 2021, when resistance most likely dropped due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 travel restrictions, which is associated with higher resistance proportions than domestically acquired campylobacteriosis. However, resistance levels for *C. jejuni* were generally still lower than before the COVID-19 pandemic, but back to pre-pandemic levels for *C. coli*.
4. Ciprofloxacin resistance in *Campylobacter* isolates from humans was again high in 2023, which is a concern for public health. It was, however, lower compared to 2019, before the COVID-19 pandemic.
5. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

Table C01 presents the aggregated MIC distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2023 from caecal samples of broilers, veal calves, pigs and faecal samples of sheep. For each sample type, resistance percentages of *C. jejuni* and *C. coli* are depicted in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products are presented in Figures C01 and C02. National surveillance data for *Campylobacter* spp. isolated from humans are shown in Figure C03 (from 2002 onwards) and in Table C03 (from 2009 onwards).

Resistance proportions

Broiler chickens 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 chicken meat.

Figure Co1 demonstrates a high similarity in resistance trends between *C. jejuni* obtained from caecal samples at slaughter and those obtained from retail meat suggesting an overlap in the bacterial population examined from the different matrixes. Resistance percentages for ciprofloxacin and tetracycline have been high with fluctuation over the years, with continuous high levels of ciprofloxacin resistance in broilers (62.5%) and chicken meat (71.4%) in 2023. The resistance levels for chloramphenicol, erythromycin and gentamicin were very low to zero as in former years.

Figure Co2 shows comparable parallel time trends in resistance for ciprofloxacin and tetracycline in *C. coli* isolates from both sources. However, after 2018 less similarity in trends is observed both in caecal and meat isolates. As in former years, gentamicin resistance in *C. coli* from broilers and poultry meat was completely absent. In *C. coli*, proportions of macrolide resistance have been fluctuating substantially over the years with resistance proportions below 10% from 2019 onwards. Resistance percentages for ciprofloxacin in broilers and poultry meat have been fluctuating on a high level since 2001 and increased even further in 2023 to extremely high proportions of resistance above 90%. Because of the relatively low number of *C. coli* isolates tested (especially from meat), these results might not be very representative.

Table Co2 shows that the proportions of resistance for tetracycline and ciprofloxacin in *C. jejuni* isolates were at high levels for isolates from chicken meat, as well as for isolates from caecal samples of broilers. Resistance levels for *C. coli* isolates from broilers and chicken meat for ciprofloxacin were even higher.

No resistance to gentamicin was detected in both *C. jejuni* and *C. coli* isolates from broilers and broiler meat. Resistance to erythromycin was detected at low levels in *C. coli* from broilers and meat thereof, whereas chloramphenicol resistance was only observed in one *C. jejuni* isolate from a broiler

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 C01. MIC distributions (in %) for *Campylobacter jejuni* (N = 252) and *C. coli* (N = 409) isolated from caecal samples of broilers, veal calves and pigs in 2023

C. jejuni, broilers and veal calves	MIC (%) distribution mg/L															R%	95% CI
(N = 252)	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024		
Chloramphenicol						23.0	60.3	11.5	4.8	0.4						0.4	0.0 - 2.2
Ciprofloxacin		40.1	7.9	3.2			0.8	14.3	23.8	8.7	1.2					48.8	42.5 - 55.2
Erythromycin					41.7	43.7	12.7	1.2							0.8	2.0	0.7 - 4.6
Ertapenem		65.9	23.0	7.9	1.6	1.2	0.4									-	-
Gentamicin			86.1	13.9												0.0	0.0 - 1.5
Tetracycline				17.9	4.8	1.6		0.4	1.2	4.8	15.5	54.0				77.4	71.7 - 82.4

C. coli, broilers, veal calves and pigs	MIC (%) distribution mg/L															R%	95% CI
(N = 409)	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024		
Chloramphenicol						4.2	32.5	45.0	17.8	0.5						0.5	0.1 - 1.8
Ciprofloxacin		40.1	16.1	2.4		0.2	2.7	11.5	15.9	9.8	1.2					41.3	36.5 - 46.3
Erythromycin					28.1	23.2	31.1	6.1	1.7				0.5	0.2	9.0	11.5	8.6 - 15.0
Ertapenem		26.2	24.4	21.5	13.2	9.0	4.4	1.2								-	-
Gentamicin			39.9	57.5	0.5					2.2						2.2	1.0 - 4.1
Tetracycline				15.6	4.2	2.0	0.7	0.2	0.5	1.2	8.3	67.2				78.2	73.9 - 82.1

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and chicken meat in the Netherlands

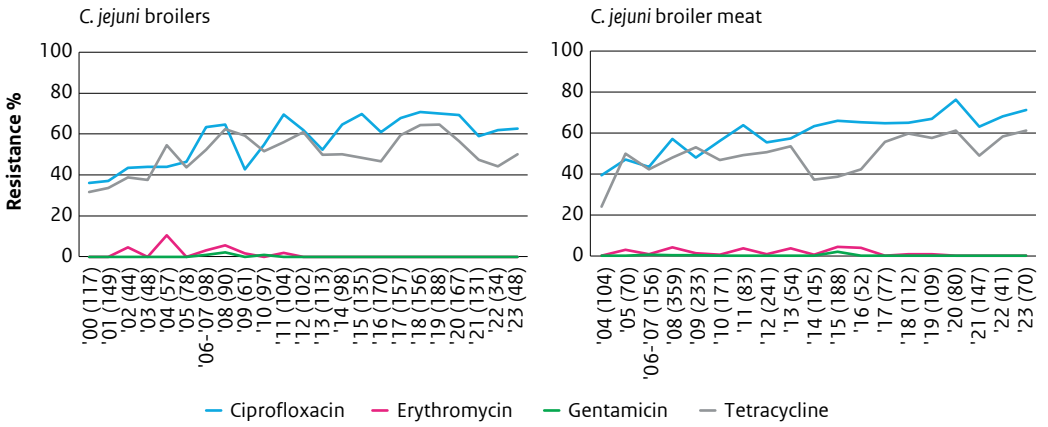


Figure C02 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands

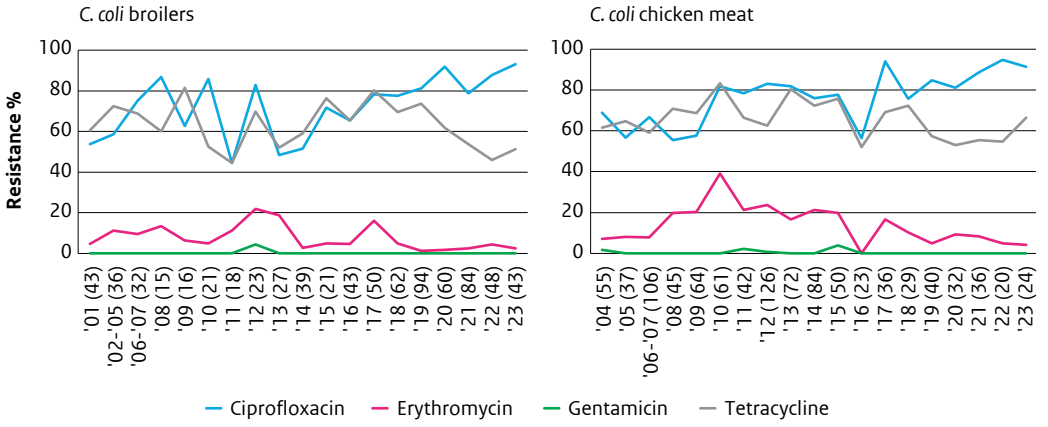


Table C02. Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of livestock and meat in 2023

	C. jejuni (R%)				*C. coli* (R%)				
	Broilers	Chicken meat	Veal calves	Sheep	Broilers	Chicken meat	Veal calves	Pigs	Sheep
	N=48	N=70	N=199	N=88	N=43	N=24	N=103	N=268	N=100
Chloramphenicol	2.1	0.0	0.0	1.1	0.0	0.0	1.9	0.0	0.0
Ciprofloxacin	62.5	71.4	46.2	11.4	93.0	91.7	78.6	18.7	8.0
Erythromycin	0.0	0.0	2.5	0.0	2.3	4.2	37.9	2.6	0.0
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	8.7	0.0	0.0
Tetracycline	50.0	61.4	85.4	10.2	51.2	66.7	98.1	75.0	24.0

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 proportions are depicted in table C02. As in former years, very high proportions of tetracycline resistance were measured for *C. jejuni* and *C. coli* from veal calves (85.4 – 98.1%) as well as for *C. coli* from pigs (75,0%). Resistance levels were also high in veal calves for ciprofloxacin in both *C. jejuni* and *C. coli* (46.2 – 78.6%). Substantially lower levels of ciprofloxacin resistance were detected in *C. coli* from pigs (18.7%) confirming the relatively lower levels observed in former years. In *C. jejuni* from veal calves resistance was completely absent for chloramphenicol and gentamicin, but low-level resistance to erythromycin was detected for the second year in row. As in former years, erythromycin resistance was frequently observed in *C. coli* from veal calves (37.9%), whereas gentamicin resistance and chloramphenicol resistance was observed at low levels. In *C. coli* from pigs resistance to erythromycin was rare and resistance against chloramphenicol and gentamicin was not detected.

Sheep

In 2023, faecal samples of sheep were collected on 160 farms as part of an ongoing national monitoring project in livestock focusing on zoonotic bacteria and antibiotic resistance. As a result a selection of 88 *C. jejuni* and 100 *C. coli* isolates from 138 different farms were collected and tested for antimicrobial susceptibility. The resistance proportions are included in table C02. Both in *C. jejuni* and *C. coli*, resistance was completely absent for erythromycin and gentamicin, whereas chloramphenicol resistance was only detected in one *C. jejuni* isolate. Resistance to ciprofloxacin (8.0 – 11.4%) and tetracycline (10.2 – 24.0%) was much lower compared to the other animals sectors included, reflecting the low antibiotic use in this sector.

Campylobacter in humans

In 2023, an estimated 4671 campylobacteriosis cases occurred in The Netherlands. Although the number of cases were higher than during the COVID-19 pandemic, they are slightly lower compared to the average of 5053 cases during 2015-2019 (range: 4794 – 5262 cases), and similar to 2022 (4757 cases). Resistance levels in isolates from human patients were determined for ciprofloxacin, tetracycline and erythromycin, and are shown in Table C03 and Figure C03. Figure C03 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.

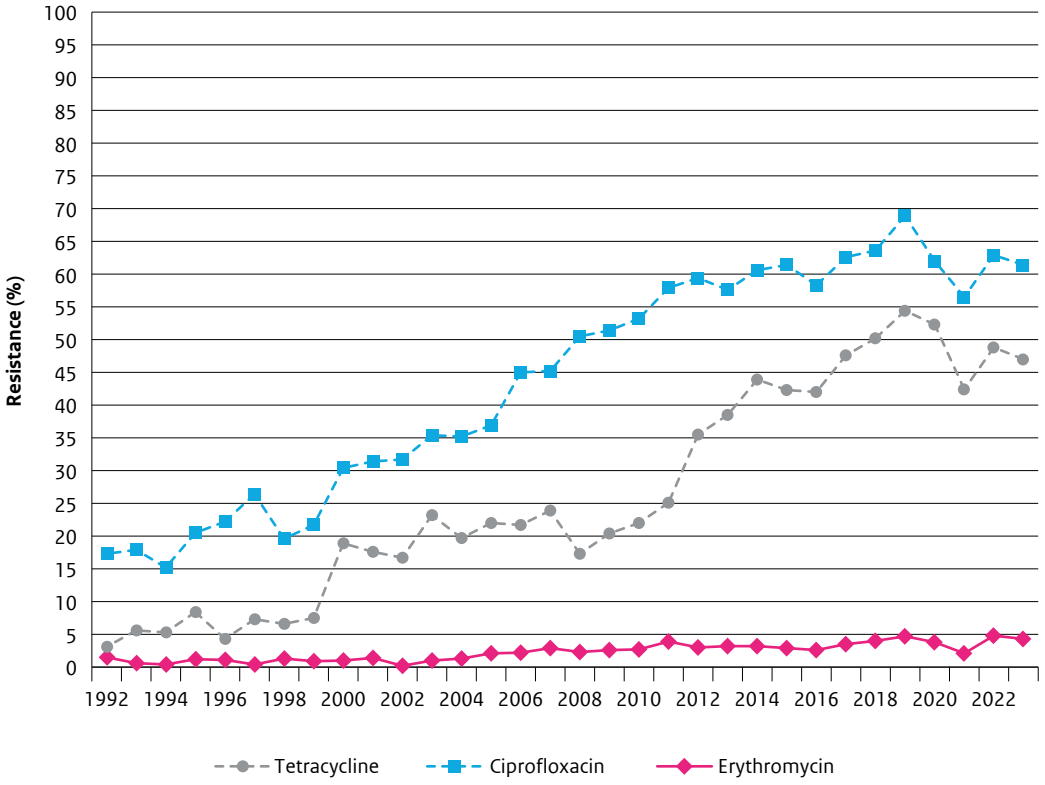
In 2023, the resistance level for ciprofloxacin in human *Campylobacter* spp. isolates was 61.4%, which is a public health concern. However, resistance is still lower compared with pre-pandemic years 2018 and 2019, with a 63.6% and 68.9% resistance for ciprofloxacin, respectively. Tetracycline resistance had a similar trend and was even slightly lower than in 2022, with a 47.0% resistance compared with 48.8%. Erythromycin resistance was 4.3% in 2023. This is slightly lower than in 2022, but higher than during 2020 and 2021.

Table C03 shows the resistance levels for human *C. jejuni* and *C. coli* isolates since 2016. Overall, the resistance levels in human *Campylobacter* spp. isolates for all three antimicrobials show an increasing trend until 2019 and a reduction in resistance levels in 2020 and 2021. In 2022 and 2023, the resistance levels for *C. coli* were back at pre-pandemic levels. For *C. jejuni*, however, the resistance levels in 2022 and 2023 were still lower than before the pandemic. Resistance proportions were higher for *C. coli* isolates than *C. jejuni* isolates.

Table C03 Resistance in *C. jejuni* and *C. coli* isolated from humans from 2016 - 2023

	C. jejuni						*C. coli*					
	Fluoroquinolone		Tetracycline		Erythromycin		Fluoroquinolone		Tetracycline		Erythromycin	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
2016	3,184	59.6	2,826	45.8	3,228	2.0	246	69.1	216	64.4	245	14.7
2017	2,965	62.0	2,642	50.0	3,026	2.6	274	77.0	256	70.3	275	22.9
2018	3,224	63.3	2,965	53.9	3,285	2.1	296	73.0	275	69.8	303	26.4
2019	3,338	66.6	3,116	54.0	3,395	1.9	400	78.3	379	73.1	402	20.7
2020	2,508	59.6	2,352	50.3	2,523	2.1	208	68.3	198	73.2	209	20.1
2021	2,904	53.2	2,768	40.2	2,954	1.3	224	69.2	215	64.7	234	15.8
2022	3,578	61.0	3,383	45.5	3,624	2.5	353	72.5	344	66.0	361	19.4
2023	3,913	60.0	3,652	44.9	3,932	2.6	358	76.5	338	69.8	363	22.6

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2023



3.1.3 Shiga-toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC)

Highlights

1. In human *STEC* O157 isolates, a decrease in proportions of resistance against ampicillin, tetracycline, trimethoprim and sulfamethoxazole compared to 2022 was observed.
2. The proportion of resistance of ciprofloxacin was higher in human STEC/EPEC non-O157 *E. coli* than in human *STEC* O157.
3. No ESBL-producing isolates were detected in *STEC* O157, but resistance to third generation cephalosporins was detected in three STEC/EPEC non-O157 *E. coli* isolates through presence of ESBL genes in two isolates ($bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}15}$). In a third isolate, a mutation in the AmpC promotor region was detected.

Shiga-toxin producing *E. 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=262) consisting of multiple STEC/aEPEC/tEPEC[1]* O157 and non-O157 serotypes were tested for susceptibility. The set consisted of 68 STEC/EPEC O157 isolates, 194 STEC/EPEC non-O157 isolates: O26 (n=29), O146 (n=23), O63 (n=20), O111 (n=10), and other O-types (n=112). All isolates were obtained from the RIVM national laboratory surveillance of STEC. Table STEC01 shows the MIC results for *E. coli* O157 isolates from humans; Table STEC02 shows resistance proportions of *E. coli* O157 and STEC/EPEC non-O157 isolates; Figure STEC01 presents the trends over time for STEC O157; Figure STEC02 presents the trends in resistance over time for STEC/EPEC non-O157.

In comparison to 2022, a decrease in resistance proportions among STEC O157 can be seen for trimethoprim, ampicillin, and ciprofloxacin (Figure STEC01). Furthermore, following a sharp increase in 2021, resistance proportions for tetracycline and sulfamethoxazole decreased further, which is in line with the strong fluctuating long-year trend. Levels of resistance for gentamicin and chloramphenicol slightly increased, remaining below 5% and in line with the trend seen from 2019 onwards. There was no resistance detected for cefotaxime and ciprofloxacin among STEC O157 in 2023. No ESBL-producing isolates were detected in 2023 among STEC O157.

[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.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2023

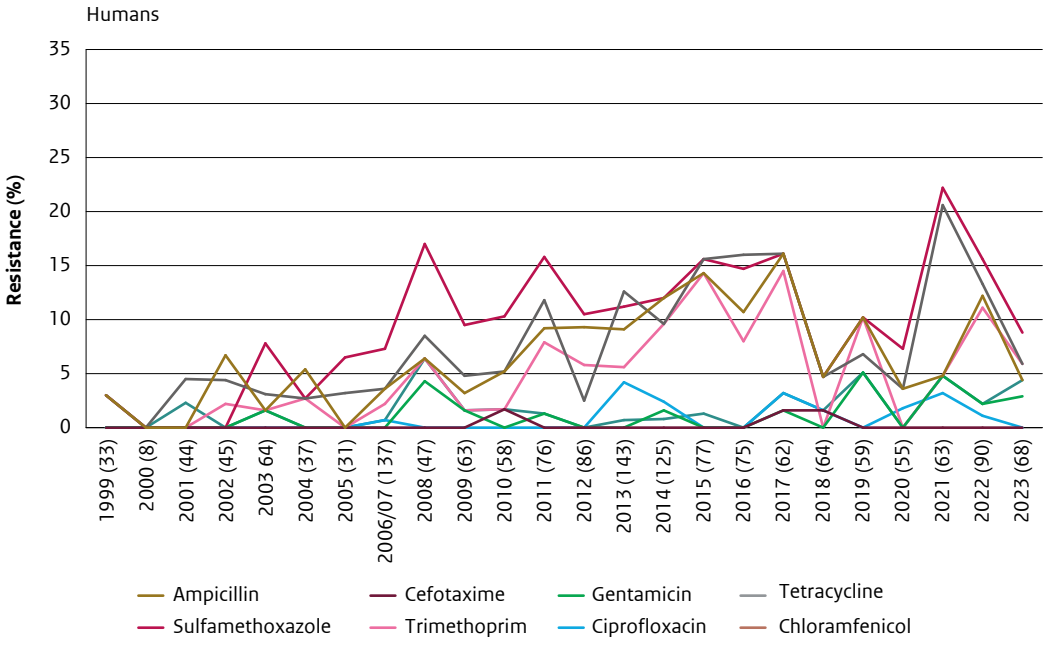


Figure STEC02 Figure STEC02 Trends in resistance (in %) of *E. coli* STEC/EPEC non-O157 isolated in the Netherlands from 2020-2023

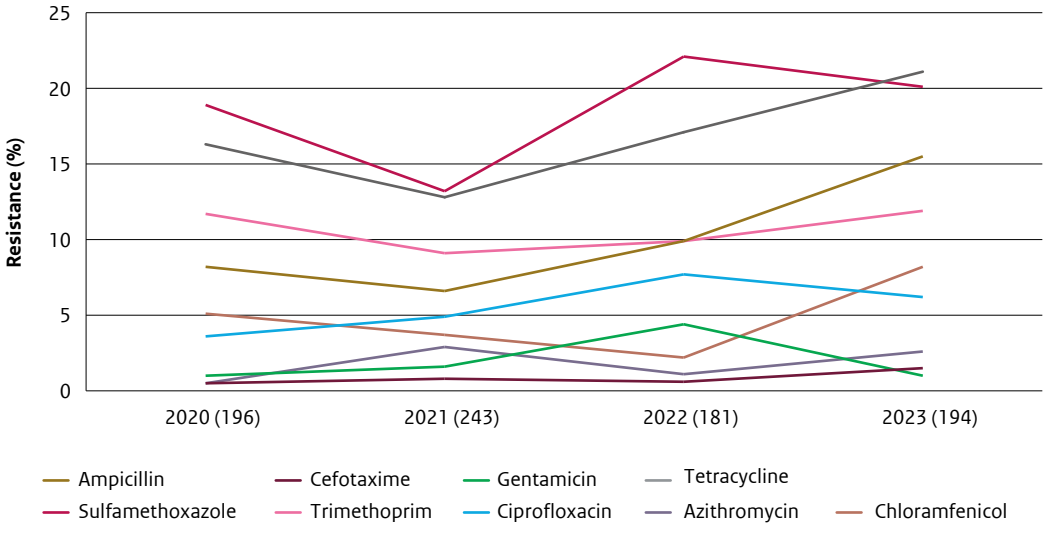


Table STEC01 MIC distribution (in %) and resistance percentages (R% incl 95% CI) for *E. coli* STEC O157 (N=68) isolated from humans the Netherlands in 2023

E. coli N = 90	MIC (%) distribution mg/L																		R%	95% CI
	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									97.1	2.9									0.0	0.0 - 5.3
Ampicillin								1.5	89.7	4.4			4.4						4.4	0.9 -12.4
Cefotaxime					100.0														0.0	0.0 - 5.3
Ceftazidime					95.6	4.4													0.0	0.0 - 5.3
Gentamicin						92.6	2.9	1.5			2.9								2.9	0.4 - 10.2
Tetracycline								75.0	19.1			1.5	4.4						5.9	1.6 - 14.4
Sulfamethoxazole										50.0	38.2	2.9					8.8		8.8	3.3 - 18.2
Trimethoprim					86.8	7.4						5.9							5.9	1.6 - 14.4
Ciprofloxacin	77.9	22.1																	0.0	0.0 - 5.3
Nalidixic acid									97.1	2.9									0.0	0.0 - 5.3
Chloramphenicol										92.6	2.9			4.4					4.4	0.9 - 12.4
Azithromycin								30.9	64.7	4.4									0.0	0.0 - 5.3
Colistin							100.0												0.0	0.0 - 5.3
Meropenem		100.0																	0.0	0.0 - 5.3
Tigecycline					97.1	2.9													0.0	0.0 - 5.3

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.

Table STEC02 shows differences in proportion of resistance between STEC O157 and STEC/EPEC non-O157 isolates. Statistical differences in the proportion of resistance is observed for ampicillin, ciprofloxacin, sulfamethoxazole and tetracycline (χ^2, p =0.018, p =0.036, p =0.034, p=0.004, respectively) with a higher resistance level for non-O157 isolates compared to O157 isolates. The proportion of multidrug resistance[2]* was significantly different (χ^2, p =0.012) at 8.8% in STEC O157 and 23.2% in non-O157 isolates, with 6 out of 68 and 45 out of 194 isolates, respectively. Furthermore, from the multidrug resistant non-O157 isolates, 19 were of the serovar O26:H11. Resistance to 3rd gen cephalosporins (cefotaxime and ceftazidime) was detected in two non-O157 isolates; O76:H21 and O26:H11. The presence of virulence genes included *stx1*, *stx2*, *eae* and *heam*, present in different combinations. Resistance marker detection in the genome sequence confirmed the presence of the ESBL genes $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}15}$. An AmpC promotor mutant was detected in a third isolate (O128:H2) with virulence genes *stx1*, *stx2* and *heam*.

Table Eco02. Resistance percentages (R%) of pathogenic *E. coli* in the Netherlands in 2023

E. coli	O157 N = 68	Other serotypes N = 194
Amikacin	0.0	0.0
Ampicillin	4.4	15.5
Cefotaxime	0.0	1.5
Ceftazidime	0.0	1.5
Gentamicin	2.9	1.0
Tetracycline	5.9	21.1
Sulfamethoxazole	8.8	20.1
Trimethoprim	5.9	11.9
Ciprofloxacin	0.0	6.2
Nalidixic acid	0.0	3.6
Chloramphenicol	4.4	8.2
Azithromycin	0.0	2.6
Colistin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals, meat and vegetables. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. *E. coli* is therefore included as indicator organism for the Gram-negative flora. As a result of less priority for including enterococci representing the Gram-positive bacteria in the surveillance, no enterococci are reported since 2017.

EFSA[2,3] 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 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 broilers by collecting samples from caeca of individual veal calves at slaughterhouses, and resistance levels were reported separately for white and rosé veal calves. From 2022 onwards, the sampling of broilers was adapted according to the updated legislation by analysing pooled samples of 10 caeca originating from 10 individual animals per flock instead of one caecal sample per flock.

It should be noted that the sampling strategies used are inherently insensitive to detect resistance at the population level, as only one randomly selected isolate from a single sample collected from one animal per epidemiological unit (herd or flock) is tested for susceptibility. The total number of isolates is intended to represent the *E. coli* population of each animal species of the entire country. One percent resistance in e.g. *E. coli* indicates that in all animals of that animal species 1% of the *E. coli* bacteria are resistant. The absence of resistance in these datasets does not exclude the possibility that resistance is present in individual animals.

3.2.1 *Escherichia coli*

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, meat and other products in the Netherlands.
The latest 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 retail as well from imported meat taken at the border control posts are examined. This includes susceptibility testing by broth microdilution according to ISO 20776-1:2019 with updated mandatory panels of antimicrobials. In this report, non-wild type susceptible isolates are classified as resistant. These isolates all harbour an acquired resistance mechanism, but may not be clinically resistant for some antibiotics.

Highlights 2023

1. In broilers, pigs and veal calves, levels of resistance stabilised for most antibiotics in the last five years, whereas resistance in dairy cattle remained traditionally low.
2. Amongst indicator *E. coli* from animals, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, and (white) veal calves.
3. Fluoroquinolone (FQ) resistance was still commonly present in indicator *E. coli* from caecal samples of broilers (25.6%) in contrast with the low prevalence observed in pigs (1.7%) and veal calves (4.3%) and the complete absence in dairy cattle.
4. Resistance to third generation cephalosporins was low (<1%) or not detected amongst (randomly isolated) indicator *E. coli* from caecal samples of all animal species.
5. For most antibiotics tested, in 2023 levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.
6. In *E. coli* isolates obtained from chicken meat sampled at retail, decreasing levels of resistance were observed with a tendency to flatten in recent years.
7. Except for the lower fluoroquinolone resistance in retail pork, resistance patterns in *E. coli* obtained from pork were comparable to broiler meat with the highest resistances monitored for ampicillin, trimethoprim, sulfamethoxazole and tetracycline. Over time, no major changes occurred in *E. coli* from pork.
8. In bovine meat, levels of resistance are traditionally low with fluctuating percentages below 5% for most antimicrobials tested. In addition, *E. coli* obtained from imported beef showed similar low levels of resistance.

Resistance levels

Table Eco01 shows resistance levels, presented as MIC-distributions, of 1182 *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 2023. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1998 to 2023 are shown in Figures Eco01a-d and information on trends in multidrug resistance in the different animals sectors is shown in Figure Eco02.
Table Eco03 presents resistance percentages of 756 *E. coli* isolates collected from raw meat (chicken meat, pork and beef) at retail and import (pork and beef) in the Netherlands in 2023. Figures Eco03a-b shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2023 isolated from fresh meat at retail of chicken and bovine.

Trend-analysis is done for broilers, pigs, dairy and veal calves (including white and rose) to estimate the trend in resistance levels in the period since 2010 until now, and the recent 5 years (2019-2023). 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 Eco01a-b and Figures Eco03a-b.

Table Eco01. MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1182) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2023

E. coli N = 1,182	MIC (%) distribution mg/L																		R%	95% CI
	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							1.6	21.4	50.2	6.8		0.1	20.0						20.1	17.8 - 22.4
Cefotaxime					99.8					0.2									0.2	0.0 - 0.6
Ceftazidime					91.2	8.5	0.1		0.1		0.1								0.2	0.0 - 0.6
Gentamicin						71.3	25.4	1.6		0.2	0.2	1.4							1.7	1.0 - 2.6
Tetracycline								69.9	5.7	0.5	0.5	0.4	23.0						23.9	21.5 - 26.5
Sulfamethoxazole										46.5	25.8	4.5	0.1	0.2	0.2	0.1	22.7		23.1	20.7 - 25.6
Trimethoprim					36.8	40.4	5.4	0.4	0.2			16.8							17.0	14.9 - 19.3
Ciprofloxacin	81.8	10.1	0.3	0.8	3.6	2.1	0.3	0.2		0.3	0.5								7.8	6.3 - 9.5
Nalidixic acid									91.8	1.4	0.3	0.2	0.6	5.7					6.8	5.4 - 8.4
Chloramphenicol										87.7	5.2	0.3	0.7	6.2					7.1	5.7 - 8.7
Azithromycin*								3.4	48.0	45.2	2.5	0.1	0.5		0.3				0.9	0.5 - 1.7
Colistin							99.5	0.5											0.0	0.0 - 0.3
Meropenem		99.6	0.4																0.0	0.0 - 0.3
Tigecycline					92.9	7.0	0.1												0.1	0.0 - 0.5
Amikacin									97.9	2.1									0.0	0.0 - 0.3

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 substantially between the different animal species (Table Eco02). 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 was observed in isolates from dairy cattle. 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, colistin, gentamicin and tigecycline. Resistance for amikacin and meropenem was completely absent.

Table Eco02 Resistance percentages (R%) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2023

Faecal samples	Broilers	Pigs	Dairy	Veal calves	
	N = 289	N = 300	N = 295	White, N = 208	Rosé, N = 91
Ampicillin	34.6	18.3	1.7	33.2	8.8
Cefotaxime	0.3	0.0	0.0	0.5	0.0
Ceftazidime	0.3	0.0	0.0	0.5	0.0
Gentamicin	4.5	0.0	0.0	3.4	0.0
Tetracycline	19.0	29.3	3.7	54.8	16.5
Sulfamethoxazole	37.4	26.0	2.7	34.1	8.8
Trimethoprim	26.6	20.3	0.3	26.4	7.7
Ciprofloxacin	25.6	1.7	0.0	5.8	1.1
Nalidixic acid	23.9	1.3	0.0	3.4	0.0
Chloramphenicol	4.5	6.3	1.0	22.6	2.2
Azithromycin	0.3	1.7	0.0	1.9	1.1
Colistin	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0
Amikacin	0.0	0.0	0.0	0.0	0.0

Fluoroquinolone resistance

Fluoroquinolones (FQ) resistance in *E. coli* from broilers was still high, but decreased from 28.0% in 2022 to 25.6% in 2023 for ciprofloxacin (Table Eco02). In *E. coli* from other animal sectors FQ resistance was low or completely absent: 5.8% in white veal calves, 1.7% in pigs, 1.1% in rosé veal calves and undetected in dairy cattle.

Resistance to fluoroquinolones in *E. coli* isolated from meat was tested in 2023 for chicken meat collected at retail, as well as for *E. coli* from bovine meat (beef) and pig meat (pork) collected at retail and import (Table Eco03). Similar to the caecal *E. coli* isolates, levels of FQ resistance were high in chicken meat (25.6%) and low in fresh retail beef (3.0%) as well as in pork (0.8%). In imported meat, FQ resistance was equally low in beef (1.5%), but noticeably higher in pork (14.9%).

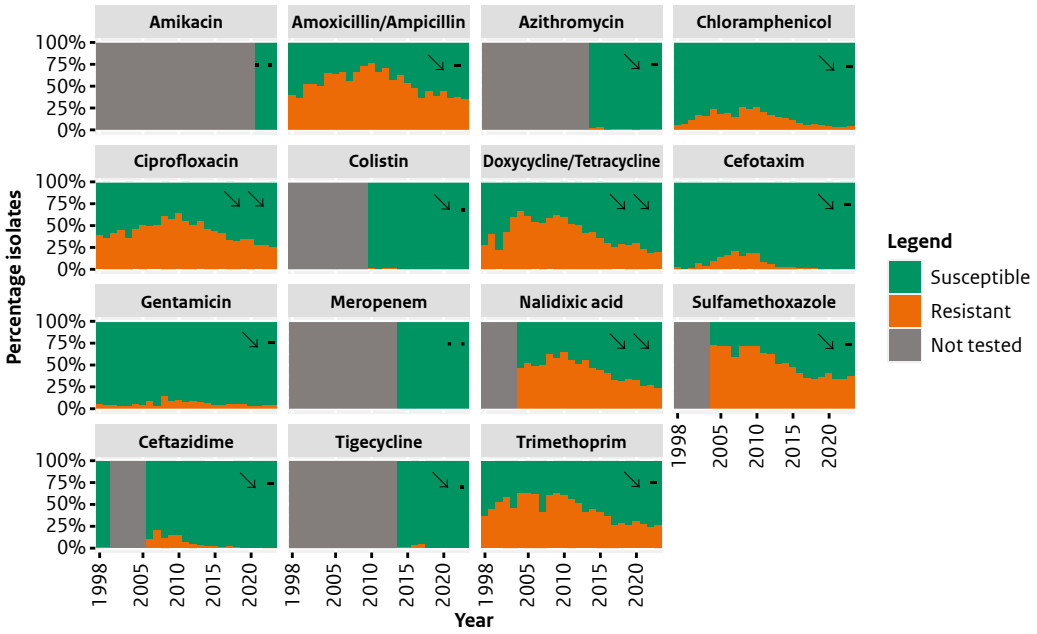
Resistance against extended-spectrum cephalosporins (cefotaxime and ceftazidime)

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 occasionally observed since 2019. In 2023, only two ESC-R *E. coli* were identified: one isolate originated from a broiler and the second isolate from a white veal calf. No ESC-resistant indicator *E. coli* were observed in randomly selected *E. coli* isolates from caecal samples of slaughter pigs, rosé veal calves and dairy cattle (Table Eco02). Amongst indicator *E. coli* obtained from meat, ESC-R was identified at low levels (0.5 – 2.2%) in alle types of meat sampled at retail and import (Table Eco03).

Broiler chickens

In 2023, resistance proportions of commensal *E. coli* isolated obtained from caecal samples of broiler chickens were similar to 2022 with relatively high levels of resistance were observed for ampicillin, (fluoro)quinolones (ciprofloxacin and nalidixic acid), sulfamethoxazole, trimethoprim and tetracycline (Figure Eco01a). Resistance to cefotaxime, ceftazidime, gentamicin and azithromycin remained low (less than 5%), where resistance to amikacin, colistin, meropenem and tigecycline was not detected amongst indicator *E. coli* (Table Eco02).

Figure Eco01a (broilers - faeces) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from faecal samples from broilers in the period 1998-2023 in the Netherlands.

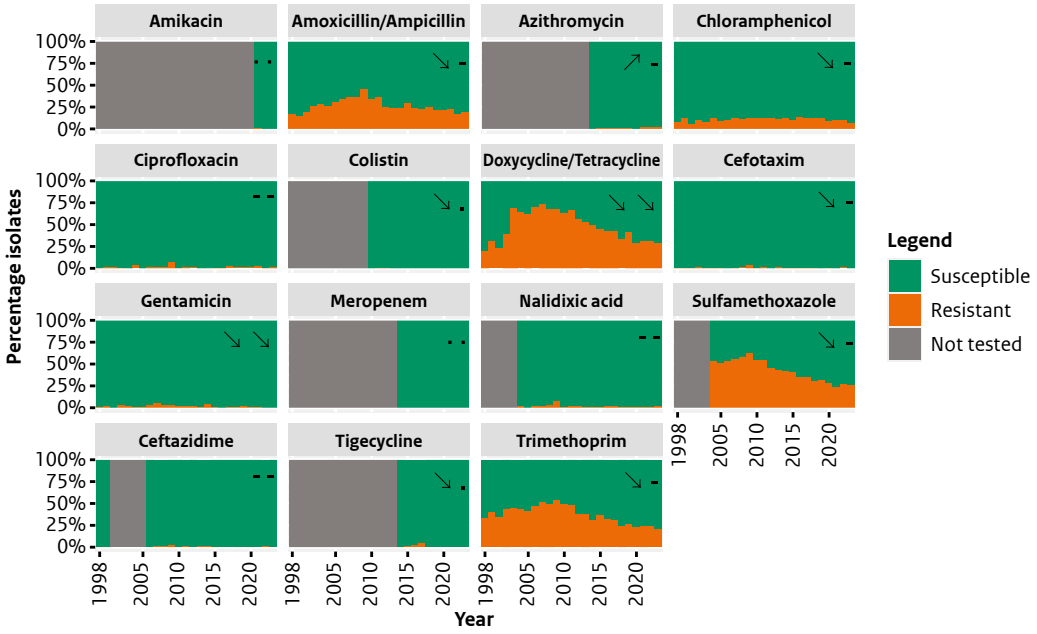


The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2023, the second symbol indicates the trend during the last 5 years (2019-2023), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing 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 first time since the start of the monitoring in 1998 (Table Eco02). The overall resistance proportion stabilised in slaughter pigs with some fluctuation in resistance between the different antibiotic classes (Figure Eco01b).
For sulfamethoxazole, tetracycline and trimethoprim resistance levels stabilised between 20% and 30% whereas resistance to ampicillin remained below 20% for the second year in row. Chloramphenicol resistance declined to 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.

Figure Eco01b (pigs - faeces) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from faecal samples from pigs in the period 1998-2023 in the Netherlands.

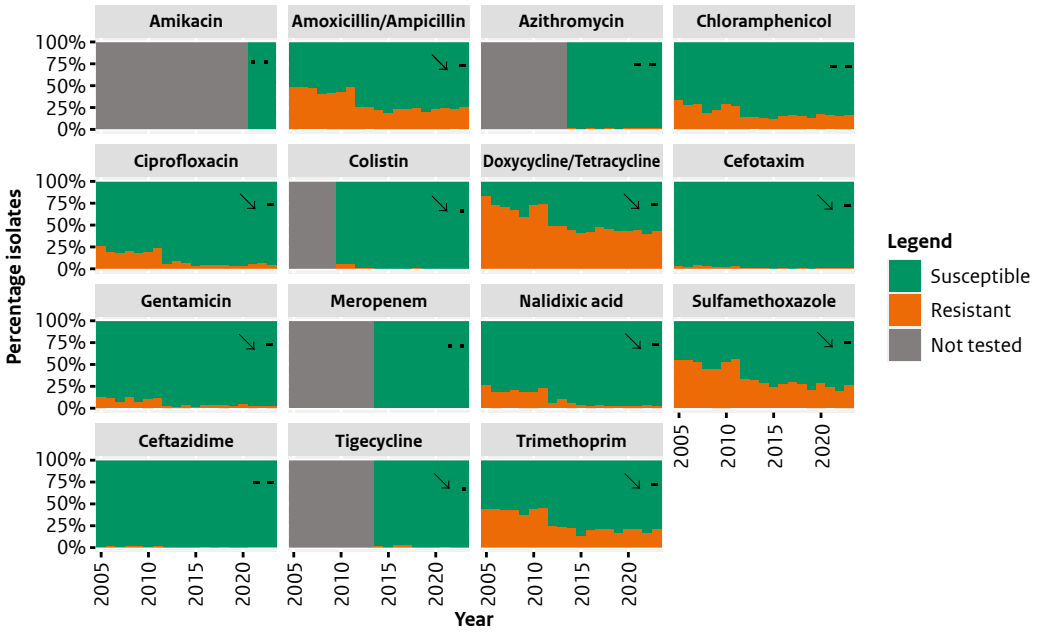


The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2023, the second symbol indicates the trend during the last 5 years (2019-2023), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing 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, because of the difference in production systems. As seen in previous years, substantially higher resistance levels were measured in isolates from white, compared to those from rosé veal calves (Table Eco02). Figure Eco01c 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 Eco01c) with large differences between the two husbandry types (Table Eco02). In 2023, resistance levels in veal calves slightly increased compared to 2022, for most antibiotic classes. In white veal calves, resistance was still high for ampicillin, tetracycline, sulfamethoxazole and trimethoprim ranging from 22.6%-54.8%. In rosé calves, resistance levels for these antibiotics were substantially lower ranging from 2.2%-16.5%. Resistance against cefotaxime, ceftazidime, gentamicin and nalidixic acid was only detected in white veal calves. In addition, no resistance was observed for amikacin, colistin, meropenem and tigecycline in both white and rosé veal calves. (TableEco02).

Figure Eco01c (veal calves - faeces) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from faecal samples from veal calves in the period 2005-2023 in the Netherlands.

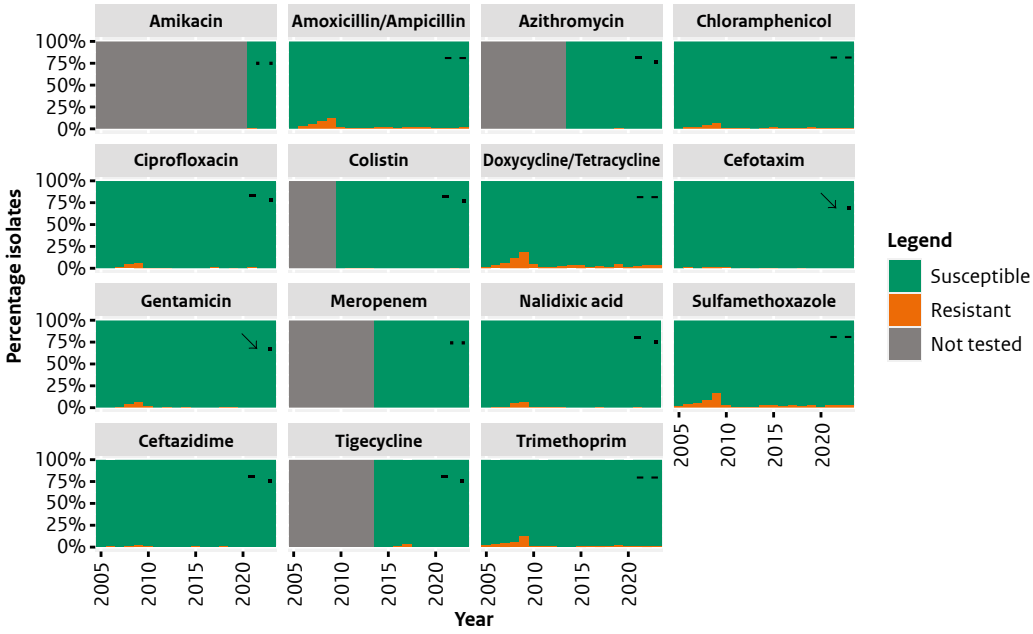


The symbols indicate thetrend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2023, the second symbol indicates the trend during the last 5 years (2019-2023), with ↗ indicating an increasing trend in resistant isolates, ↘ indicating a decreasing 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 slightly fluctuating but traditionally low compared to pigs, broilers and veal calves (Table Eco02), reflecting the low use of antibiotics in dairy farming. As in previous years resistance to the 3rd generation cephalosporins was not detected.

Figure Eco01d (dairy cows - faeces) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from faecal samples from dairy in the period 2005-2023 in the Netherlands.



The symbols indicate the trend inpercentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2010-2023, the second symbol indicates the trend during the last 5 years (2019-2023), 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.

Multidrug resistance

Data to determine multidrug resistance 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), fluoroquinolones (ciprofloxacin), phenicols (chloramphenicol), macrolides (azithromycin) and polymyxins (colistin). The data with the determined level of multidrug resistance over the years are shown in Figure Eco02.

In general, the level of multidrug resistance (showing resistance to three or more classes of antimicrobials) stabilised in the last five years. The level of multidrug resistance amongst *E. coli* in broilers (30.8%) was similar to the years 2021-2022. In pigs, a gradual long-term decrease resulted in in a level of multidrug resistance below 20%. In veal calves, the level of multidrug resistance is fluctuating in the last decade between 20.1%-28.1%. As in former years, multidrug resistance *E. coli* in dairy cattle was extremely low (1.4%) compared to the other animals species reflecting the low antibiotic use in this animal sector.
In ten years' time, proportions of complete susceptibility (pan-susceptible) *E. coli* isolates increased. As a result, the percentage of pan-susceptible isolates ranged from 42.9% (broilers) to 52.6% (pigs) and 54.8% in veal calves in 2023. Traditionally, the proportion of pan-susceptible isolates was very high in dairy cattle with 96.3%.

Figure Eco02 Percentage (%) of indicator *E. coli* isolates, isolated from faecal samples from broilers and pigs (1998-2023), dairy and veal calves (2005-2023), 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.

E. coli in raw-meat

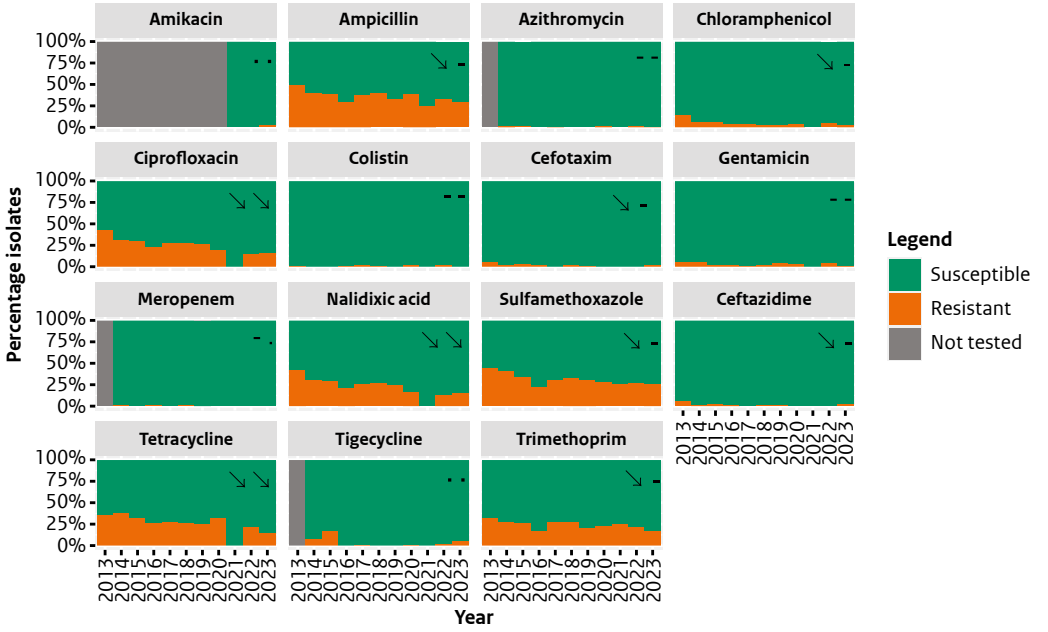
Table Eco03 presents resistance percentages of *E. coli* isolated from fresh chicken meat, bovine meat (beef) and pig meat (pork) sampled at retail as well as imported beef and pork collected at border control posts by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail comprises meat produced in The Netherlands, but also in other EU countries. Amongst *E. coli* isolates from retail meat, overall resistance levels were the highest in chicken meat, followed by pork. Whereas, resistance in *E. coli* isolates obtained from beef was traditionally low. Resistance to third generation cephalosporins was infrequently found in all types of meat samples included.

Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw meat in the Netherlands in 2023

Products	Chicken Fresh, retail N = 183	Pork Fresh, retail N = 129	Pork Fresh, import N = 94	Bovine Fresh, retail N = 165	Bovine Fresh, import N = 184
Ampicillin	29.0	17.1	24.5	4.8	0.5
Cefotaxime	2.2	0.8	2.1	0.6	0.5
Ceftazidime	1.6	0.0	2.1	0.0	0.0
Gentamicin	1.1	1.6	2.1	0.6	1.1
Tetracycline	14.8	23.3	21.3	4.2	4.3
Sulfamethoxazole	25.7	18.6	17.0	5.5	1.6
Trimethoprim	17.5	17.8	14.9	4.2	0.5
Ciprofloxacin	16.4	0.8	14.9	3.0	1.6
Nalidixic acid	15.3	0.0	9.6	2.4	0.5
Chloramphenicol	2.7	4.7	8.5	1.2	0.0
Azithromycin	0.5	0.0	1.1	0.6	0.0
Colistin	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	4.9	9.3	6.4	5.5	3.3
Amikacin	2.2	2.3	1.1	0.6	2.2

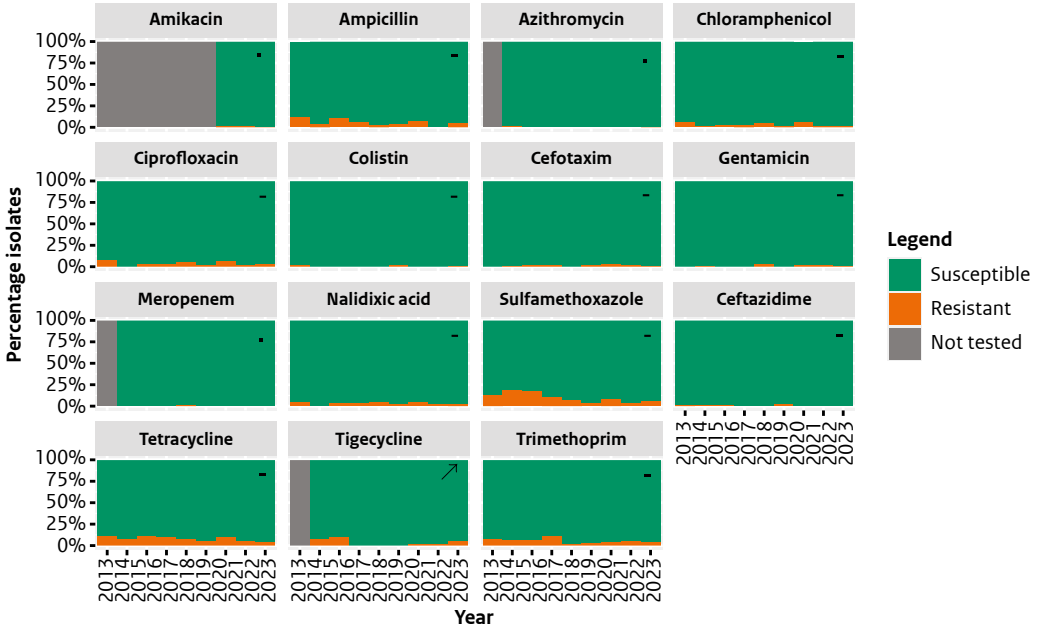
Figure Eco03a-b shows the trends in resistance of indicator *E. coli* from fresh chicken meat and beef collected at retail. In *E. coli* isolates from chicken meat, decreasing levels of resistance are observed with a tendency to flatten in the more recent years. Except for the lower FQ resistance in retail pork, resistance patterns in *E. coli* obtained from pork were comparable to broiler meat (Table Eco03) with the highest resistances monitored for ampicillin, trimethoprim, sulfamethoxazole and tetracycline. Over time, no major changes occurred in *E. coli* from pork. In bovine meat, levels of resistance are traditionally low with fluctuating percentages below 5% for most antimicrobials tested. In addition, *E. coli* obtained from imported beef showed similar low levels of resistance.

Figure Eco03a (chicken - meat retail) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from chicken retail meat in the period 2013-2023 in the Netherlands.



The symbols indicate the trend in percentage of resistant isolates per antibiotic, the first symbol indicates the trend during the period 2013-2023, the second symbol indicates the trend during the last 5 years (2019-2023), 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 Eco03b (beef - meat retail) Percentage (%) of resistant indicator *E. coli* isolates against different antibiotics, isolated from beef retail meat in the period 2013-2023 in the Netherlands.



The symbols indicate the trend in percentage of resistant isolates per antibiotic during the period 2019-2023, 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.

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.

3 European Food Safety A, European Centre for Disease P, Control. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA J. Mar 2020;18(3):e06007.

4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae and MRSA in food-producing animals and meat in the Netherlands in 2023

In this chapter, monitoring of organisms of specific interest is described, either due to their resistance mechanism or their potential to spread between the Dutch livestock and human populations. These include various species and resistance against antimicrobials which are classified by the World Health Organisation (WHO) List of Medically Important Antimicrobials[1], as 'highest priority critically important antimicrobial': Gram-negative bacteria which are resistant to third and fourth generation cephalosporins, carbapenems or colistin. Furthermore, the monitoring of the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock and food is described here.

Highlights

1. As in previous years, ESC-resistant *E. coli* were rarely detected (<1%) amongst randomly selected *E. coli*.
2. In dairy cattle, the prevalence of selectively isolated ESC-resistant *E. coli* significantly increased over the past 5 years from 10.3% in 2019 to 17.9% in 2023.
3. A significant increase in the prevalence of selectively isolated ESC-resistant *E. coli* was also observed in broilers over the past 4 years. This is partially attributed to a change in the sampling method.
4. In comparison to domestically produced pork, imported pork products have a higher ESC-resistant *E. coli* prevalence (5.1% vs 0.3%), while no ESC-resistant *E. coli* was detected in imported beef.
5. Whole Genome Sequencing of ESC-resistant *E. coli* showed that genetic clustering is mostly seen within linked production sectors, although some spill-over appears to occur.
6. The prevalence of ESBL-producing *Salmonella* isolated from humans was low (1.4%). No, ESBL-producing *Salmonella* isolates were identified in samples from livestock and meat.
7. In 2023, no carbapenemase-producing Enterobacteriaceae were detected in samples from livestock, companion animals and meat.
8. The prevalence of *mcr* encoding *E. coli* was low in livestock and meat.
9. In 2023, 4.5% of the investigated sheep farms was tested positive for MRSA.

4.1 ESC-resistant Enterobacteriaceae

Third and fourth generation cephalosporins or 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 Enterobacteriaceae 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 livestock and meat. Since the implementation of whole genome sequencing (WGS) in EU legislation(2020/1729/EU) in 2021, all ESC-resistant *E. coli* from livestock and meat are analysed by WGS and compared over the years at the end of this section.

4.1.1 Randomly isolated ESC-resistant *E. coli* from livestock

Random isolation of commensal *E. coli* from caecal samples of broilers, slaughter pigs, veal calves and dairy cows is described in Chapter 3. 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 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].

Figure ESBL01 shows the trends of randomly isolated ESC-resistant *E. coli* from 1998 until 2023. Over the past 15 years, ESC resistance has reduced to a level where in 2019, no randomly isolated ESC-resistant *E. coli* had been detected. Since then, 2 to 5 ESC-resistant isolates have been detected each year in all livestock samples combined. Table ESBL01 includes the number of isolates for each group of animals, and the results of WGS analysis. In 2023, one randomly selected *E. coli* was isolated from a veal calf that contains the CTX-M-32 gene. Furthermore, one *E. coli* was isolated from broiler chickens that contains the CTX-M-55 gene.

Figure ESBL01 Trends in ESC resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows

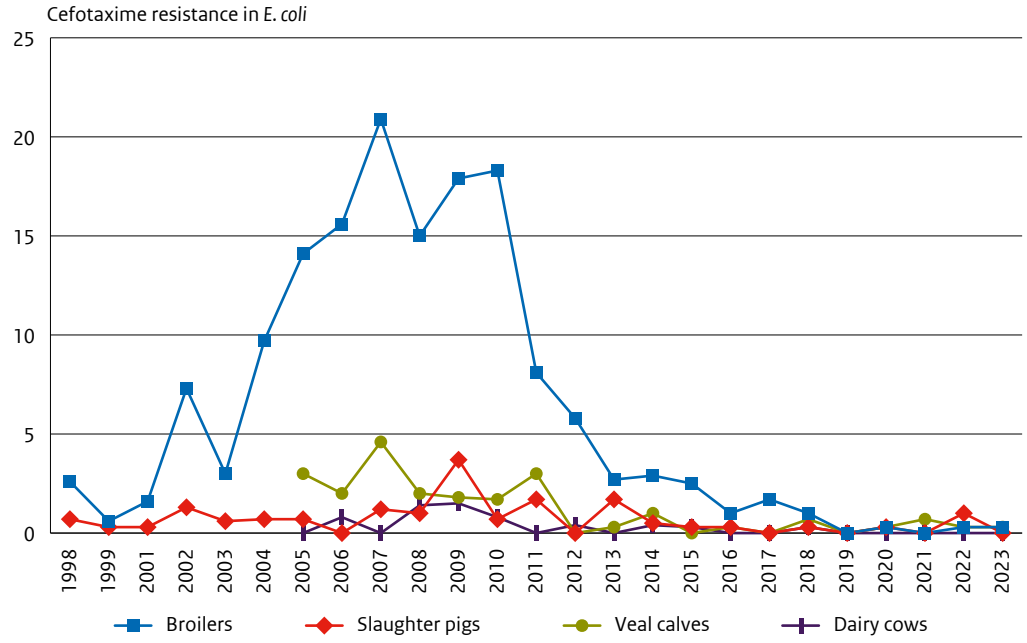

Table ESBL01 ESBL/pAmpC-genes and chromosomal AmpC mutations found in *E. coli* isolates with reduced susceptibility to ESC derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2023

ESBLs isolated from							ESBL-genes detected											
Year	[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-52c	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
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
Total	**277**	**34**	**35**	**8**	**6**	**360**	**142**	**19**	**3**	**40**	**2**	**45**	**12**	**44**	**24**	**31**	**19,921**	**1.8**

a All were bla$_{CTX-M-1}$, only in 2011 one bla$_{CTX-M-3}$ gene was found in an isolate from a veal calf.
b One combination of bla$_{SHV-12}$ together with bla$_{TEM-52}$ occured in 2012 in one broiler isolate.
c In broilers, three combinations were found: in 2008: bla$_{CTX-M-1}$ with bla$_{CTX-M-2}$; in 2009: bla$_{CTX-M-1}$ with bla$_{SHV-12}$ and bla$_{CTX-M-1}$ with bla$_{SHV-12}$ and bla$_{CMY-2}$.
d In dairy cows, one combination of bla$_{CMY-42}$ with bla$_{TEM-190}$.
n.t.: not tested

4.1.2 Selectively isolated ESC-resistant *E. coli* from livestock and food products

The randomly selected ESC-resistant *E. coli* described above provide insight into the total prevalence of ESC-resistance in the livestock 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 (EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal samples, version 7, December 2019: https://www.eurl-ar.eu/protocols.aspx).

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 (EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from meat samples, version 7, December 2019: https://www.eurl-ar.eu/protocols.aspx).

Putative 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 3 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 4.1.1.

Results of selective isolation and molecular typing of ESC-resistant *E. coli* from livestock

The selective isolation of ESC-resistant *E. coli* has an increased sensitivity and is expected to result in a higher prevalence than the randomly selected ESC-resistant *E. coli* described in 4.1.1. Table ESBL02 shows the number of suspected ESC-resistant *E. coli* that were isolated in 2023 and the number of isolates that were confirmed via WGS as ESBL/pAmpC producing *E. coli* or AmpC promotor mutants. The trends over time of confirmed ESBL/pAmpC producing *E. coli* or AmpC promotor mutants are depicted in Figure ESBL02. The results of the molecular typing per livestock category are presented in Table ESBL03. The results of this molecular typing of ESC-resistance have previously been used to assess the relative attribution of livestock species and the environment to ESC-resistance in the human population[5].

A steep decline of ESC-resistant *E. coli* was realized in **broiler chickens** between 2014 and 2020 from 66.8% to 10.1% of the tested samples that were positive for ESBL/pAmpC *E. coli* or AmpC-mutants. 2021 was the first year in which this decline had halted, and in 2022 a new method was introduced based on a change in the EU legislation, in which the caecal contents of 10 broilers are pooled, instead of testing the contents of a single animal. While this change was hypothesized to be responsible for the increase in prevalence to 15.7% in 2022, a further increase to 20.4% was detected in 2023. Analysis was performed on the prevalence data using a generalized linear model to assess if this increase is statistically significant. The model does not show a significant increase over the past 5 years, but over the past 4 years there has been an increase. These differences in the outcome is attributed to the fact that there was still a reduction seen in the prevalence between 2019 and 2020, after which the prevalence has been increasing again.

As in previous years, only 2 of the 59 ESC-resistant isolates were AmpC promotor mutants. A decade ago, CMY-2 and CTX-M-1 were the most prevalent ESBL/pAmpC genes in broilers, but CMY-2 was now found in only 4 isolates (6.8% of ESC-resistant *E. coli*). CTX-M-1 and SHV-12 were the most prevalently isolated genes in 2023 at 25.4% and 22.0% respectively. For several years, CTX-M-15 and CTX-M-55 have been present at approximately 10% of the population of ESC-resistant *E. coli*.

The prevalence of ESC-resistant *E. coli* in **slaughter pigs** has been stable over the past few years between 15% and 20% of samples being positive for ESC-resistant *E. coli.* CTX-M-1 is the most prevalent plasmid encoded ESBL gene, detected in 30.4% of the ESC-resistant *E. coli.* It is currently unknown why AmpC promotor mutants are most prevalent in pigs, but this has been seen over the past decade and in 2023 these were present in 41.3% of ESC-resistant *E. coli.* In addition, two novel AmpC mutants were detected, noted in Table ESBL03 as related to EC-18. AmpC mutations in the gene rather than the promotor have been described in literature to be responsible for ESC-resistance but these are currently not detected by Resfinder[6].

Both in **rosé and white veal calves**, a rise in the ESC-resistant *E. coli* was measured between 2015 and 2016. Further studies into factors that may drive the persistence of ESC-resistant *E. coli* in veal farms are ongoing but so far have not been able to identify factors that contribute here[7]. Since 2015, the prevalence in rosé veal calves has fluctuated quite a lot between 11.0% and 29.3%, while in 2023 the prevalence was 23.9%. A part of these fluctuations may be caused by the fact that the rosé veal sector is relatively smaller in size and is therefore represented by a lower number of samples than the white veal calves and other sectors. Over the past decade, CTX-M-1 and CTX-M-15 have been the most prevalent ESBL genes in rosé veal calves, and in 2023 these were present in 27.3% and 40.9% respectively of the ESC-resistant *E. coli.* In white veal calves, the rise in prevalence of ESC-resistant *E. coli* continued until 2018 at which point 47.6% of samples were positive. Since then, the lowest prevalence was 30.9% in 2022, but rose again to 38% in 2023. As for the rosé veal calves, in white veal CTX-M-1 and CTX-M-15 have been most prevalent over time, and these were 22.9% and 20.5% of ESC-resistant *E. coli* in 2023. Over the past 6 years, CTX-M-32 has increased from 1.9% in 2018 to 21.7% of ESC-resistant *E. coli* in 2023.

Until 2022, **dairy cattle** was the sector in which the ESC-resistant prevalence was the lowest of all livestock sectors. In 2022, 18.3% of the samples was positive, which was 17.9% in 2023. The same linear model described above for broiler chickens was used to assess the prevalence data over the past 5 years. This shows that there has been a significant increase compared to previous years. CTX-M-1 and the AmpC promotor mutants were the most commonly detected mechanisms and the absolute numbers of detection for these mechanisms has been relatively stable. The current increase in prevalence of ESC-resistant *E. coli* is due to a rise in CTX-M-15, which was not detected in 2014 and first detected as 9.1% of the ESC-resistant *E. coli* population in 2015. Since then, this mechanism is present in 39.6% of ESC-resistant *E. coli* from dairy cattle in 2023.

A novel seasonal analysis approach using the data on the selective culture method for ESC-resistant *E. coli* in the Netherlands from 2014-2022 has re-examined the data for broiler chickens, slaughter pigs, dairy cattle and veal calves. This analysis indicates an increase in the prevalence of ESC-resistant *E. coli* in the summer and autumn seasons compared to winter and spring in dairy cattle and veal calves, but not in broiler chickens and slaughter pigs (Brouwer *et al.* accepted for publication at Science in One Health). The reason for the differences between livestock sectors, and the mechanism that drives the increase in summer and autumn have not been studied yet.

In 2023, an in-depth study by RIVM and WFSR into potentially zoonotic bacteria has also analysed multiple manure samples from **sheep** farms. Out of 159 of the tested farms, ESC-resistant *E. coli* were detected on 24 of the farms (15.1%). A total of 39 isolates, 1 to 4 ESC-resistant *E. coli* isolates per farm were selected for confirmation by WGS. CTX-M-15 was the most prevalent gene detected in 23 of the 39 isolates (59%), followed by SHV-12 (n=5, 12.8%) and AmpC promotor mutants (n=4, 10.2%) while other genes were all detected in less than 10% of the ESC-resistant isolates.

Table ESBL02 Proportion of *E. coli* isolates showing resistance to ESC derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves, and dairy cows collected in 2023

	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	290	59	20.3	57	19.7	2	0.7
Pigs	300	46	15.3	25	8.3	21	7.0
Veal calves white	208	83	39.9	79	38.0	4	1.9
Veal calves rosé	92	22	23.9	22	23.9	0	0.0
Dairy cows	295	53	18.0	47	15.9	6	2.0
Total	**1185**	**263**	**22.2**	**230**	**19.4**	**33**	**2.8**

Figure ESBL02 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 2014-2022 determined by using selective isolation

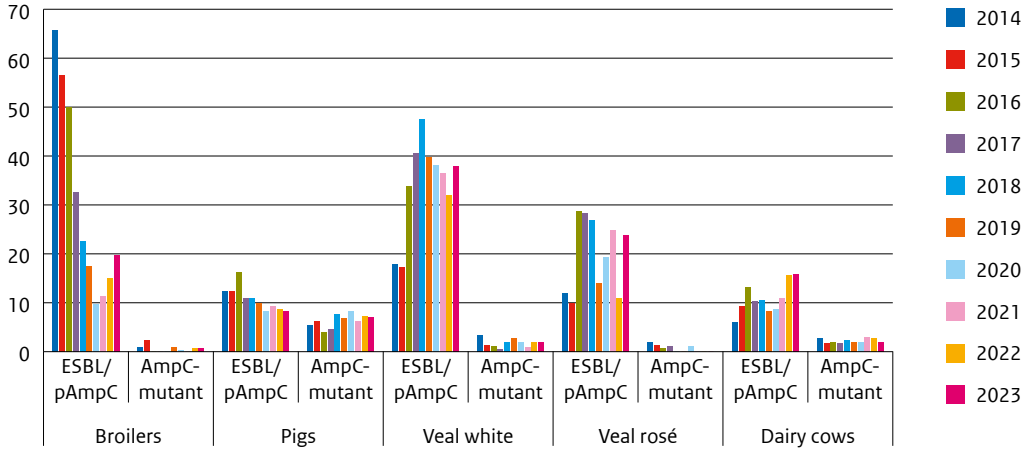

Table ESBL03 Beta-lactamases identified in *E. coli* derived from selective culturing of faecal samples of broilers, slaughter pigs, veal calves and dairy cows in 2023

		Broilers	Slaughter pigs	Veal calves		Dairy cows	Total
				White	Rosé		
CTX-M-1 group	CTX-M-1	15	14	19	6	8	62
	CTX-M-1var			1			1
	CTX-M-15	7	2	17	9	21	56
	CTX-M-32	2	3	18	2	1	26
	CTX-M-55	[6]	1	6	2	2	17
CTX-M-2 group	CTX-M-2	1	1	1			3
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						0
	CTX-M-14		1	1		2	4
	CTX-M-27				1	1	2
	CTX-M-65			1		2	3
TEM	TEM-52B	5				1	6
	TEM-52Bvar		1				1
	TEM-52C	[4]		5	1		10
SHV	SHV-12	13	1	6	1	5	26
CMY	CMY-2	4	1	2		3	10
DHA	DHA-1					1	1
Chromosomal *ampC*	-42 C>T	2	19	4		6	31
	EC-18*		2				2
Total		**59**	**46**	**83**	**22**	**53**	**263**

In 2 isolates from pigs, a novel mutation in the ampC-gene is expected to be resposible for the cefotaxime resistant phenotype.
In 1 isolates from pigs, a novel allele of TEM-52B was detected.
In 1 isolates from veal, a novel allele of CTX-M-1 was detected.

Results of selective isolation and molecular typing of ESC-resistant *E. coli* in fresh meat and vegetables

Selective isolation of ESC-resistant *E. coli* was performed from food for human consumption, including samples from fresh meat, poultry, fish and fresh herbs from domestic produce and imported goods from outside the EU. Similar to the selective isolation of ESC-resistant *E. coli* from livestock, described above, the selective isolation is more sensitive than the non-selective isolation method described in chapter 3.

The number of detected ESC-resistant *E. coli*, the number of confirmed ESBL/pAmpC-producing *E. coli* and the number of *E. coli* containing a chromosomal AmpC promotor mutation are included in Table ESBL04. A comparison of the prevalence of these *E. coli* in comparison to previous years is shown in Figure ESBL03 for the categories of food samples of which sufficient numbers were tested through the years. Similarly as described for the samples from livestock above, for these samples from meat, AmpC-promoter mutants were indicated separately in the figure. These results indicate that AmpC-promoter mutants are detected in much lower frequency from meat samples than from livestock samples. The results of the WGS that was performed to confirm the genotype of the isolates are described in Table ESBL05. Further samples are included in this table for ESC-resistant *E. coli* that were isolated from smaller numbers of samples, ESC-resistant *E. coli* that were isolated through non-selective culturing, and products imported from outside of the EU.

Table ESBL04 Prevalence of ESC-resistant *E. coli* isolates from raw meat, poultry, aquaculture and fresh herbs in the Netherlands in 2023

Animal source	N screened	N ESC-R suspected	N ESC-R confirmed	% ESBL/ pAmpC positive	N AmpC promotor mutants	% AmpC promotor mutants
Beef	453	6	6	1.3	0	0.0
Veal	184	5	5	2.7	0	0.0
Pork	291	1	1	0.3	0	0.0
Chicken	185	27	25	13.5	1	0.5
Turkey	7	0	0	0.0	0	0.0
Imported pork	99	5	5	5.1	0	0.0
Imported beef	228	0	0	0.0	0	0.0
Imported aquaculture/ wild caught fish	330	8	8	2.4	0	0.0
Imported exotic meat	22	0	0	0.0	0	0.0
Fresh herbs	55	2	0	0.0	0	0.0
Total	**1,799**	**52**	**50**	**2.8**	**1**	**0.1**

Figure ESBL03 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 2014-2023 determined by using selective isolation

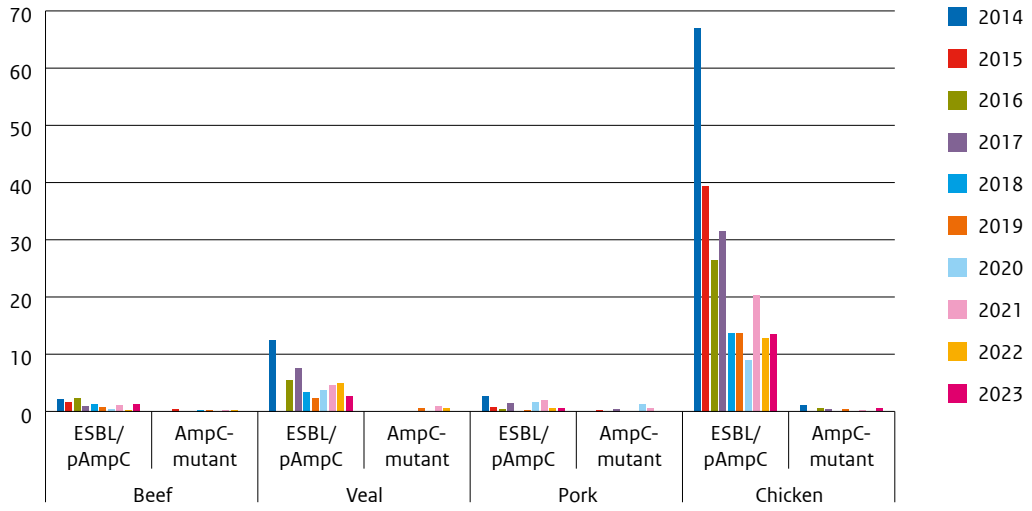

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products and aquaculture in the Netherlands in 2023

	Beta-lactamase gene	Chicken	Pork	Beef	Veal	Imported pork	Imported aquaculture	Total
CTX-M-1 group	CTX-M-1	8	1	4	2	2		17
	CTX-M-15				2	4	3	9
	CTX-M-32			1				1
	CTX-M-55	2					1	3
	CTX-M-55; CMY-2						1	1
CTX-M-8 group	CTX-M-8				1			1
CTX-M-9 group	CTX-M-14					2		2
	CTX-M-27			1			1	2
TEM	TEM-52B	2						2
	TEM-52C	1						1
SHV	SHV-12	11						11
CMY	CMY-2	1						1
DHA	DHA-1						2	2
Chromosomal *ampC*	-42 C>T	1						1
Total		**26**	**1**	**6**	**5**	**8**	**8**	**54**

** These genes do not show 100% identity to previously described genes.*

In **beef**, the prevalence of ESC-resistant *E. coli* was 1.3% of the analyzed samples in 2023, which is within the expected range based on the data since 2014. Four out of six isolates were CTX-M-1, which is the most prevalent ESBL gene in beef.

One ESC-resistant *E. coli* containing CTX-M-1 was detected in **pork**, resulting in 0.6% prevalence in 2023, the same as 2022.

Chicken meat has the highest prevalence of ESC-resistant *E. coli*, but just as for the sampling at slaughterhouses, the prevalence on the meat has greatly decreased since 2014. In 2023, 26 isolates were confirmed, from 14% of the samples. Similarly to the samples from slaughterhouses, CMY-2 was most prevalent in 2014 but was only found in 1 isolate in 2023. SHV-12 and CTX-M-1 are the most prevalent genes found in ESC-resistant *E. coli* from chicken in 2023.

No ESC-resistant *E. coli* were detected in **turkey**, but since these only represented 7 samples in 2023, it is impossible to draw any conclusions on the real prevalence.

The prevalence of ESC-resistant *E. coli* in **veal** was 2.7% in 2023, somewhat lower than in previous years. The ESBL genes detected in the 5 isolates include CTX-M-1, CTX-M-15 and CTX-M-8.

In line with the new regulations on testing of imported meat in EU legislation (2020/1729/EU), **imported pork** and **imported beef** were examined in 2023. Imported pork originated from Chili, UK and Australia and ESC-resistant *E. coli* was cultured from 5.1% of the samples. A total of 8 *E. coli* isolates were cultured from 5 individual samples, of which 4 contained CTX-M-15, 2 contained CTX-M-1 and 2 contained CTX-M-14. No ESC-resistant *E. coli* was isolated from imported beef, originating from Argentina, Australia, Brazil, Canada, Japan, Namibia, New-Zealand, Paraguay, USA, Uruguay and UK.

From **imported aquaculture and wild caught fish**, 2.4% of samples were positive for ESC-resistant *E. coli* from tilapia, shrimp and Nile perch, originating from India, Kenya and Vietnam. These isolates included a large variety of ESBL/pAmpC genes, including CTX-M-1, CTX-M-15, CTX-M-27, CTX-M-55, CMY-2 and DHA-1. The origin of imported shrimp is mainly from India, Vietnam, Nigeria, and Ecuador, Pangasius almost all from Vietnam and Tilapia originates for almost for 90% from China, Vietnam and Indonesia.

In samples of **fresh herbs**, two suspected batches were reported but subsequent testing did not confirm these as ESBL/AmpC producing *E. coli.* Furthermore, in samples of **imported exotic meat**, no ESC-resistant *E. coli* were detected.

Results of genomic comparisons of ESC-resistant *E. coli* from food and livestock

Since 2021, all ESC-resistant *E. coli* isolated from food and livestock 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[8]. Only clusters containing more than 4 isolates, of which at least 1 isolate was collected in 2023, 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[9].

A total of 1156 *E. coli* genomes from 2021, 2022 and 2023 were compared, from which 164 genomes were shown to be part of 22 clonal lineages. The AmpC chromosomal promotor mutants were detected in 3 clones (total 17 isolates), of which 2 clones were only detected in pigs and one detected in dairy cattle, white veal and sheep.

The ESBL-gene TEM-52 was found in 1 clone (4 isolates), in white veal calves and broilers. The ESBL-gene SHV-12 was found in 3 clones (total 22 isolates), 1 detected in broilers and pigs, 1 detected in broilers and white veal, and 1 detected in white veal, rosé veal, dairy cattle and sheep. The ESBL-gene CTX-M-1 was found in 3 clones (total of 17 isolates), of which 1 was detected in broilers, white veal, dairy cattle, pork and beef. 1 was detected in broilers and chicken meat, and 1 was detected in broilers, rosé veal and chicken meat. The ESBL-gene CTX-M-55 was found in 2 clones (total of 10 isolates), of which 1 was detected in broilers, white veal, dairy cattle, chicken meat and lamb meat, and the other in broilers, pigs and dairy cattle.

The ESBL-gene CTX-M-15 was found in 8 clones (total 62 isolates), of which the majority of isolates of all clones were detected in dairy cattle, rosé or white veal (total 50 isolates). These clones were sporadically detected in samples from broilers, sheep, pork and chicken meat. Through the years, some increase of these isolates was present (respectively 16, 21, and 25 isolates).

The ESBL-gene CTX-M-32 was found in 2 clones (total 32 isolates). Clone ST398 (4 isolates) was detected in white veal and broilers (respectively 1, 2 and 1 isolates over the years). Clone ST14919 was detected in white veal (n=24), rosé veal (n=2), dairy cattle (n=1) and pig (n=1). In 2021, the clone was detected in 3 isolates, followed by 6 isolates in 2022, and 19 isolates in 2023.

Analysis of the WGS data shows that the clusters that are detected are mostly linked within a specific production sector, with few isolates detected in other production sectors, similar to previous years. The highly related clones described above containing CTX-M-15 and CTX-M-32 are of specific concern because they appear to be increasing over time. These isolates are present mostly in the veal and dairy sectors, in which an increase in prevalence was also detected. As the isolates included in the WGS analysis all represent an individual flock of animals, an increased detection of highly related clones over time indicates that these bacteria are spreading throughout the production chain. In-depth analysis will be performed to determine which co-selection mechanisms could contribute to this transmission.

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 paragraph 3.1.1 for a full description.

Table ESBL06 indicates the resistance genes per serovar that were detected in *Salmonella* isolates collected in 2023 using WGS. In Table ESBL07, the beta-lactamase genes are indicated at group level per year in order to make comparisons of the resistance mechanisms that are present over time.

In 2023, all ESC-resistant *Salmonella* isolates that were analyzed originated from human samples. As seen in previous years, genes from the CTX-M-1 group (CTX-M-55 and CTX-M-15) and the CTX-M-9 group (CTX-M-65) were amongst the most prevalent genes detected.

Table ESBL06 Beta-lactamases identified in *Salmonella* isolates in 2023 (n=20, all human)

Serovar	CTX-M-15	CTX-M-55	CTX-M-8	CTX-M-14b	CTX-M-27	CTX-M-65	SHV-12	CMY-2	TEM-52b	DHA-12	Total
Agona	1										1
Anatum							1				1
Apeyeme		1									1
Braenderup	1										1
Enteritidis			1						1	1	3
Give		1									1
Heidelberg		1									1
Infantis						2					2
Kentucky		1		1	1						3
Muenster		1									1
Schwarzengrund		2									2
Typhimurium		1						1			2
Typhimurium (monophasic)								1			1
Total	**2**	**8**	**1**	**1**	**1**	**2**	**1**	**2**	**1**	**1**	**20**

Table ESBL07 Beta-lactamases identified in *Salmonella* isolates collected in 2007-2023

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	1,514	3.1
2008	25	12		1	1	13	1		6	2			61	2,149	2.8
2009	12	4			2	3		1	9				31	2,232	1.4
2010	8	3			1	2		3	4				21	1,715	1.2
2011	5	3			1	1		2	13				25	1,444	1.7
2012	14	5			2	2			10	1			34	1,795	1.9
2013	1	3		5	4	5	1		36				55	1,369	4.0
2014	6			2	3	1			21				33	1,688	2.0
2015	13	2			6	1			12				34	1,761	1.9
[f]2016	7				15	2			10		1		36	2,117	1.7
[g]2017	3				23			1	3		1		31	1,697	1.8
[g]2018	2		1	1	8				2				14	1,718	0.8
2019	4				11			1	3				19	1,880	1.0
2020					4				2				6	1,310	0.5
2021	2				5			1	2				10	1,264	0.8
[h]2022	4	2		1	6			2	32				47	1,503	3.1
2023	10			1	4	1		1	2			1	20	2,139	0.9
Total	**125**	**47**	**1**	**11**	**96**	**48**	**4**	**16**	**169**	**3**	**2**	**1**	**524**	**29,295**	**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.

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

Passive screening

Based on the outcomes of the 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. No meropenem resistant isolates were detected amongst these bacteria in 2023.

Active screening

To screen for the presence of Carbapenemase producing *Enterobacteriaceae* (CPE), faecal samples of livestock are cultured overnight in BPW (1 gram sample in 9 ml BPW) and cultured the next day on two chromogenic agar plates (ChromID CARBA and ChromID OXA, Biomerieux). After incubation, plates are inspected visually for growth of CPE suspected colonies and identified by MALDI-TOF. In 2023, no CPE were identified using this culture method.

To enhance the sensitivity of the screening, all 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. Samples were grown overnight in Buffered Peptone Water (BPW) with 0.25 mg/L ertapenem and 50 mg/L vancomycin. After incubation, five individual samples were pooled in lysis buffer, next DNA was isolated with a bead method. A multiplex Real-Time PCR (In house) that can detect the most prevalent carbapenemase gene families (KPC, NDM, VIM, IMP and OXA-48) was used. PCR screening was extended with 2 additional carbapenem resistance genes (IMI and FRI) which are more frequently found in *Enterobacter cloacae* complex isolates obtained from imported seafood. Recently, an Enterobacter isolate carrying an IMI gene was found in a feed mill in Sweden[10].
If Real-Time PCR gave suspicious or positive results, a step-wise analysis was performed to confirm the results:

1. Singleplex Real-Time PCR-tests were performed on purified DNA of the 5 individual samples of the pool;
2. If PCR was positive, the original faecal sample and corresponding broth culture of suspected positive samples were inoculated for bacterial isolation on commercial selective plates (ChromID CARBA and ChromID OXA, Biomerieux, for *Enterobacteriaceae*) and on HIS plates with 0.125 mg/L ertapenem (selective for *Shewanella* spp);
3. DNA was isolated from bacterial isolates grown on the selective agar plates, Real-Time PCR was repeated and genes were confirmed with Sanger sequencing.

The screening of 1185 faecal samples (collected from 290 broilers, 300 slaughter pigs, 300 veal calves and 295 dairy cattle) resulted in seven OXA-48-like positive faecal samples (0.6%) which is similar to 2021 with 0.8% and 2022 with 0.9% OXA-48-like positive samples, respectively. Positive samples were only of bovine origin: veal calves (n=3) and dairy cattle (n=4). In four samples the presence of OXA-48-like carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: OXA-181 (n=2), OXA-252 (n=1); OXA-547 (n=1). Three samples were tested positive for OXA-48 group in the enrichment broth with PCR, but culturing of *Shewanella* was negative. 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[11], these genes

were considered of environmental origin and not a public health risk. PCR screening for the remaining six carbapenem resistance genes was negative for all samples. Most importantly, no carbapenemase-producing *Enterobacteriaceae* were detected in faecal samples from livestock in the Netherlands in 2023. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2024.

4.2.2 Monitoring in companion animals

Carbapenemase producing *Enterobacteriaceae* (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[12,13], Spain[14], France[15], UK[16], Portugal[17] and Switzerland[18]. 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 sample from a dog, 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,* harboring 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, 106 faecal samples from dogs and 110 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.

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 (BioMerieux). 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 (BioMerieux). In addition, total DNA of the enrichment broth was isolated for molecular screening by PCR for the targets NDM[19], KPC[20], IMP[21], VIM[21], OXA-group-23, -24, -51, -58[22] and OXA-group-48[23].

All samples were tested negative with selective culturing, but in three faecal samples of a dogs were tested positive for OXA-10 with PCR. However, OXA-10 enzymes belong to class D β-lactamases and lack carbapenemase activity. These genes are mainly found in Gram-negative bacteria that do not belong to the order *Enterobacterales*. Since, bacterial culturing was negative this could not be confirmed. As a consequence, these samples were considered negative for CPE. In conclusion, no CPE-suspected isolates were found in the other 106 faecal dog samples nor in 110 faecal cat samples. These outcomes 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 2024.

4.2.3 Monitoring in imported seafood, seaweed and herbs

In 2023, 268 batches of frozen fish originating from fish farms in South-East Asia and Africa as well as 62 batches of shrimps, mainly from farms in Africa and Asia, were screened for the presence of CPE by WFSR through selective culturing. In addition, 58 batches of imported herbs were screened for the presence of CPE. In four of these analysed seafood samples CPE were detected. These isolates were further analyzed for the presence of carbapenems genes by Whole genome sequencing. In two *Enterobacter cloacae* complex isolates NDM-1 was identified, next to one *Enterobacter cloacae* complex isolate with FRI, and for the first time NDM-1 was identified in one *Escherichia coli* isolate. Our findings confirm earlier reports on the presence of CPE in imported seafood in the Netherlands[24,25] 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 monitoring of imported food will be continued in 2024.

4.3 Colistin resistance

In 2023, active screening for the presence of *mcr*-genes in caecal samples was continued using selective culturing and PCR. For this purpose, purified DNA of pooled BPW + 2mg/L colistine cultures (five samples per pool) from a total of 1185 faecal samples of Dutch livestock were tested with 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 (https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/396_*mcr*-multiplex-pcr-protocol-v3-feb18.pdf). 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 were tested, followed by direct culturing of the original BPW broth on MacConkey agar with 2 mg/L colistin.

As a result of the screening, *mcr*-1 positive *E. coli* were identified in three samples all obtained from broilers (1.0%). In addition, *mcr*-9.1 was detected in one *E. coli* isolate obtained from a white veal calf. No colistin resistant indicator *E. coli* were identified in livestock and meat.

4.4 MRSA

MRSA surveillance in livestock and humans

MRSA causes healthcare- and community-associated infections and asymptomatic carriage in humans. During the last two decades, MLST clonal complex (CC) 398 has emerged in livestock and persons in contact with livestock in many countries, including The Netherlands. This type 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[26]. During the last decade, however, the number of persons colonized or infected with LA-MRSA in The Netherlands who did not have direct contact with livestock, seems to be increasing[27]. In 2018, a project on surveillance of MRSA in humans, livestock, and meat products was started. This project is a collaboration between NVWA, RIVM, WBVR and WFSR. MRSA isolates obtained from animals, dust from livestock farms, farmers and their family members and meat are compared with isolates collected in the Dutch national MRSA surveillance in humans. For the Dutch national MRSA surveillance, medical microbiology laboratories send MRSA isolates

from carriers and from infected persons to the RIVM. The objective of this project is to assess possible changes in the rate or nature of MRSA transmission between animals and humans. Below are the findings obtained within this surveillance project.

Prevalence of MRSA on livestock farms, in caecal samples and in isolates from meat

For the MRSA surveillance in livestock, each year one animal sector is monitored at farm level. For the year 2023 sheep farms and persons working and/or living on these farms were sampled.

Sheep farms

In 2023, a total of 156 sheep farms were investigated. Nasal samples were taken from three animals per farm aged > 12 months. A total of 468 nose swabs were examined. One or more samples from 7 farms were MRSA-positive, resulting in a farm prevalence of 4.5 (95%CI:2.2-9.0) (Table MRSA01). The sample prevalence was 2.4% (11/468).

Persons working/living on sheep farms

Persons living and/or working on the sheep farms were requested to take a nasal swab on a voluntary basis. In total 80 persons living and/or working on 52 farms volunteered to send in a nasal swab. Five persons (6.3 %; 95%CI 2.7-13.8 %) on three farms (5.8%) were MRSA-positive. A prevalence of 6.3% is higher than the prevalence in the population at large (<1%)[28]. On one farm, where two persons tested positive, one sample from a sheep was also MRSA positive. Whole genome sequence data show that the isolates from the sheep and the farmer differ by only 18 alleles, suggesting transmission between sheep and farmer. On the farms with the other three MRSA positive persons the samples of the sheep were negative. The results of sheep farms are summarized in Table MRSA01 together with data from 2018/2019 (broiler farms), 2020 (pig farms), 2021 (dairy farms), 2022 (rabbit farms) and 2022 veal calf farms. In the study period 2018 – 2023 MRSA prevalence varied substantially between the sectors. The MRSA prevalence on sheep farms is relatively low, while the prevalence of persons living/working on these farm was higher than expected. All positive persons reported that they had also contact with livestock other than sheep (dairy cattle, beef cattle, dairy goats, pigs, poultry or horses), which might also contribute to the higher prevalence of MRSA carriage compared to the general population.

Table MRSA01 Number of MRSA found on farms and in persons working/living on these farms from 2018-2023

Year	Animal	MRSA positive farms (n)	Total (n)	Prevalence (%)	95%CI	Humans (n)	Total (n)	Prevalence (%)	95%CI
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

In the Netherlands, the prevalence of MRSA (all MRSA types including LA-MRSA) carriage in individuals in the population at large is low and has varied between 0.1% and 0.8% during the last decade depending on the methods used and the population studied[28-30]. In a Dutch study among persons living in a livestock-dense area, but without professional livestock contact, 0.6% carried MRSA and the prevalence of LA-MRSA was 0.4%[31]. Despite a relatively low MRSA farm prevalence, prevalence among the studied group of sheep farmers is much higher compared to MRSA prevalence described in the general population.

MRSA from caecal samples of pigs and broilers

In 2023, 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 41/300 pigs and 1/290 faecal samples from broilers. Caecal samples from cattle and veal calves were not investigated in 2023 (Table MRSA02).

Table MRSA02 Number of MRSA isolated from caecal samples collected in 2023

Year	2021			2022			2023		
	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)
Pigs	15	100	15	48	300	16	41	300	13.7
White veal calves	31	70	44	58	207	28	-	ni	-
Rosé veal calves	0	32	0	2	100	2	-	ni	-
Dairy cows	1	102	1	1	300	0.3	-	ni	-
Broilers	1	102	1	-	no	-	1	290	0.3

ni = not investigated

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 in 2023 was comparable to the prevalence found in previous years (2021 and 2022). The prevalence found in broilers was very low and this was also consistent with previous years.

MRSA on meat

MRSA was also found on meat. From 2018 to 2023, 4529 retail meat samples were analyzed and 412 (9.1%) were MRSA-positive (Table MRSA03). The prevalence of MRSA on veal increased from 3.8% in 2020 to 13.6% in 2023, while the prevalence on beef is lower: 2.1% in 2018 and 3.9% in 2023 (Table MRSA03). Poultry meat is contaminated with MRSA at a rate of approximately 10%. The prevalence of MRSA on mutton and goat meat was 4.6%.

Generally it is thought that meat consumption is not an important transmission route for humans, especially if the meat is heated before consumption. In some studies performed abroad, however, food handling has been implicated as a transmission route[32,33].

Table MRSA03 Number of MRSA found on meat (products) from 2018-2023

Year	Sample	Sheep/ goats meat	Pork	Beef	Poultry-meat	Veal	Other
2023	Positive (n)	7	20	17	20	25	1
	Total (N)	151	291	432	192	184	22
	Prevalence (%)	**4.6**	**6.9**	**3.9**	**10.4**	**13.6**	**4.5**
2022	Positive (n)	*no data*	13	9	18	15	3
	Total (N)		180	147	194	164	21
	Prevalence (%)		**7.2**	**6.1**	**9.3**	**9.1**	**14.3**
2021	Positive (n)	37	*no data*	*no data*	28	18	4
	Total (N)	299			324	261	20
	Prevalence (%)	**12.4**			**8.6**	**6.9**	**5.0**
2020	Positive (n)	1	3	*no data*	45	2	0
	Total (N)	46	57		248	52	4
	Prevalence (%)	**2.2**	**3.5**		**16.5**	**3.8**	**0.0**
2019	Positive (n)	*no data*	25	11	50	*no data*	*no data*
	Total (N)		296	286	251		
	Prevalence (%)		**8.4**	**3.8**	**19.9**		
2018	Positive (n)	*no data*	8	3	29	*no data*	*no data*
	Total (N)		135	140	132		
	Prevalence (%)		**5.9**	**2.1**	**22.0**		

Comparison of animal-related MRSA GG0398 isolates to isolates from the Dutch national surveillance

A total of 811 animal-related MRSA GG0398 isolates were sequenced and compared to the sequences of 1758 MRSA GG0398 isolates from humans from the Dutch national surveillance. Comparing the strains on a molecular level by whole genome MLST (wgMLST) showed that isolates originating from animals, meat and humans grouped together. Remarkably, one branch comprised of only MRSA GG0398 from humans, and these isolates were often carrier of a human-related virulence factor called Panton Valentine Leucocidin (PVL) which was confined to only this group of isolates. These persons had no known link to animals/farms. As there were no clusters containing animal isolates only and some animal-related isolates were closely related to human isolates, it is likely that transmission between animals and humans occurred. In addition, isolates derived from different animal species differed sometimes by less than 16 wgMLST alleles, which also points to recent transmission events or the presence of a common source. PVL-positive MRSA GG0398 were human-associated and not livestock-associated anymore and human-to-human transmission seems probable.

Resistance levels of MRSA from livestock and meat

In 2023, susceptibility testing of MRSA was performed on a subset of isolates originating from caeca of pigs (n=41) and broilers (n=1) as well as isolates obtained from nasal swabs (n=11) and meat (n=5) from sheep. MRSA isolates were tested for antimicrobial susceptibility with broth microdilution according to ISO standards using commercially available Sensititre plates (Thermofisher Scientific, panel EUST). The MIC-values were interpreted with ECOFFs as advised by EUCAST. Resistance percentages of isolates from pigs (caeca) and sheep (nasal swabs) are depicted for each type of sample in Table MRSA04. Due to the low number of isolates, isolates from broilers and sheep meat samples are not included.

Table MRSA04 Resistance percentages (R%) of MRSA isolated from pigs (caecal samples) and sheep (nasal swabs) in 2023

Antibiotic	Pigs caeca (N=41)	Sheep (N=11)
Cefoxitin	100.0	100.0
Chloramphenicol	51.2	18.2
Ciprofloxacin	2.4	0.0
Clindamycin	34.1	45.5
Erythromycin	24.4	36.4
Fusidic acid	0.0	0.0
Gentamicin	24.4	36.4
Kanamycin	17.1	45.5
Linezolid	0.0	0.0
Mupirocin	0.0	0.0
Penicillin	100.0	100.0
Quinupristin/dalfopristin	12.2	9.1
Rifampicin	0.0	0.0
Streptomycine	2.4	9.1
Sulfamethoxazole	0.0	0.0
Tetracycline	100.0	100.0
Tiamulin	12.2	9.1
Trimethoprim	46.3	54.5
Vancomycin	0.0	0.0

As expected for MRSA, all isolates tested resistant against (benzyl)penicillin and cefoxitin. In addition all isolates showed resistance to tetracycline. This is in line with the known high levels of tetracycline resistance in LA-MRSA. Very high to high levels of resistance were observed in isolates both from pigs and sheep for trimethoprim, clindamycin, erythromycin and gentamicin. Also, kanamycin resistance was high in isolates from sheep, but moderate in pigs. On the contrary, chloramphenicol resistance was high in isolates from pigs, but moderate in sheep. Finally, no resistance was detected against the following human antibiotics: linezolid, mupirocin, rifampicin, or vancomycin.

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