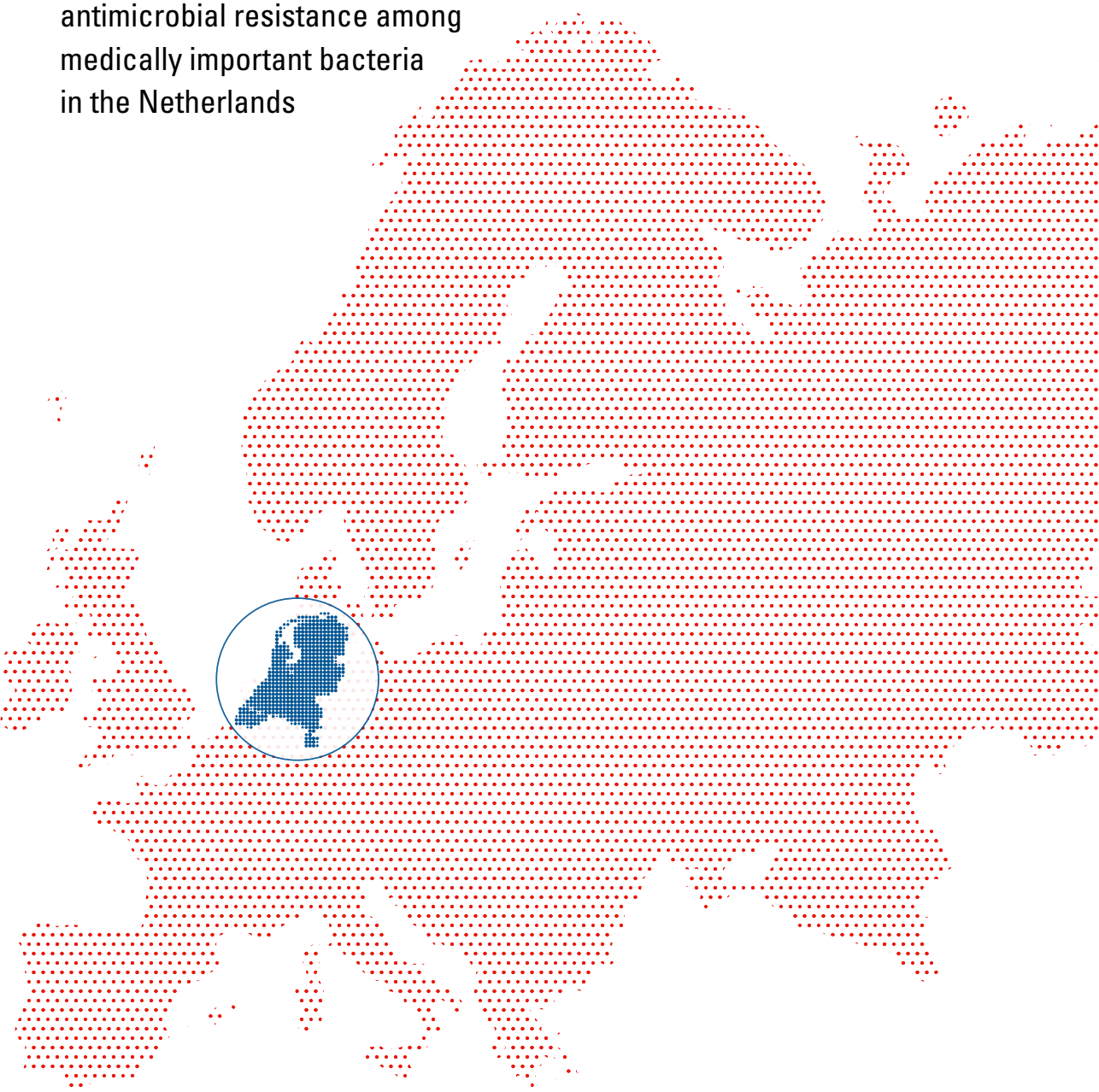
NethMap 2019

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

NethMap 2019

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2018

June 2019

Synopsis

NethMap/MARAN-report

The number of bacteria that are resistant to antibiotics is increasing worldwide. In the Netherlands, that number is basically remaining stable and it is not at such a high level as in many other countries. Nevertheless, there is reason to be concerned and alert. The resistance of some bacterial species to some antibiotics is increasing slowly. Particularly in the case of *Klebsiella pneumoniae*, a common intestinal bacterium, several antibiotics have been becoming less effective over the past five years. These bacteria can cause harmless infections, such as bladder infections, and resistance is making them more difficult to treat. Consequently, certain types of antibiotics that are considered a last resort are having to be used more often.

To prevent resistance, it is important to use antibiotics properly and only when necessary. General practitioners prescribed the same number of courses of antibiotics as in the previous years. The overall use of antibiotics in hospitals is continuing to increase, though.

Approximately the same amounts of antibiotics were prescribed for animals in 2018 as in 2017. With respect to 2009, the reference year, the use of antibiotics has dropped by over 63%. Almost no antibiotics that are important in treating infections in humans have been used for animals in recent years. The number of resistant bacteria in animals has remained roughly the same. However, the number of ESBL-producing intestinal bacteria has dropped further in almost all animal species that are used for food production. This number is only continuing to increase in veal calves. ESBLs are enzymes that can break down commonly used antibiotics such as penicillins.

This is shown in the annual report NethMap/MARAN 2019, in which various organisations jointly present data on antibiotic use and resistance in the Netherlands, for both humans and animals.

In recent years, extra measures have been taken in the Netherlands to combat antibiotic resistance. These measures go further than the healthcare system because resistant bacteria also occur in animals, in foodstuffs and in the environment (One Health). Among other things, 'regional care networks' have been set up to encourage cooperation between various care professionals and to minimize the risk of resistant bacteria being transferred.

Keywords:
Antibiotic resistance, bacteria, antibiotic use, infection

Publiekssamenvatting

NethMap/MARAN-rapport

Wereldwijd neemt het aantal bacteriën die resistent zijn tegen antibiotica toe. In Nederland blijft dat aantal over het algemeen stabiel en is het minder hoog dan in veel andere landen. Toch blijft er reden voor zorg en alertheid. Bij sommige bacteriesoorten neemt de resistentie tegen sommige antibiotica wel langzaam toe. Vooral bij *Klebsiella pneumoniae*, een veel voorkomende darmbacterie, werken de laatste 5 jaar meerdere antibiotica steeds vaker minder goed. Deze bacteriën kunnen onschuldige infecties zoals een blaasontsteking veroorzaken en zijn door de resistentie moeilijker te behandelen. Ook moeten dan vaker soorten antibiotica worden gebruikt die alleen als laatste redmiddel worden gebruikt.

Om resistentie te voorkomen is het belangrijk om antibiotica op de juiste manier te gebruiken en alleen als het nodig is. Huisartsen schreven in het afgelopen jaar even veel antibioticakuren voor als de jaren daarvoor. In ziekenhuizen blijft het totale antibioticagebruik wel stijgen.

Voor dieren is in 2018 is ongeveer evenveel antibiotica voorgeschreven als in 2017. Ten opzichte van 2009, het referentiejaar, is het gebruik met ruim 63 procent verminderd. Voor dieren zijn de afgelopen jaren bijna geen antibiotica gebruikt die belangrijk zijn om infecties bij de mens te behandelen. Het aantal resistente bacteriën bij dieren is ongeveer gelijk gebleven. Wel is het aantal ESBL-producerende darmbacteriën verder afgenomen bij bijna alle diersoorten die voor de voedselproductie worden gebruikt. Alleen bij vleeskalveren blijft het aantal stijgen. ESBL zijn enzymen die veelgebruikte antibiotica kunnen afbreken, zoals penicillines.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2019. Hierin presenteren diverse organisaties gezamenlijk de gegevens over het antibioticagebruik en -resistentie in Nederland, zowel voor mensen als voor dieren.

In Nederland zijn de afgelopen jaren extra maatregelen genomen om antibioticaresistentie te bestrijden. Deze maatregelen reiken verder dan de gezondheidszorg omdat resistente bacteriën ook bij dieren, in voeding en in het milieu voorkomen (One Health). Onder andere zijn ‘regionale zorgnetwerken’ opgezet om de samenwerking tussen verschillende zorgprofessionals te stimuleren en de kans dat resistente bacteriën worden overgedragen zo klein mogelijk te houden.

Kernwoorden:
Antibioticaresistentie, bacteriën, antibioticagebruik, 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. The document was produced on behalf of the SWAB by the Studio of the RIVM.

NethMap can be ordered from the SWAB secretariat, c/o Secretariaat SWAB p/a Leids Universitair Medisch Centrum (LUMC), afdeling Infectieziekten C5-P t.a.v. SWAB, Postbus 9600 2300 RC Leiden or by email to secretariaat@swab.nl
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Contents

1 Introduction

This is NethMap 2019, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2018 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 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 resistance surveillance data. Based on the national AMR surveillance system (ISIS-AR), trends in antimicrobial resistance are monitored using routine antibiotic susceptibility testing data from microbiology laboratories in the Netherlands. Furthermore, the CIb subsidizes specific 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 Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) which aims to mitigate large-scale outbreaks of AMR in hospitals and nursing homes and to prevent spread to other health care facilities through early warning and reporting. Together these constitute the basis of the surveillance of resistance reported in NethMap and 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 2019 extends and updates the information of the annual reports since 2003. Since the introduction of a revised format five years ago, reflected in both a different format as well as more concise information, we have tried to further improve and highlight the most important trends. The appearance of highly resistant microorganisms (HRMO's) receives attention in a separate chapter. The reader is encouraged to visit www.isis-web.nl for tailored overviews of resistance development. Likewise, the Antimicrobial Stewardship Monitor program is gaining footage in an increasing number of hospitals and described for the fourth consecutive year.

In April 2018, the Ministry of Health sent out a letter describing the progress of actions against antimicrobial resistance in the Netherlands which were initiated in 2015. One of the major targets set to be achieved in human healthcare is the improvement of the national surveillance systems concerning antimicrobial resistance, healthcare-associated infections and antibiotic usage. In addition, ten regional healthcare networks for AMR were set up to improve regional collaboration to control antimicrobial resistance across disciplines and healthcare domains. The networks will continue their activities for the coming four years with a subsidy provided through the RIVM. In the coming years the results of these improvements and regional approach will be reflected in NethMap.

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.

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.

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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 2019) and the veterinary sector (MARAN 2019).

2.1 Most important trends in antimicrobial use

In outpatients

- In 2018 total systemic antibiotic use in outpatients remained stable with 10.05 DDD/1,000 inhabitant days (DID).
- No major shifts in antibiotic use in outpatients have been observed except for beta-lactamase sensitive penicillins.
- The large decrease of beta-lactamase sensitive penicillins was probably driven by shortages in pheneticillin throughout 2018.

In hospitals

- The inpatient use of antibiotics in 2017 slightly increased to 85.7 when expressed as DDD/100 patient-days and increased to 340.2 when expressed as DDD/100 admissions, probably indicating further intensification of the use of antibiotics in hospitals or trend towards higher antibiotic dosing strategies in Dutch hospitals.
- The use of beta-lactamase resistant penicillins increased most and reached a level of 9.6 DDD/100 patient-days.
- The use of fluoroquinolones decreased with 0.4 to 8.7 DDD/100 patient-days, mainly driven by reduction in use of ciprofloxacin.
- The use of first-, second- and third-generation cephalosporins has increased with 0.7, 0.1 and 0.5 DDD/100 patient-days, respectively.
- Carbapenem use has increased from 1.8 DDD/100 patient-days in 2016 to 2.0 DDD/100 patient-days in 2017.

- There are large differences in total antibiotic drug use between Dutch hospitals (range 43-166 DDD/100 patient-days). General hospitals used the least antibiotics (84.0 DDD/100 patient-days), whereas large teaching hospitals reported the highest overall antibiotics use (88.8 DDD/100 patient-days).
- The use of antimycobacterials increased with 1.8 DDD/100 patient-days in 2017 and has now reached a level of 4.3 DDD/100 patient-days.
- The use of antimycotics for systemic use has decreased from 14.2 in 2016 to 13.6 DDD/100 patient-days (-0.6 DDD/100 patient-days) in 2017.
- Antibiotic use expressed as days of therapy (DOT)/100 patient-days informs on patient level exposure to antibiotics. In the future, the course of the ratio between the DDD and DOT per 100 patient-days could provide more information on, for instance, potential dose inflation or extension of indications.
- PREZIES data showed that as in 2017, for surgical prophylaxis, cefazolin was used in 61% of cases in 2018. Use for medical prophylaxis was more diverse.

In long-term care facilities

- The mean use of antibiotics in SWAB long-term care facilities varies from year to year. In 2017, the mean of total antibiotic use for systemic use was 52.9 DDD/1,000 residents/day (range 17-117 DDD/1,000 residents/day).
- The mean use of total antibiotics use for systemic use in SNIV long-term care facilities was 39.4 DDD/1,000 residents/day.
- The most frequently used antibiotics for prophylactic use was nitrofurantoin (35%) and for treatment of infections ciprofloxacin and amoxicillin with clavulanic acid with 13% and 8%, respectively.

2.2 Most important trends in antimicrobial resistance

Several surveillance programs have been active in the Netherlands over the years to monitor antimicrobial resistance in important pathogens in different settings. In addition, a number of specific surveillance programs exist that focus on the monitoring of specific pathogens, or even specific resistance mechanisms. These programs often include susceptibility testing, confirmation of important resistance mechanisms and molecular typing. For instance, all *Neisseria meningitidis* isolates cultured in the Netherlands are submitted to a reference laboratory for further analysis. In table 2.2.1 an overview is provided of surveillance programs that are included in NethMap 2019.

Table 2.2.1 Overview of Surveillance programs included in NethMap 2019.

Surveillance program[1]	Origin of isolates	availability	Sources 2018	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in major pathogens					
ISIS-AR	GP, Hospital, Nursing homes	2008-	47 laboratories	Decentral testing	Various methods used in routine susceptibility testing
Specific surveillance program aimed at resistance surveillance in specific pathogens					
Neisseria meningitidis	Hospital	1994-	Nationwide	Central testing	Gradient testing
Neisseria gonorrhoeae	SHC centers	2006-	90% of SHC center attendees	Decentral testing	Gradient testing
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Agar dilution and BACTEC-Mgit 960
(liquid breakpoint)					
Influenza antiviral drugs	community, GP, nursing home, hospital	2005-	NIVEL GP sentinels, SNIV nursing home sentinels, hospital/ regional laboratories	central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Neuraminidase enzym inhibition assay; for established molecular markers sequencing and/or single nucleotide polymorphism (SNP) PCR
Resistance among anaerobic pathogens	Hospital	2010-	1 lab	Central testing	Gradient testing
Clostridium difficile	Hospital, nursing homes	2005-	23 hospitals	(de)central testing	Agar dilution testing and PCR ribotyping
Azole resistance in Aspergillus fumigatus	Hospital	2011-	5 University hospitals + 5 teaching hospitals	Central testing	EUCAST microbroth dilution methodology
MRSA	GP, hospital, nursing homes	2008-	Nationwide	Central testing	MLVA, next-generation sequencing
CPE	GP, hospital, nursing homes	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, next-generation sequencing
CPPA	GP, hospital, nursing homes	2016-	Nationwide	Central testing	Gradient testing, multiplex PCR, next-generation sequencing

[1] *ISIS-AR: Infectious Disease Surveillance information system on Antibiotic Resistance;*
STI: Sexually Transmitted Infections; NIVEL: Netherlands Institute for health services research; GP: General practitioner; SNIV: National sentinel surveillance network for infectious diseases in nursing homes; WHO-CC: World Health Organisation Collaborating Centre; MRSA: methicillin-resistant Staphylococcus aureus; CPE: carbapenemase-producing Enterobacterales; CPPA: carbapenemase-producing Pseudomonas aeruginosa

In GPs

- For most antimicrobials, there are no statistically significant and clinically relevant shifts in resistance levels since 2014.
- For isolates from urine cultures a distinction was made for patients aged below and above 12 years of age in accordance with age categories used in the urinary tract infection guidelines of the Dutch College of General Practitioners (NHG). In general, resistance rates in the older age group were slightly higher than in the younger age group, except resistance of *K. pneumoniae* for co-amoxiclav which was higher in the age group below 12 years.
- In *E. coli* and *K. pneumoniae*, there was a significant and clinically relevant increase in resistance to co-amoxiclav in both age groups. However, this is likely due – at least in part – to underestimation of resistance in earlier years because of a change in susceptibility testing methods, such as the introduction of a new testpanel for the VITEK2 automated system in 2016. Still, its role in empiric therapy for urinary tract and intra-abdominal infections should be reconsidered.
- The percentage of highly resistant microorganisms (HRMO) and multidrug-resistance remained was ≤6% in all *Enterobacterales*.
- Resistance levels for *E. coli* were comparable between the regional cooperative networks for the selected antimicrobials. For *K. pneumoniae* regional differences were more pronounced.
- Resistance to the antibiotics to treat tuberculosis remained stable over the last 5 years.
- In gonococci, no resistance to ceftriaxone, the current first-line treatment was found. Resistance to azithromycin continued to increase, from 2% in 2012 to 11% in 2018.

In hospitals

- Compared to 2013, overall resistance rates for many antimicrobials were similar, with a few exceptions, for which statistically significant and clinically relevant increasing or decreasing trends were observed:
  - In all hospital departments, a statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* and in *K. pneumoniae*. However, this is likely due – at least in part – to underestimation of resistance in earlier years because of a change in susceptibility testing methods, such as the introduction of a new test-panel for the VITEK2 automated system in 2016. Still, its role in empiric therapy for urinary tract and intra-abdominal infections should be reconsidered.
  - ESBL percentages overall have increased over the years in *E. coli* and *K. pneumoniae*, but are still low compared to other countries in Europe. In intensive care units the percentage is highest, with 13% *K. pneumoniae* being ESBL-positive. For *E. coli* on ICU there is no rise, being stable at 7%. The rise in ESBL-positivity overall is much higher in *K. pneumoniae.*
  - Outpatient departments: In *E. coli*, a significant and clinically relevant increase was seen in multidrug resistance to 7%. In *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed in resistance for cefotaxime/ceftriaxone, ceftazidime, ciprofloxacin, trimethoprim and multidrug resistance (from 3% to 6%).
  - Unselected hospital patient departments: Resistance levels ≥20% were found for amoxicillin/ampicillin, trimethoprim and co-trimoxazole in *E. coli* and *P. mirabilis*, for co-amoxiclav in *E. coli* and *K. pneumoniae*, and for fosfomycin in *E. cloacae* complex. A significant and clinically relevant increase in resistance was observed for ceftazidime in *E. coli* and *K. pneumoniae*. A statistically significant and clinically relevant increase in resistance was observed for meropenem/imipenem in *Acinetobacter spp.* from 1% in 2014 to 4% in 2018.

  - Intensive Care Units: In *E. coli* and *K. pneumoniae*, resistance to ceftazidime increased in the last five years from 4% to 6% and 7% to 12%, respectively.
  - Blood isolates from inpatient departments: In *K. pneumoniae*, statistically significant and clinically relevant increases in resistance to various empiric therapy combinations were observed.
- The percentage of HRMO was highest among *E. coli* and *K. pneumoniae* and mostly ≥10% in *K. pneumoniae*.
- In 2018, a significant and clinically relevant increasing trend in ESBL in *E. coli* and *K. pneumoniae* was observed for general practitioner patients, in outpatient departments and in hospital inpatient departments excluding Intensive Care Unit (ICU), with the most outspoken results for *K. pneumoniae*. The prevalence of ESBLs was correlated with the complexity of care, with highest percentages in the ICU´s.
- The MRSA prevalence in blood culture isolates remained low, 1%.
- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing has remained stable around 0.8% over the past five years. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was low (0.05% and 0.52%). Of the isolates submitted to the CIb, the most frequently identified carbapenemase encoding genes in *Enterobacterales* were genes encoding for OXA-48, NDM, VIM and KPC. In pseudomonas this was the *bla*VIM gene.
- The proportion of vancomycin-resistance in infection-related isolates with *E. faecium* in various healthcare settings varies around 1% and has not changed in the previous five years. Vancomycin resistance in *E. faecium* does possibly not impose an extra burden on morbidity and mortality compared to vancomycin-susceptible *E. faecium*, if accounting for underlying diseases. Constant evaluation of the infection control measures to contain outbreaks is needed.
- Azole resistance in *Aspergillus fumigatus* stabilized at 14.7% in university hospitals, and was 7.8% in large teaching hospitals.
- Data on antimicrobial susceptibility of anaerobic bacteria is limited. To gain more insight in resistance in anaerobic bacteria a more extensive surveillance program will be needed.

2.3 Antibiotic use and resistance in animals

Antibiotic use

- Sales of antimicrobial veterinary medicinal products (AMVPs) in 2018 (179 tonnes) showed a decrease of 1.1 % compared to 2017 (181 tonnes).
- In all sectors (dairy cattle, other cattle, veal valves, pigs and turkeys) except in broilers a slight reduction in consumption has been realized.
- The use of highest prioritized antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation and fluoroquinolones) in livestock is further reduced compared to previous years.

Antimicrobial resistance

- In 2018, the proportion ESBL-suspected *Salmonella* isolates was 0.9%, among seven different serovars, mainly isolated from human samples. Cefotaxime resistance was detected in one *Salmonella* Infantis isolate obtained from poultry meat. In 2018 no carbapenemase producing *Salmonella* were found.
- Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for ciprofloxacin and tetracycline and increased slightly in 2018, compared to 2017.

Resistance to macrolides was rarely detected amongst these *Campylobacter* isolates. Ciprofloxacin resistance in *Campylobacter* isolates from human patients remained at a high level (with a further increase in 2018). Resistance to erythromycin, representing macrolides used in human medicine for campylobacteriosis, remained at a low level.

- Among commensal *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat. In 2018, these resistance levels stabilized (or increased for ampicillin) in broilers and veal calves and slightly decreased in pigs. In dairy cattle the resistance proportions remained at a constant low level. The proportion of commensal *E. coli* resistant to extended spectrum cephalosporins was very low in faecal samples from broilers, pigs, dairy cattle and veal calves.
- Selective culturing of ESBL/AmpC producing *E. coli* from broilers showed an ongoing decrease in the proportion of samples positive (prevalence) from 66% in 2014 to 23% in 2018. After a peak in the prevalence of ESBL/AmpC producing *E. coli* from rosé veal calves in 2016, little fluctuation was seen since then. However, in white veal calves since in 2016 a steady increase is still ongoing. The prevalence of ESBL/AmpC producing *E. coli* in Dutch retail meat has further decreased to 3.9% in 2018.
- No carbapenemase-producing *Enterobacterales* were found in livestock. Only $bla_{\text{OXA-48}}$-like genes were detected in fifteen caecal samples of different livestock species all associated with *Shewanella spp.*
- In 2018, the colistin resistance gene, *mcr*-1 was identified incidentally in *E. coli* from different livestock species. *mcr*-4 was rarely detected in veal calves. No *mcr* genes were detected in *Salmonella*.
  The finding of *mcr*-1 positive *E. coli* on poultry meat indicates a higher level in retail meat from chicken and turkey, related to imports from neighbouring countries. A significant higher prevalence of *mcr*-1 was detected in German broilers.
- The data on usage are to a large extend reflected in the resistance data of 2018, where proportions of resistant *E. coli* stabilized in most livestock species. In broilers the proportion of samples (caeca and meat) positive for ESBL/AmpC-producing *E. coli* was again lower than in previous years. In contrast to broilers, in 2018 the prevalence of ESBL-carriers again increased in white veal calves. This shows that the measures implemented in Dutch livestock production to reduce the overall antibiotic use and to stop the use of 3rd-generation cephalosporins have been effective in reducing ESBL/AmpC-contamination of food-products. But, they have not been sufficiently effective in the veal calf sector, where ESBL occurrence increased. As in previous years, carbapenemase producing *Enterobacterales* or the colistine resistance gene *mcr*, were not detected or found at low levels, respectively.

2.4 Implications for therapy

Overall, no major shifts in resistance rates have occurred in the Netherlands in 2018. The only major exception, as already found in 2017, is the decrease in susceptibility to co-amoxiclav. Although this is primarily due to a change in testing methods, this resistance to co-amoxiclav limits its usefulness in (empiric) therapy, i.e. for urinary tract infections and intra-abdominal infections.
Although there is no major shift in general, there are significant differences in susceptibility by patient category. In particular for patients on the ICU resistance levels are generally higher. Routine culturing with antibiograms remains mandatory to tailor therapy to the individual patient. If broad spectrum therapy is initially chosen, antibiograms should be used to narrow down antimicrobial therapy to prevent even further emergence of resistance and culture repeated if indicated. In Dutch hospitals,

antimicrobial stewardship programs are contributing significantly in optimizing antimicrobial therapy. Of note, EUCAST susceptibility breakpoints are based on the use of certain dosing regimens (to be found at www.eucast.org). The use of alternative (lower) dosing regimens should be used with care. Of importance, resistance rates reported in NethMap are for one isolate per patient, and only the first one. Resistance of bacteria in the individual patient, especially those that stay longer in the hospital, is often significantly higher than reported here. On the other hand, resistance may be overestimated in GP, since cultures are usually only performed after failure of initial therapy.
In the summary below, some of the most important implications for therapy are provided, based on the general trends of resistance. As implications differ by category of patient and indication of use, the summary is organized as such. 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 UTI. Non-susceptible rates can be higher than resistance rates in some cases.

In GPs

- Resistance to nitrofurantoin and fosfomycin are still below 2% in *E. coli* indicating suitable use for urinary tract infections. However, this is likely an underestimation for fosfomycin, as current testing systems overestimate susceptibility for fosfomycin. High resistance rates and intrinsic resistance make fosfomycin unsuitable for *Klebsiella* therapy.
- Co-amoxiclav resistance in *E. coli* and *K. pneumoniae* are high, and its usefulness in the treatment of urinary tract infection in some patient categories is becoming more and more limited.
- Clindamycin (inducible) resistance in *S. aureus* has risen to more than 10%, this should be taken into account when empiric clindamycin therapy is considered.
- Resistance percentages are now available per region, these indicate that there are differences in susceptibility between regions for some antibiotics. These differences should be taken into consideration.

In hospitals

Outpatient departments

- The levels of resistance preclude empirical treatment with oral agents for complicated UTI; culture, antibiograms and tailored therapy are necessary.
- Resistance levels are stable in most species, but there is constant rise over the years in resistance of *K. pneumoniae* to many antimicrobial agents.

Unselected hospital patient departments

- Resistance in *K. pneumoniae* stabilised when compared to 2017; resistance is now 9% to ceftazidime, 10% for cefotaxime/ceftriaxone and the %HRMO is now 11%. Patients suspected for *K. pneumoniae* infection have a high risk of non-adequate treatment.
- For other *Enterobacterales*, it is encouraging to see that resistance to most antimicrobials did not change markedly or was lower. The only major exception was the rise in resistance to co-amoxiclav, as a result of new testing methods. The % resistance in *E. coli* is now 36% and in *K. pneumoniae* it is 22%. This renders the drug unsuitable for empiric therapy, unless it is combined with a second drug, for instance an aminoglycoside.
- For *P. aeruginosa* resistance declined for all antibiotics. If ciprofloxacin is considered as empiric therapy, combination with a second antipseudomonal should be considered.

- Combination therapy of a beta-lactam with an aminoglycoside is still the best suitable options for empirical treatment in serious infections with Gram-negative bacteria, unless a quinolone is specifically desired to cover specific pathogens.
- Overall, susceptibility of *S. aureus* is stable, with the exception of the rise of macrolide resistance and clindamycin inducible resistance. The 12% resistance for clindamycin indicates that culture and susceptibility testing are mandatory before starting treatment with this drug.

Intensive care patients

- Similar to other wards, the level of resistance in *K. pneumoniae* is the main treatment challenge. The %HRMO in this group has risen to 15% in 2018. The %HRMO in *E. coli* was stable at 10% when compared to 2017. Since species identification in Dutch laboratories is now usually very fast for positive cultures (within hours) due to the almost universal use of the MALDI-TOF and susceptibility still commonly requires overnight cultures, identification can have significant consequences for (empiric) therapy. Resistance in *Enterobacterales* in general were similar and often even lower than in the previous year(s).
- Local resistance levels vary significantly, including from time to time. Tailored therapy and culture remain the mainstay of therapy.

Specific micro-organisms

- In 2018 the level of azole resistance in *Aspergillus fumigatus* was stable at 14.7% in university hospitals. Monotherapy of azoles is no longer advised for empiric therapy and guidelines for empiric therapy have been renewed following this development.
- No resistance to metronidazole for *C. difficile* was found in 2018. However, in 2017 a clinical isolate of *C. difficile* PCR ribotype 014 with MIC=8 mg/L to metronidazole was detected. The emergence of metronidazole resistance needs attention in the near future.
- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing has remained stable (around 0.8%) over the past five years.
- In 2018, 306 unique carbapenemase-producing *Enterobacterales* isolates were obtained from 266 persons (mean age 60 years and 53% male). In 2017, this was 233 CPE isolates from 201 persons.
- Hospitalization abroad for at least 24 hours during the previous two months was the most common risk factor for the presence of CPE.
- In 2018, four outbreaks with carbapenemase-producing *Enterobacterales* (2017: 3 outbreaks) were reported to the Early warning and response meeting for Hospital-acquired Infections and Antimicrobial Resistance (SO-ZI/AMR).

2.5 Antimicrobial stewardship

Since 2014, following the recommendation of the Dutch Health Care Inspectorate (IGZ) in response to the statement of the SWAB to contain antimicrobial resistance, hospitals have established anti-microbial stewardship teams (A-teams) that are responsible for the implementation of an antimicrobial stewardship program. The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by antimicrobial stewardship teams in hospitals and 2) the quality of antimicrobial use in hospitals.

The most important development concerning stewardship teams are:
- All surveyed hospitals have an A-team
- Increasingly, nurses and infectious disease specialists are part of A-teams
- There is a steadily increase in budget provided to A-team, although still less than indicated by the staffing standard

SWAB has continued the development and implementation of an antimicrobial stewardship monitor with the aim to provide benchmarked feedback reports based on automated data extraction. Results will follow in 2019. This year, point prevalence data on quality of antimicrobial use in hospitals from 2017/2018 were analyzed. Most hospitals identified lower respiratory tract infection as an important target for improvement. Most hospitals have started improvement interventions based on the results of the point prevalence survey.

2.6 Implications for public health and health policy

Antibiotic resistance is a serious threat to public health in Europe, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death.
Especially, the global rise of carbapenem-resistant *Enterobacterales* (CRE) is alarming and represents an increasing threat to healthcare delivery and patient safety.
Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that in Europe in 2017, carbapenem resistance in *E. coli* remained rare (<0.1%), and most countries reported low levels for *K. pneumoniae*. On the other hand, compared to these low numbers, a small group of countries reported considerably higher carbapenem resistance percentages above 10% for *K. pneumoniae*, which were mostly countries with high resistance percentages to other antibiotic groups as well. As a result, in these settings, only a limited number of therapeutic options are available such as colistin, often leading to more toxicity and side-effects. Furthermore, colistin resistance may develop in patients treated with this drug, which poses a substantial public health risk. To provide updated and more detailed information on the distribution of these resistance patterns, recently the carbapenem- and/or colistin-resistant *Enterobacterales* (CCRE) project was launched in the Netherlands as part of the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net). Furthermore, for *K. pneumoniae*, more than one third of the isolates reported to EARS-Net for 2017 were resistant to at least one of the surveyed antimicrobial group, but no relevant significant increasing trends were noted for any of the groups including combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides. In *E. coli*, an increasing EU/EEA trend for resistance to third-generation cephalosporins and fluoroquinolones between 2014 and 2017 was observed.
In the Netherlands, the prevalence of carbapenem resistance among *Enterobacterales* remained rare. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was low (0.05% and 0.52%) and there was no significant increase in the last years. On the other hand, a gradually increasing trend in ESBL-*Enterobacterales* was observed in all healthcare settings, which was most outspoken for *K. pneumoniae*. Additionally, in hospitals the percentage of HRMO among *K. pneumoniae* was increasing and usually ≥10% in all departments, as well as increasing resistance trends for *K. pneumoniae* to the different groups of antimicrobials under surveillance. These increases are not likely to be attributable to outbreaks in healthcare settings, as the rise is more widespread among various patient groups

including general practices. Since these developments lead to a growing use of last-resort antibiotics, this requires ongoing attention.

In 2015 the Minister of Health initiated a One Health-approach with actions to combat antimicrobial resistance in the Netherlands.[1] This integrated One Health-approach aims at measures for all relevant domains, including human health care, the veterinary sector, the food chain, the environment and international involvement. In April 2018, the Ministry of Health published a letter on the progress of this approach.[2] In 2018, multiple initiatives and projects were further developed. First, the ten Regional Cooperative Networks concerning antimicrobial resistance, started in 2017, continued their set up and are fully operative from May 2019 onwards. The target of these networks is to stimulate regional collaboration between all relevant stakeholders in healthcare settings, concerning the control of antibiotic resistance and HRMOs, infection prevention measures, antibiotic use, patient flows, and more. Various initiatives within the networks to reach these goals have been developed in the previous years, including the organization of a regional coordinating team. Secondly, since surveillance is an essential pillar in the fight against antimicrobial resistance, further improvement of the national surveillance of antimicrobial resistance, healthcare-associated infections and antimicrobial use is being targeted. The project "Eenheid van Taal – Antimicrobial Resistance" aims to implement standardized communication of microbiological, clinical and epidemiological data between stakeholders. It kicked off successfully in 2017 in a pilot setting and in 2018, a number of additional "leader" microbiological labs were enrolled into the implementation of the project. Since April 2019, the first lab routinely submits its data on antimicrobial resistance testing to the national surveillance program (ISIS-AR) by using "Eenheid van Taal". Lastly, in 2018 a point prevalence study in nursing homes has been performed to investigate the prevalence of HRMOs among residents, in combination with interactive feedback and advise to improve infection prevention and hygiene measures in the institutions if necessary. The results of the study will be published in the coming months.

Conclusions

The data presented in NethMap 2019 demonstrate that the ongoing implementation of the national approach is needed to combat antibiotic resistance. It is encouraging to see that resistance is not rising or even going down in many important species. Carbapenem resistance and multidrug resistance in *Enterobacterales* is of major concern, and needs close attention. With adequate surveillance systems the impact of these measures on 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. Some surveillance systems and reference laboratory functions may need more attention. For instance, national surveillance of Enterococci is missing at the moment, and surveillance of resistance in anaerobic bacteria is only based on data from one lab and therefore not considered representative for the Netherlands.

[1] *https://www.rijksoverheid.nl/documenten/kamerstukken/2015/06/24/kamerbrief-over-aanpak-antibioticaresistentie*

[2] *https://www.rijksoverheid.nl/documenten/kamerstukken/2018/04/26/kamerbrief-over-voortgang-aanpak-antibioticaresistentie*

3
Use of antimicrobials

3.1 Outpatient antibiotic use

Methods

Dutch data on outpatient antibiotic use are annually obtained from the SFK (Foundation for Pharmaceutical Statistics, the Hague) and are expressed in numbers of Defined Daily Doses (DDD) for each ATC-5 code. The SFK collects dispensing data from 90% of the Dutch community pharmacies (serving 91.5% of the Dutch population) and extrapolates the data to 100%. These data include prescriptions from general practitioners as well as prescriptions from outpatient clinics and dentists. Data are presented as DDD per 1,000 inhabitants per day (DID).

Results

Total outpatient use of antibiotic for systemic use remained stable with 10.05 DID versus 10.06 DID in 2018 and 2017 respectively (Table 3.1.1). In 2018, the use of penicillins with extended spectrum increased with 0.08 DID, resulting in a level of 2.02 DID, mostly driven by increased amoxicillin use (Figure 3.1.1 and Figure 3.1.2A). In addition, the use of macrolides, mainly represented by azithromycin, increased with 0.05 DID to a level of 0.87 DID (Figure 3.1.2B). As in 2016 and 2017, the use of tetracyclines decreased again in 2018. The use of nitrofurantoin remained stable, whereas the use of fosfomycin, which started increasing since 2009, steadily increased further to 0.06 DID in 2018 (Figure 3.1.1). In 2018, a remarkable decrease was seen in use of beta-lactamase sensitive penicillins, which dropped to 0.07 (-0.15 DID) (Figure 3.1.1). The use of lincosamides has increased over the past 10 years, mainly driven by increased clindamycin use.

Discussion

Total outpatient antibiotic use in the Netherlands remained stable in 2018. Decreased tetracycline prescribing probably reflects a delayed reaction to the adaptation of the national treatment guideline 'acute cough'. Since 2012, amoxicillin is the preferred antibiotic for the indication pneumonia, because of increasing resistance of *S. pneumoniae* for doxycycline. However, the decrease doxycycline use is not entirely compensated by the increase in amoxicillin use, and additionally, the total use of antibiotics often used for respiratory tract infections has been decreasing over the years. The stabilisation in the

use of nitrofurantoin is promising, as this is a valuable first-line treatment for uncomplicated urinary tract infection. In the meantime, fosfomycin became second choice for cystitis in non-pregnant women in 2013, hence fosfomycin prescribing has increased in recent years.

The observed, large decline in use of beta-lactamase sensitive penicillins was probably caused by shortages in pheneticillin throughout 2018. In some cases prescribers might have chosen to prescribe macrolide antibiotics or penicillins with extended spectrum instead. Unnecessary use of broad-spectrum antibiotics is worrisome as it could lead to increased antimicrobial resistance in the future.

Table 3.1.1 Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2009-2018 (source: SFK).

ATC Group*	Therapeutic group	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	2.67	2.67	2.60	2.49	2.33	2.23	2.25	2.10	1.98	1.94
J01CA	Penicillins with extended spectrum	1.89	1.81	1.91	1.94	1.99	1.94	2.13	2.08	1.94	2.02
J01CE	Beta-lactamase sensitive penicillins	0.39	0.37	0.35	0.33	0.31	0.30	0.23	0.24	0.22	0.07
J01CF	Beta-lactamase resistant penicillins	0.38	0.38	0.39	0.41	0.41	0.44	0.43	0.46	0.46	0.49
J01CR	Penicillins + beta-lactamase-inhibitors	1.74	1.80	1.82	1.82	1.67	1.55	1.56	1.52	1.42	1.42
J01D	Cephalosporins & carbapenems	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.21	0.20	0.20	0.19	0.17	0.16	0.14	0.14	0.13	0.13
J01EE	Sulphonamides + trimethoprim	0.35	0.35	0.34	0.33	0.29	0.28	0.28	0.28	0.29	0.30
J01FA	Macrolides	1.33	1.31	1.34	1.34	1.22	1.18	1.20	1.17	1.17	1.22
J01FF	Lincosamides	0.12	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.23
J01GB	Aminoglycosides	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.86	0.85	0.82	0.80	0.76	0.79	0.77	0.75	0.73	0.73
J01XE	Nitrofuran derivatives	1.17	1.23	1.31	1.38	1.37	1.40	1.40	1.39	1.36	1.35
J01XX01	Fosfomycin	0.01	0.01	0.01	0.01	0.02	0.03	0.04	0.05	0.05	0.06
	Others	0.04	0.05	0.05	0.05	0.04	0.04	0.04	0.02	0.05	0.04
J01	**Antibiotics for systemic use (total)**	**11.21**	**11.23**	**11.37**	**11.34**	**10.83**	**10.58**	**10.72**	**10.44**	**10.06**	**10.05**

** From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2009-2018 (source: SFK).

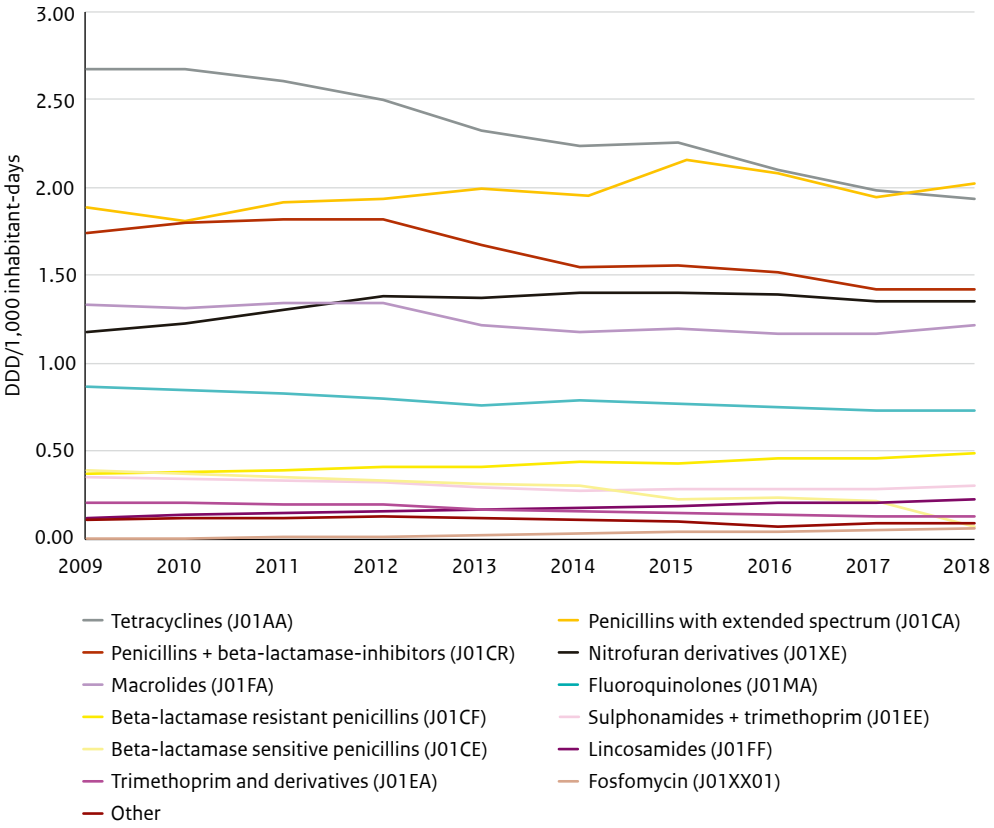
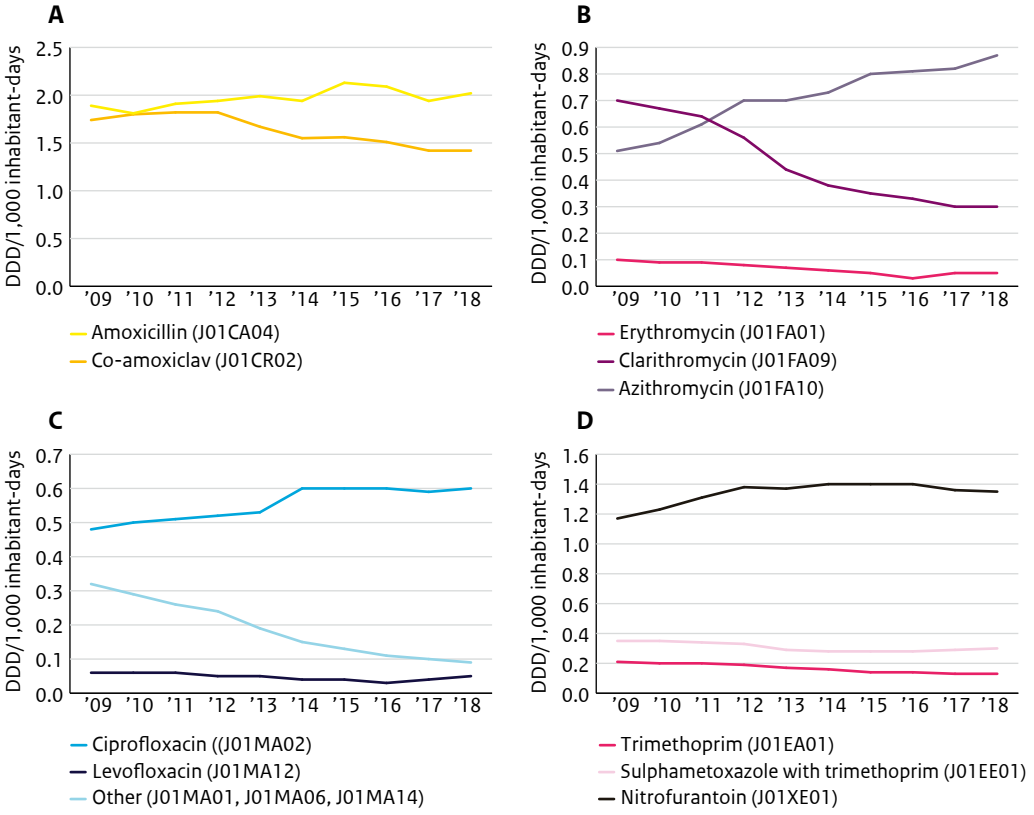


Figure 3.1.2 A-D Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2009-2018 (source: SFK).



3.2 Hospital care

3.2.1 Hospital antibiotic use in DDD

Methods

Data on the use of antibiotics in Dutch hospitals in 2017 were collected by means of a questionnaire distributed to all Dutch hospital pharmacists. DDD assigned per ATC-code and route of administration by the WHO were extracted from the Dutch drug database (Z-index) on unit and product level, and used to calculate total antibiotic use as total amount of DDD per ATC-code. [1] Use of antibiotics is expressed as DDD/100 patient-days and in DDD/100 admissions. The number of patient-days is calculated by subtracting the number of admissions from the number of bed-days to compensate for the fact that in bed-days statistics both the day of admission and the day of discharge are counted as full days. Hospital extrapolated data, expressed in DDD/1,000 inhabitants per day, as used for the international antibiotic surveillance of the ECDC, are also reported. Hospital consumption data and corresponding hospital statistics were used to estimate total hospital consumption in the Netherlands. Methods are further described in Kwint et al.[2] Data on annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS). In addition, Dutch hospitals collected detailed data on antibiotic usage (according to the methodology proposed by the ECDC), combined with the PREZIES prevalence study on healthcare associated infections.[3] All patients admitted to the hospital had to be included, with the exception of patients on psychiatric wards and in the haemodialysis centre. Only systemic antibacterials (ATC-code J01) were included, with a maximum of three concomitant substances per patient.

Results

Data over 2017 were received from 67 hospitals, together with the annual number of bed-days and admissions. The inpatient use of antibiotics increased with 1.6 DDD/100 patient-days to 85.7 DDD/100 patient-days in 2017. Furthermore, total inpatient use of antibiotics, when calculated as DDD/100 admissions, increased with 14.1 from 326.1 to 340.2 (Table 3.2.1.1). Total use of antibiotics for systemic use, when calculated as DDD/1,000 inhabitant-days, decreased with 0.025 from 0.967 to 0.942 (Table 3.2.1.2).

The use of beta-lactamase resistant penicillins and cephalosporins increased in 2017. Although in 2016 an increase was seen in the use of penicillins in general, in 2017 the use of penicillins with extended spectrum decreased (-0.7 DDD/100 patient-days) and was back at 10.2 DDD/100 patient-days. Another notable decrease (-0.4 DDD/100 patient-days) was seen in the use of fluoroquinolones (Figure 3.2.1.1). This decrease is mainly driven by reduction in use of ciprofloxacin (-0.3 DDD/100 patient-days). The use of first-, second- and third-generation cephalosporins increased with 0.7, 0.1 and 0.5 DDD/100 patient-days, respectively. The use of meropenem increased to 1.9 DDD/100 patient-days (+0.2 DDD/100 patient-days) (Figure 3.2.1.2).

Although total antibiotic drug use in the Netherlands is low in general, a large variation is seen between Dutch hospitals (Figure 3.2.1.3 and Figure 3.2.1.4). Considering site of care, in 2017, general hospitals used the lowest amount of antibiotics (84.0 DDD/100 patient-days), whereas large teaching hospitals reported the highest overall antibiotic use (88.8 DDD/100 patient-days).

The use of combinations of penicillins with a beta-lactamase inhibitor is still the highest in general hospitals, with 23.3% versus 13.7% and 14.7% in large teaching and university hospitals, respectively. As in 2016, the use of second-generation cephalosporins is the highest in large teaching hospitals. Carbapenems, third generation cephalosporins (Figure 3.2.1.5) and glycopeptides are primarily used in university hospitals, whereas most of the use of combinations of penicillins, penicillins with extended spectrum, nitrofuran derivatives and lincosamides comes from general hospitals. The increase in use of beta-lactamase resistant penicillin in 2017 is caused by an increase in use within all types of hospitals, especially in large teaching hospitals (Figure 3.2.1.6). Decreased use of fluoroquinolones was observed in large teaching and general hospitals, whereas in university hospitals the use of fluoroquinolones, mostly ciprofloxacin, increased (Figure 3.2.1.7).

In table 3.2.1.3 use of antimycotics (J02), antimycobacterials (J04) and antivirals (J05) in university hospitals is provided from the years 2008 to 2017, expressed in DDD/100 patient-days. In 2017, in particular the use of antimycobacterials has increased, with 1.76 DDD/100 patient-days, and has now reached a level of 4.31 DDD/100 patient-days. The use of antimycotics for systemic use has decreased from 14.23 in 2016 to 13.63 DDD/100 patient-days (-0.6 DDD/100 patient-days) in 2017.

In 2018, PREZIES data were received from 38 hospitals, including 4845 patients of which 1685 received antibiotics, with a total of 2219 prescriptions. Antibiotic use divided by surgical versus medical prophylaxis and hospital versus community acquired infections is depicted in Figure 3.2.1.8. As in 2017, for surgical prophylaxis, cefazolin was used in 61% of cases in 2018. Use for medical prophylaxis was more diverse. Antibiotic use for hospital and community acquired infections in 2018 is comparable to the distribution in 2017.

Discussion

In 2017, antibiotic use in hospitals slightly increased when expressed as DDD/100 patient-days and as DDD/100 admissions. This could indicate further intensification of the use of antibiotics in hospitals or trend towards higher antibiotic dosing strategies in Dutch hospitals. Moreover, there is a large variation in total antibiotic use between Dutch hospitals and shifts are observed between different subgroups of antibiotics, e.g. in use of fluoroquinolones. In addition, the use of cephalosporins and carbapenems continued to rise in 2017. However, little is known about possible changes in hospital and patient characteristics which could influence the quality of our data surveillance to a certain extent.
The observed increase in use of antimycobacterials is not fully understood. According to the Dutch National Institute for Public Health and the Environment (RIVM) the number of tuberculosis infections decreased in 2017.[4] However, at the same time the number of latent tuberculosis infections increased. [5] Increased use of immunosuppressant therapy for diverging indications compel increased need for prophylactic treatment of latent tuberculosis. Increased rifampicin use could also be the result of more frequent use as combination therapy for *S. aureus* infections.

Table 3.2.1.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2008-2017 (source: SWAB).

ATC Group*	Therapeutic group	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	1.7	1.6	1.7	1.8	1.7	1.7	1.9	1.9	2.0	2.0
J01CA	Penicillins with extended spectrum	6.5	7.6	7.3	7.3	7.6	8.0	8.4	9.2	10.9	10.2
J01CE	Beta-lactamase sensitive penicillins	1.3	1.6	1.5	1.5	1.7	1.9	2.4	2.4	2.5	2.5
J01CF	Beta-lactamase resistant penicillins	6.4	6.6	6.8	6.7	7.1	8.1	8.7	7.7	8.7	9.6
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	16.2	16.5	16.0	15.8	15.0	14.8	14.5	14.3	14.6	14.7
J01DB	First-generation cephalosporins	2.6	3.0	3.0	3.5	3.6	3.7	4.4	4.6	4.6	5.3
J01DC	Second-generation cephalosporins	3.0	3.6	3.4	3.7	4.1	4.7	5.0	5.3	5.8	5.9
J01DD	Third-generation cephalosporins	3.2	3.5	3.7	3.9	4.4	5.0	5.7	5.5	5.9	6.4
J01DH	Carbapenems	1.0	1.1	1.2	1.4	1.5	1.7	1.6	1.7	1.8	2.0
J01EA	Trimethoprim and derivatives	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.3
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.4	2.0	2.0	1.9	1.8	1.9	1.9	1.8	2.1	2.4
J01FA	Macrolides	2.7	2.6	2.7	2.9	2.8	2.6	2.9	2.7	3.0	2.8
J01FF	Lincosamides	2.1	2.4	2.3	2.3	2.2	2.3	2.3	2.4	2.4	2.4
J01GB	Aminoglycosides	3.9	4.2	4.1	3.9	3.3	3.5	3.6	3.7	3.7	3.6
J01MA	Fluoroquinolones	8.8	9.3	9.0	9.2	8.9	8.6	9.0	8.4	9.1	8.7
J01XA	Glycopeptides	1.1	1.3	1.3	1.3	1.4	1.5	1.6	1.6	1.6	1.7
J01XB	Polymyxins	0.2	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2
J01XD	Imidazole derivatives	1.7	1.8	1.9	2.2	2.3	2.6	2.6	2.6	2.8	3.0
J01XE	Nitrofuran derivatives	1.2	1.1	1.2	1.2	1.2	1.3	1.6	1.4	1.7	1.7
J01XX	Other antibacterials	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3
	Others**	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
J01	**Antibiotics for systemic use (total)**	**66.8**	**70.9**	**70.3**	**71.3**	**71.3**	**74.7**	**78.6**	**77.9**	**84.1**	**85.7**
	expressed in DDD/100 admissions:										
J01	**Antibiotics for systemic use (total)**	**344.7**	**321.3**	**315.9**	**306.4**	**295.7**	**307.8**	**326.0**	**330.1**	**326.1**	**340.2**

** From the 2017 edition of the Anatomical Therapeutic Chemical (ATC) classification system*
*** J01DI, J01DF, J01EC and J01XC*

Table 3.2.1.2 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1,000 inhabitant-days), 2008-2017 (source: SWAB).

ATC Group*	Therapeutic group	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	0.023	0.025	0.027	0.026	0.024	0.022	0.023	0.025	0.022	0.021
J01CA	Penicillins with extended spectrum	0.101	0.111	0.110	0.103	0.100	0.099	0.101	0.118	0.125	0.117
J01CE	Beta-lactamase sensitive penicillins	0.019	0.023	0.023	0.020	0.023	0.023	0.028	0.028	0.029	0.029
J01CF	Beta-lactamase resistant penicillins	0.086	0.093	0.097	0.089	0.093	0.100	0.105	0.097	0.102	0.103
J01CR	Penicillins + beta-lactamase-inhibitors	0.229	0.241	0.256	0.223	0.211	0.199	0.187	0.186	0.171	0.159
J01DB	First-generation cephalosporins	0.034	0.040	0.042	0.045	0.049	0.047	0.052	0.055	0.053	0.065
J01DC	Second-generation cephalosporins	0.045	0.051	0.055	0.050	0.052	0.055	0.058	0.065	0.066	0.067
J01DD	Third-generation cephalosporins	0.040	0.047	0.050	0.050	0.057	0.062	0.066	0.067	0.068	0.067
J01DH	Carbapenems	0.011	0.014	0.015	0.018	0.019	0.020	0.019	0.021	0.020	0.021
J01EA	Trimethoprim and derivatives	0.007	0.007	0.009	0.006	0.005	0.004	0.003	0.003	0.003	0.003
J01EE	Sulphonamides + trimethoprim	0.029	0.030	0.030	0.026	0.024	0.024	0.022	0.021	0.024	0.023
J01FA	Macrolides	0.037	0.039	0.041	0.037	0.038	0.034	0.034	0.034	0.034	0.030
J01FF	Lincosamides	0.029	0.033	0.035	0.032	0.031	0.032	0.028	0.030	0.028	0.027
J01GB	Aminoglycosides	0.048	0.055	0.058	0.054	0.044	0.045	0.044	0.046	0.043	0.037
J01MA	Fluoroquinolones	0.139	0.129	0.138	0.127	0.124	0.116	0.112	0.112	0.106	0.097
J01XA	Glycopeptide antibacterials	0.012	0.015	0.016	0.017	0.017	0.018	0.018	0.019	0.019	0.019
J01XB	Polymyxins	0.008	0.009	0.006	0.003	0.002	0.003	0.002	0.003	0.002	0.001
J01XD	Imidazole derivatives	0.025	0.026	0.030	0.027	0.029	0.030	0.030	0.032	0.032	0.034
J01XE	Nitrofuran derivatives	0.016	0.017	0.018	0.015	0.018	0.016	0.018	0.018	0.018	0.019
J01XX08	Linezolid	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.000
	Other antibiotics	0.003	0.003	0.003	0.002	0.003	0.001	0.001	0.001	0.000	0.003
J01	**Antibiotics for systemic use (total)**	**0.941**	**1.008**	**1.061**	**0.971**	**0.963**	**0.950**	**0.953**	**0.982**	**0.967**	**0.942**

** From the 2017 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.2.1.1 Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days) at ATC-4 level, 2008-2017 (source: SWAB).

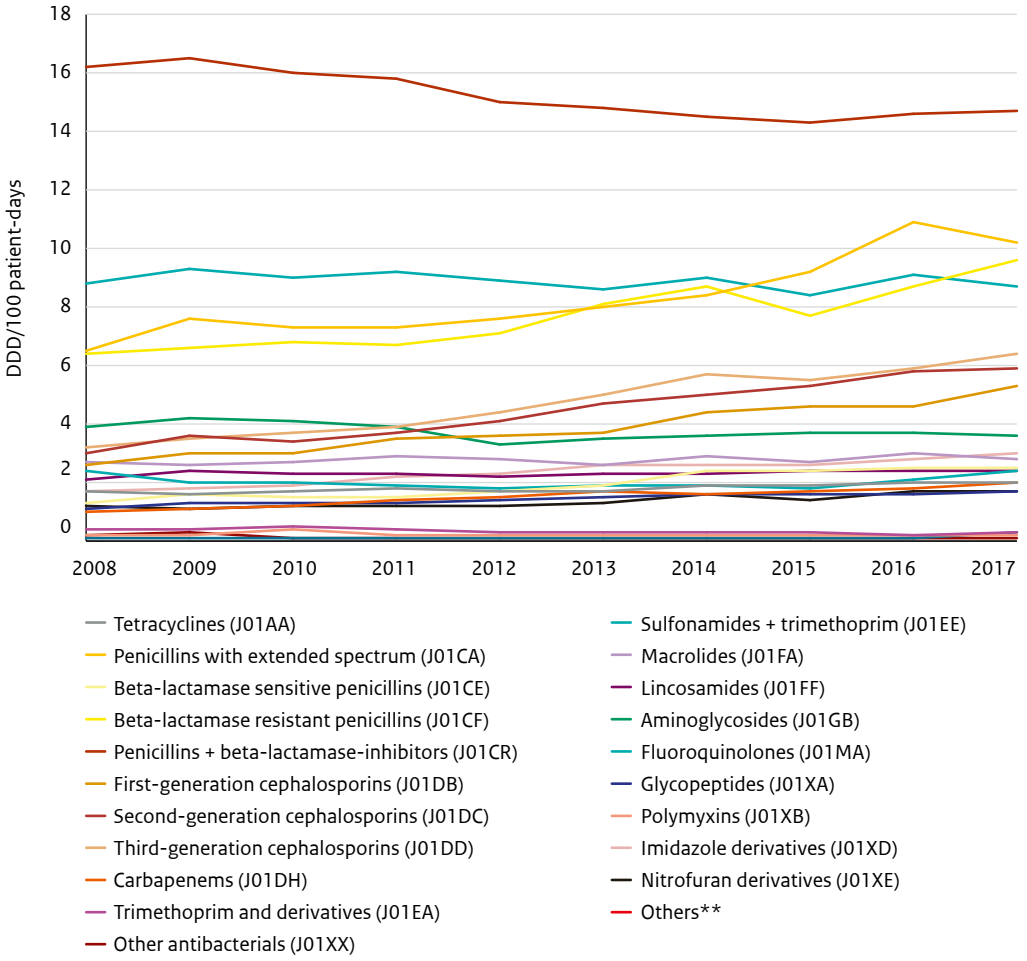


Figure 3.2.1.2 Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC-5 level, 2008-2017 (source: SWAB).

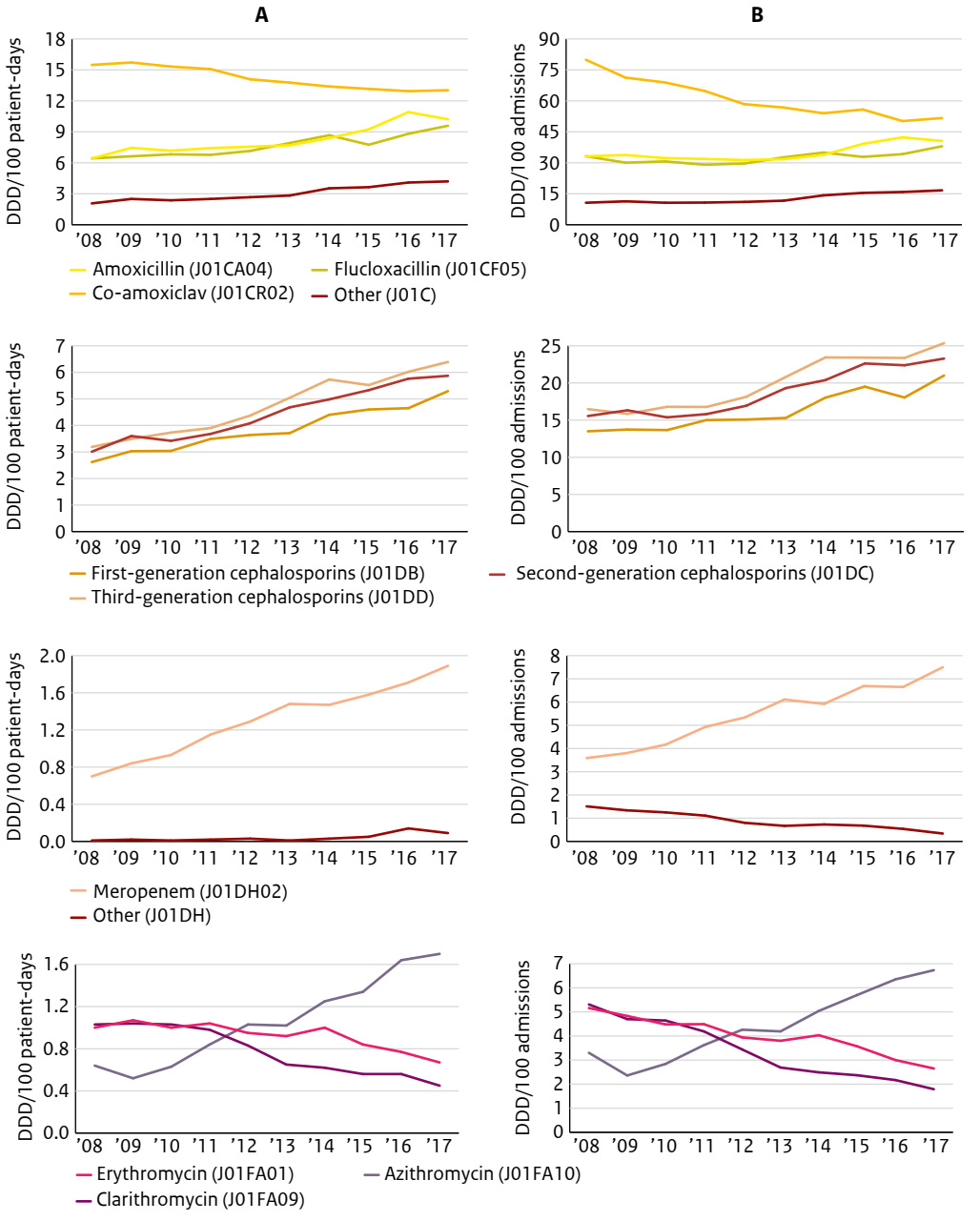


Figure 3.2.1.2 (continued) Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC-5 level, 2008-2017 (source: SWAB).

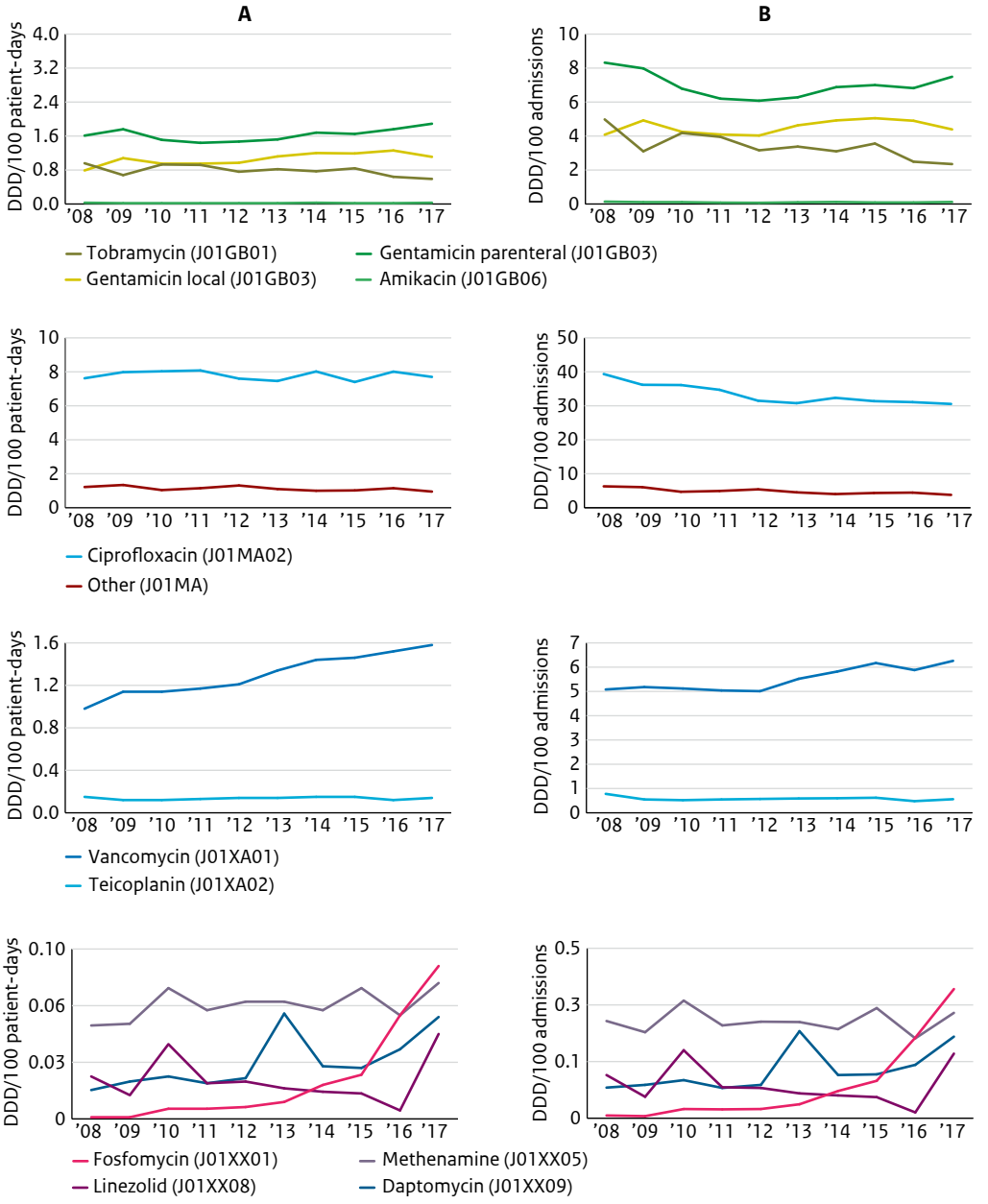


Figure 3.2.1.3 Total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals in 2017 (source: SWAB).

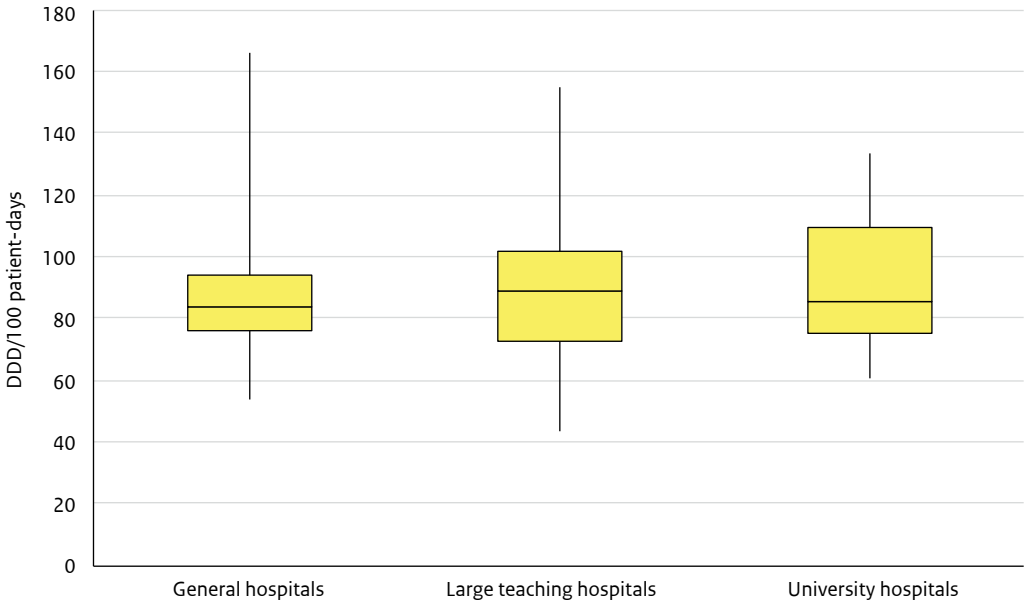


Figure 3.2.1.4 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2017 (source: SWAB).

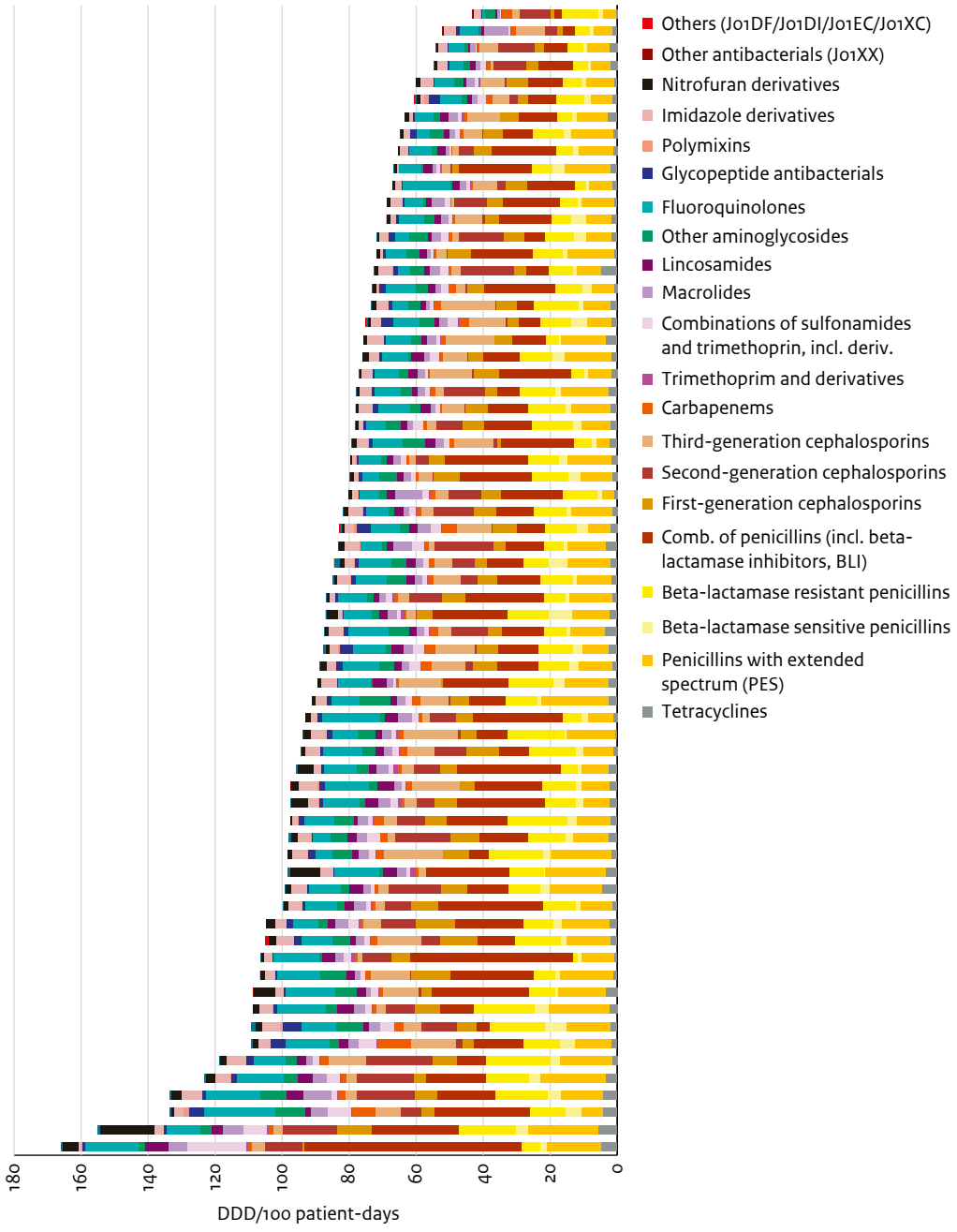


Figure 3.2.1.5 Use of 1st, 2nd and 3th-generation cephalosporins in university, large teaching and general hospitals at ATC-5 level in 2017 (source: SWAB).

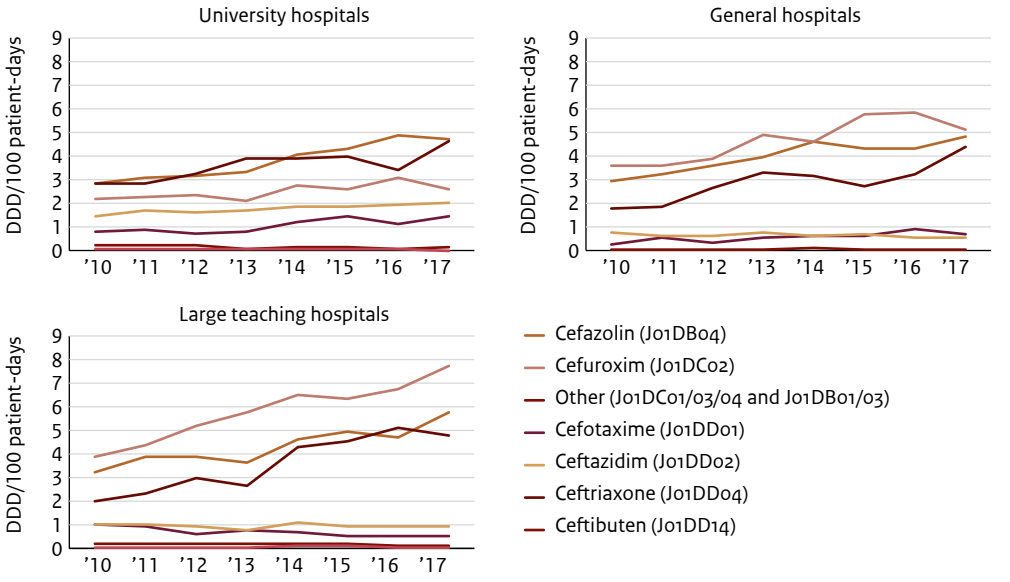


Figure 3.2.1.6 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2017 (source: SWAB).

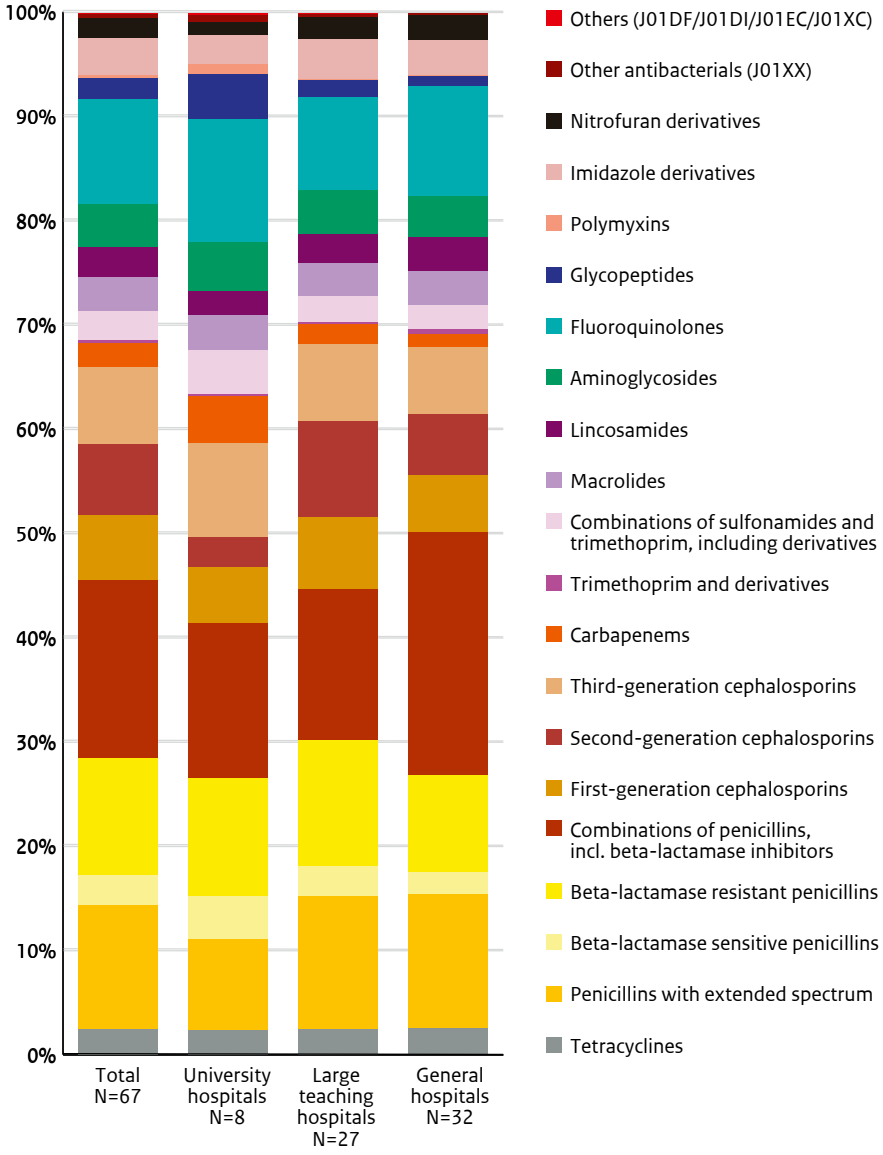


Figure 3.2.1.7 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2008-2017 (source: SWAB).

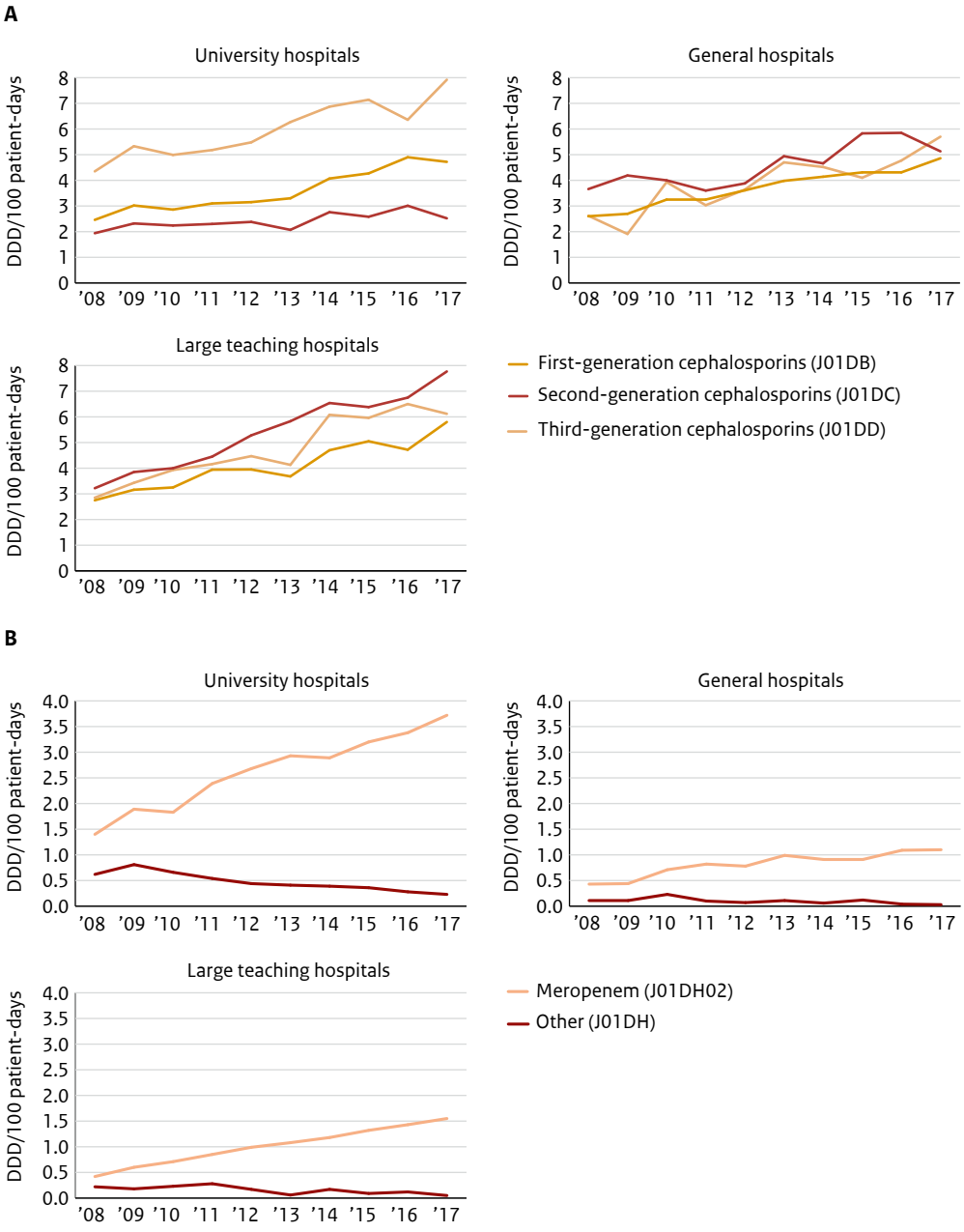
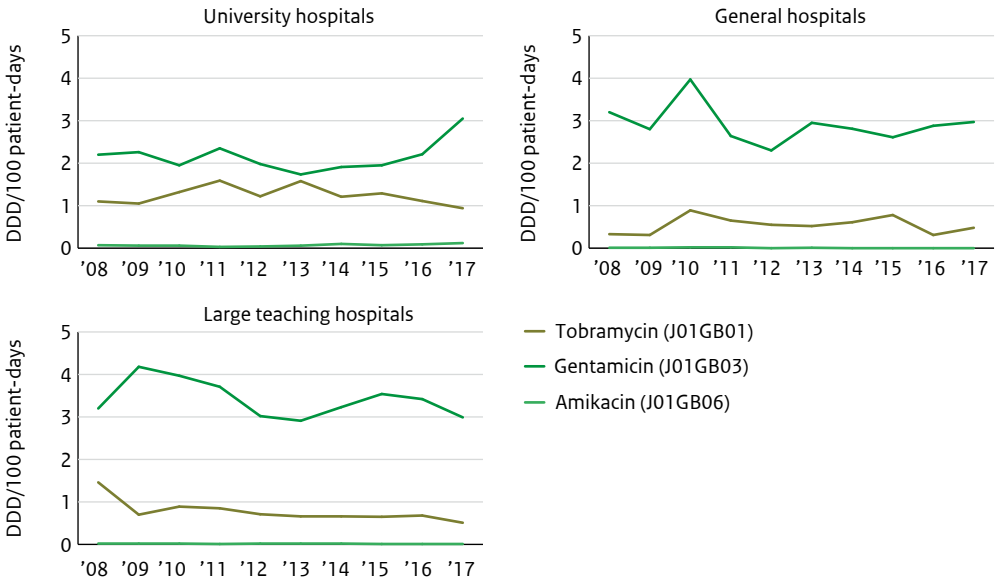


Figure 3.2.1.7 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2008-2017 (source: SWAB).

C



D

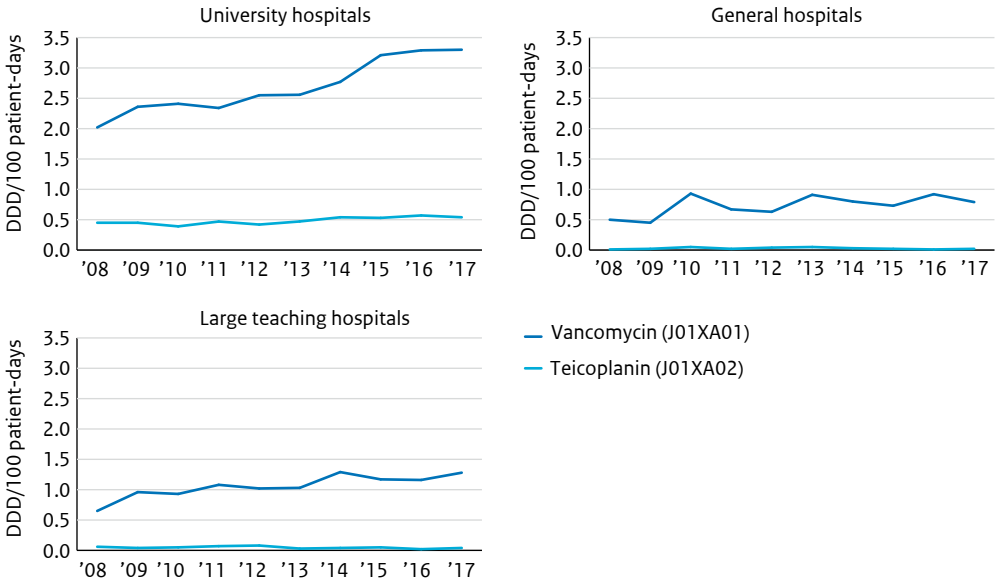


Figure 3.2.1.7 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2008-2017 (source: SWAB).

E

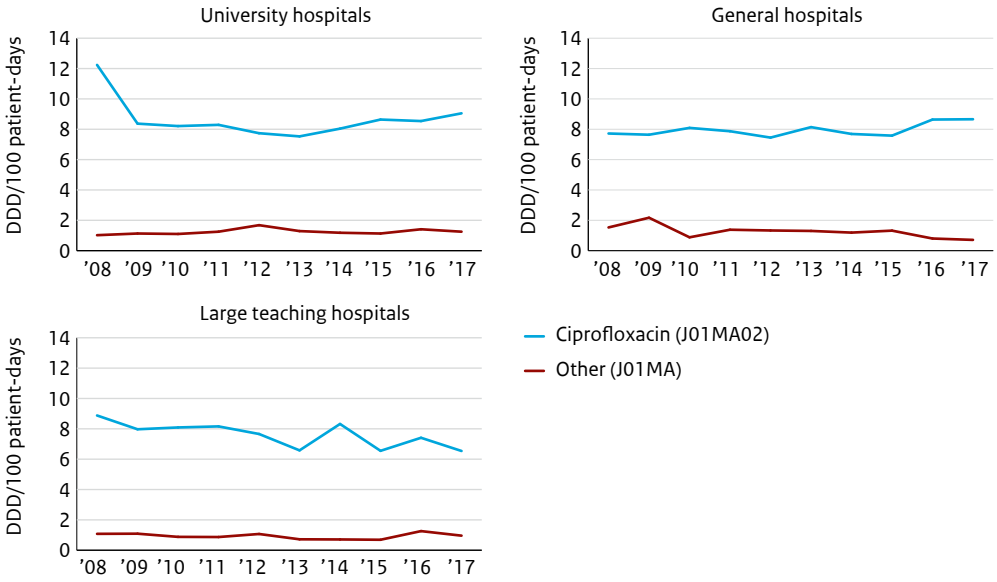
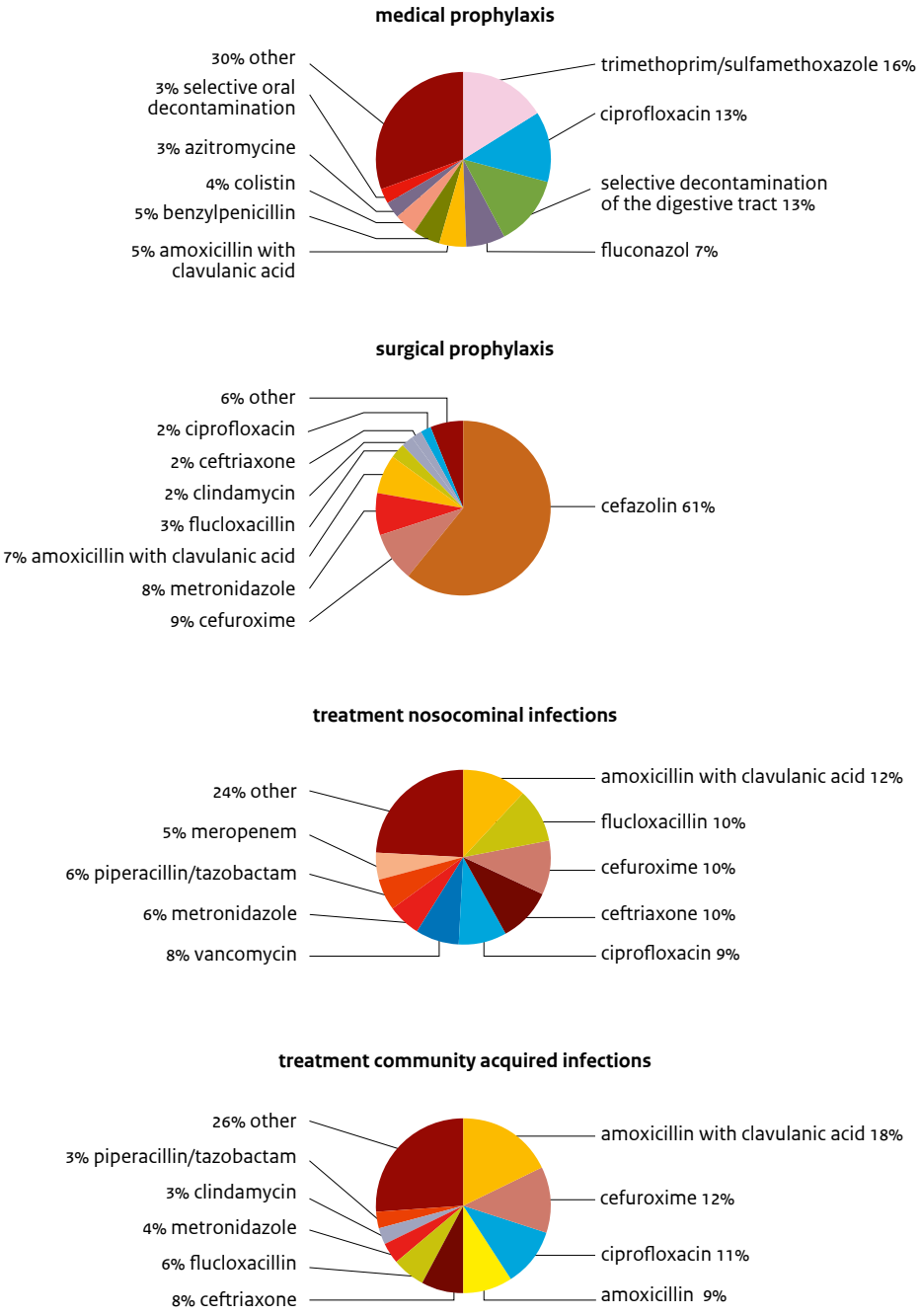


Table 3.2.1.3 Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2008-2017 (source: SWAB).

ATC Group*	Therapeutic group	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J02AA01	Antibiotics (amphotericin B)	1.12	1.35	1.65	1.77	2.43	3.01	3.46	4.17	4.34	4.80
J02AB02	Imidazole derivatives (ketoconazole)	0.11	0.08	0.15	0.09	0.10	0.06	0.24	0.34	0.04	0.08
J02AC	Triazole derivatives	6.36	6.72	6.31	5.83	6.25	6.29	7.15	7.55	9.22	7.80
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.40	0.61	0.56	0.57	0.55	0.71	0.61	0.64	0.64	0.96
J02	**Antimycotics for systemic use (total)**	**7.98**	**8.77**	**8.66**	**8.26**	**9.33**	**10.06**	**11.47**	**12.70**	**14.23**	**13.63**
J04AA	Aminosalicylic acid and derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J04AB	Antibiotics (mainly rifampicin)	1.34	1.27	1.41	1.56	1.24	1.43	1.39	1.33	1.13	1.69
J04AC	Hydrazides (mainly isoniazide)	0.29	0.40	0.34	0.30	0.40	0.57	0.56	0.35	0.30	0.67
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.12	0.14	0.01
J04AK	Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol)	0.31	0.34	0.37	0.26	0.31	0.16	0.28	0.19	0.15	0.66
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.01	0.02	0.04	0.07	0.11	0.15
J04BA	Drug for treatment of leprosy (dapson)	0.39	0.33	0.45	0.49	0.62	0.70	0.60	0.70	0.71	1.13
J04	**Antimycobacterials for systemic use (total)**	**2.33**	**2.35**	**2.58**	**2.62**	**2.57**	**2.88**	**2.87**	**2.76**	**2.55**	**4.31**
J05AB	Nucleosides excl. Reverse transcriptase inhibitors (J05AB)	2.00	2.22	2.02	2.18	2.24	2.33	2.71	2.76	2.97	2.99
J05AD	Phosphonic acid derivatives (J05AD)	0.11	0.13	0.10	0.10	0.15	0.12	0.16	0.14	0.20	0.20
J05AE	Protease inhibitors (J05AE)	0.92	0.75	0.78	0.55	0.81	0.63	0.40	0.33	0.30	0.31
J05AF	Nucleoside reverse transcriptase inhibitors (J05AF)	0.74	0.64	0.67	0.63	0.69	0.54	0.59	0.71	0.52	0.70
J05AG	Non-nucleoside reverse transcriptase inhibitors (J05AG)	0.25	0.23	0.22	0.14	0.18	0.16	0.18	0.23	0.22	0.26
J05AH	Neuraminidase inhibitors (J05AH)	0.05	n.a.#	0.21	0.42	0.19	0.49	0.16	0.30	0.43	0.31
J05AR	Antivirals for the treatment of HIV, combinations (J05AR)	0.52	0.55	0.76	0.69	0.91	0.89	0.94	0.95	0.99	1.12
J05AX	Other antivirals (J05AX)	0.06	0.06	0.15	0.17	0.24	0.29	0.22	0.33	0.46	0.72
J05	**Antivirals for systemic use (total)**	**4.65**	**4.59**	**4.91**	**4.89**	**5.41**	**5.47**	**5.37**	**5.75**	**6.09**	**6.60**

** from the 2017 edition of the Anatomical Therapeutic Chemical (ATC) classification system*
 # Total use not to be assesed because of alternative distribution during the pandemic

Figure 3.2.1.8 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2018 (source: PREZIES).



3.2.2 Hospital antibiotic use in days of therapy (DOT)

Methods

Electronic prescriptions for antibiotics on patient level were extracted from Dutch hospital electronic prescribing systems over 2017. From these data the number of DOT was calculated and expressed as DOT/100 patient-days, taking day of discharge into consideration. The method for calculation of the number of patient-days is described in chapter 3.2.1. To compare these results to antibiotic use expressed in DDD a ratio diving the number of DDD/100 patient-days by the numbers of DOT/100 patient-days per ATC4-code was calculated.

Results

Data over 2017 was evaluated for 11 hospitals (5 large teaching hospitals and 6 general hospitals) and number of DOT/100 patient-days for antibiotics restricted to in-hospital use is shown in table 3.2.2.1. Especially the use of penicillins with extended spectrum, beta-lactamase sensitive en resistant penicillins, combination of penicillins, including beta-lactamase-inhibitors and aminoglycosides expressed as DOT/100 patient-days was lower compared to their use expressed as DDD/100 patient-days, resulting in a DDD/DOT-ratio >1. The DDD/DOT-ratios for carbapenems, first- and second generation cephalosporins were also above 1. In contrast, the number of DOT/100 patient-days for third-generation cephalosporins and nitrofuran derivates exceeded the number of DDD/100 patient-days and resulted in the lowest DDD/DOT-ratios (<1).

Discussion

Antibiotic use expressed as DOT/100 patient-days informs on patient level exposure to antibiotics. Differences observed between antibiotic use expressed as DDD/100 patient-days and DOT/100 patient-days might be explained by differences in DDD and the actual prescribed daily antibiotic dose that is given in clinical practice. For penicillins, aminoglycosides, carbapenems and cephalosporins probably higher doses, that exceed the actual DDD, are given to individual patients. A DDD/DOT-ratio <1 could reflect the use of lower antibiotic dosages as compared to the assigned DDD by the WHO or prophylactic antibiotic use. In the future, the course of the ratio between the DDD and DOT per 100 patient-days could provide more information on, for instance, potential dose inflation or extension of indications.

Table 3.2.2.1 Antibiotic use in hospitals expressed as days of therapy (DOT) /100 patient-days, DDD/100 patient-days and ratio DDD/DOT at ATC-4 level in 2017.

ATC Group*	Therapeutic group	DDD/100 patient-days	DOT/100 patient-days	Ratio DDD/DOT
J01AA	Tetracyclines	1.97	1.36	1.45
J01CA	Penicillins with extended spectrum	10.22	3.88	2.63
J01CE	Beta-lactamase sensitive penicillins	2.50	1.46	1.71
J01CF	Beta-lactamase resistant penicillins	9.59	3.03	3.16
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	14.73	11.24	1.31
J01DB	First-generation cephalosporins	5.29	4.31	1.23
J01DC	Second-generation cephalosporins	5.87	4.51	1.30
J01DD	Third-generation cephalosporins	6.39	8.11	0.79
J01DH	Carbapenems	1.98	1.24	1.60
J01EA	Trimethoprim and derivatives	0.27	0.31	0.88
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.38	1.82	1.31
J01FA	Macrolides	2.82	3.15	0.90
J01FF	Lincosamides	2.43	2.14	1.13
J01GB	Aminoglycosides	3.62	1.03	3.51
J01MA	Fluoroquinolones	8.65	8.17	1.06
J01XA	Glycopeptides	1.72	1.16	1.48
J01XB	Polymyxins	0.24	0.11	2.30
J01XD	Imidazole derivatives	3.00	2.80	1.07
J01XE	Nitrofuran derivatives	1.73	2.06	0.84

** From the 2017 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

3.3 Long-term care facilities

Methods

All hospital pharmacists participating in the SWAB surveillance of antibiotic use in hospitals were asked to provide the antibiotic consumption data from long-term care facilities their pharmacy is serving for 2017. In addition over 2017, long-term facilities from SNIV network of RIVM were also asked to provide the antibiotic consumption data. For each facility the amount of DDD/1,000 residents/day was calculated, while assuming occupancy of 100%, and their weighted mean, capacity based, was calculated.
In long-term care facilities of the SNIV network of RIVM, in 2018 a prevalence study was performed comparable to the intramural methods described above.[6] Dutch long-term care facilities participating in SNIV collected detailed data on antibiotic usage (according to the methodology proposed by the ECDC), combined with the SNIV prevalence study on healthcare associated infections. All residents admitted to somatic, psychogeriatric and geriatric revalidation departments 48 hours before the registration date, and present in the long-term care facilities on the registration date, were included. Only systemic and topical antibacterials were included, with a maximum of four concomitant substances per patient.

Results

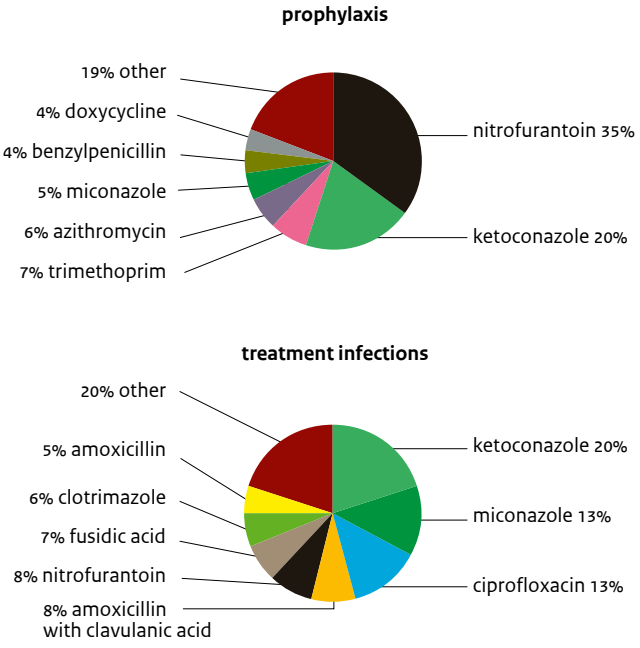
The antibiotic use of 10085 residents of long-term facilities was included in data analysis for 2017.
The size of long-term facilities varied from 63 to 2555 residents per home, with a mean of 593 residents. In comparison to 2016, the mean antibiotic use in long-term care facilities decreased by 4.3 DDD/1,000 residents/day to 52.9 DDD/1,000 residents/day. The use varied highly with a minimum of 17.4 and a maximum of 117.6 DDD/1,000 residents/day. Especially, the use of penicillins with extended spectrum, flouroquinolones and nitrofuran derivates decreased, all with ~1 DDD/1,000 residents/day, to 4.6, 6.9 and 8.3 DDD/1,000 residents/day, respectively. In contrast to 2016, the use of tetracyclines decreased by 0.9 DDD/1,000 residents/day to 4.0 DDD/1,000 residents/day (Table 3.3.1). Within the long-term care facilities of the SNIV network of RIVM (n=10) the overall total of antibiotic use for systemic use expressed as DDD/1,000 residents/day was 39.4 in 2017.
Figure 3.3.1 depicts antibiotics used in the prevalence study performed in 36 long-term care facilities of the SNIV network of RIVM in 2018. A total of 3486 residents were participating with a total of 228 prescriptions. For prophylaxis nitrofurantoin is used the most (35% of total prophylactic use).

Table 3.3.1 Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, expressed as DDD/1,000 residents/day, 2011-2017 (source: SWAB).

ATC group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	5.4	6.0	6.2	4.7	3.9	4.9	4.0
J01CA	Penicillins with extended spectrum	4.5	6.6	4.3	5.1	5.0	5.6	4.6
J01CE	Beta-lactamase sensitive penicillins	0.3	0.2	0.5	0.5	0.7	0.3	0.6
J01CF	Beta-lactamase resistant penicillins	2.5	3.7	1.7	1.4	2.3	1.8	2.2
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	18.8	18.8	19.5	16.3	17.9	16.1	15.5
J01DB	First-generation cephalosporins	0.0	0.0	0.0	0.1	0.1	0.0	0.2
J01DC	Second-generation cephalosporins	0.2	0.1	0.2	0.1	0.2	0.1	0.3
J01DD	Third-generation cephalosporins	0.5	1.0	0.6	0.6	0.8	0.4	0.5
J01DH	Carbapenems	0.1	0.0	0.0	0.0	0.1	0.0	0.1
J01EA	Trimethoprim and derivatives	2.2	2.3	2.4	1.9	1.4	1.6	1.6
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	3.2	2.5	1.7	1.5	1.6	1.1	1.2
J01FA	Macrolides	1.8	2.1	1.8	1.8	2.1	2.4	2.8
J01FF	Lincosamides	3.1	4.0	2.4	2.0	2.6	3.7	2.9
J01GB	Aminoglycosides	0.1	0.1	0.0	0.2	0.2	0.1	0.3
J01MA	Fluoroquinolones	10.3	10.7	8.3	8.4	8.9	8.2	6.9
J01XA	Glycopeptides	0.1	0.1	0.1	0.1	0.2	0.1	0.2
J01XB	Polymyxins	0.3	0.2	0.0	0.0	0.1	0.2	0.0
J01XD	Imidazole derivatives	0.1	0.1	0.0	0.1	0.1	0.1	0.1
J01XE	Nitrofuran derivatives	9.5	11.0	11.1	10.4	11.4	9.6	8.3
J01XX	Other antibacterials	0.5	0.6	0.4	0.2	0.5	0.8	0.8
	Others**	0.4	0.1	0.0	0.0	0.0	0.0	0.0
J01	**Antibiotics for systemic use (total)**	**63.8**	**70.3**	**61.1**	**55.3**	**60.0**	**57.2**	**52.9**

** From the 2017 edition of the Anatomical Therapeutic Chemical (ATC) classification system*
*** J01DI, J01DF, J01EC and J01XC*

Figure 3.3.1 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2018 (source: SNIV).



Discussion

Although the antibiotic use in long-term care facilities decreased in 2017, compared with previous years, more or less the same pattern of usage is seen. Amoxicillin with clavulanic acid, fluoroquinolones and nitrofuran derivatives are still the most widely used antibiotics in long-term care facilities. Nevertheless, the observed decline in the use of combinations of penicillins with beta-lactamase-inhibitors in 2017 is promising. The high use of nitrofurantoin is not surprising, as urinary tract infections are one of the most common infections among elderly patients. With respect to broad spectrum antibiotics, the high use of fluoroquinolones is especially worrisome.

The overall total antibiotic use in 10 SNIV long-term care facilities, which provided data expressed as DDD/1,000 residents/day is lower compared with the overall total antibiotic use in SWAB long-term care facilities. This could be explained by smaller numbers of residents in SNIV long-term care facilities compared with SWAB long-term care facilities, whereby precision of data decreases.

The results of the point prevalence study in the long-term care facilities of the SNIV show a somewhat different pattern of usage compared with SWAB surveillance data. SNIV data are based on prescriptions on an index day, whereas overall use is based on DDD's collected over 365 days.

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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 laboratories in The Netherlands, including underlying minimal inhibitory concentration (MIC) values and disk zone diameters, are collected in the Infectious Diseases Surveillance Information System for Antibiotic 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 2018, 47 laboratories were connected to ISIS-AR, all conducting antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 47 laboratories, 32 provided complete data over the five most recent years (2014 to 2018). Four of these laboratories exclusively served university hospitals, 26 laboratories served non-university hospitals, general practitioners and long-term care facilities and two laboratories only served general practitioners and long-term care facilities. For most analyses in the current report we selected only data from these 32 laboratories, to avoid bias in time trends of resistance percentages due to incomplete data. We calculated resistance percentages and linear time trends over the five most recent years (2014 to 2018) for the most prevalent pathogens in combination with their main antimicrobial treatment options. For calculation of resistance percentages for pathogens for which we did not calculate time trends (for details see paragraph on time trends below) we used data from 40 laboratories for which at least complete data for the year 2018 were available (six serving university hospitals, 32 serving non-university hospitals, general practitioners, and long-term care facilities, and two serving general practitioners and long-term care facilities only). For *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus* isolates from general practitioners' patients we conducted an extra analysis to calculate resistance to a selection of antibiotics in 2018 by regional cooperative network (for more information on regional cooperative networks see https://www.ggdghorkennisnet.nl/thema/antibioticaresistentie/nieuws/8176-factsheet-regionale-zorgnetwerken-antibioticaresistentie).

For this analysis we used data from a subset of 34 non-university laboratories for which at least complete data for the year 2018 were available.

Selection of isolates

We calculated resistance levels and, if applicable, time trends by site; i.e. general practice, outpatient departments, inpatient departments (excl. intensive care units), intensive care units, urology departments (inpatient and outpatient separately), and long-term care facilities. For a selection of antibiotics we calculated resistance in isolates from general practitioners' patients by regional cooperative network. For general practices (chapter 4.2) and long-term care facilities (chapter 4.4) we selected urinary isolates for analysis of resistance in Enterobacteriaceae and *P. aeruginosa*, and wound,pus, orskin isolates for analysis of resistance in *Staphylococcus aureus*. For outpatient departments (chapter 4.3.1), inpatient departments (excl. intensive care units, chapter 4.3.2), and intensive care units (chapter 4.3.3), we calculated resistance levels based on isolates from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound,pus, orskin. Additionally, we conducted a separate analysis for blood isolates from inpatients (incl. patients from intensive care units, chapter 4.3.4). For urology departments (chapter 4.3.5) we selected only urinary isolates. Finally, in chapter 4.3.6, we performed a separate analysis on respiratory pathogens (*Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*), separately for general practitioners' patients and hospital patients. For this analysis we selected isolates from higher respiratory tract and lower respiratory tract, and in the analysis on hospital patients additionally blood and cerebrospinal fluid.
To avoid bias due to repeated sampling in the calculation of resistance levels and time trends, we selected for each chapter the first isolate per species per patient per year. We excluded data on non-diagnostic samples, and only calculated resistance levels for pathogens for which at least 100 isolates were available for analysis. Furthermore, to avoid bias due to selective testing of antibiotics, 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 each pathogen-agent combination if at least 50% of laboratories could be included.

Calculation of resistance levels

We calculated the percentage of resistant isolates ('R'). To avoid bias due to differences in breakpoint guidelines and expert rules used in the participating laboratories, we conducted these calculations using reinterpreted crude test values according to EUCAST breakpoints, version 8.1. However, reinterpretation of test values does not take into account differences in testing methods that result in higher or lower test values. In 2016 a new testpanel for Gram-negative bacteria was introduced for the VITEK2 automated system (Biomérieux), which is the automated system used by most laboratories. In this testpanel resistance to co-amoxiclav is tested according to EUCAST guidelines, using a fixed concentration (2 mg/L) of clavulanic acid, irrespective of the concentration of amoxicillin. Before the introduction of the new panel, resistance was tested according to the guidelines from Clinical and Laboratory Standards Institute (CLSI), using a fixed 2:1 ratio between amoxicillin and clavulanic acid. The use of a fixed clavulanic acid concentration results in higher MIC values for co-amoxiclav, which subsequently influences resistance in Gram-negative bacteria from 2016 onward to higher levels than before. The magnitude of this effect may vary, depending on the microorganism.

Furthermore, for co-amoxiclav the 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 resistance breakpoint of >32 mg/L. Therefore, in chapters 4.2 through 4.4 we only present resistance to co-amoxiclav according to the breakpoint for non-uncomplicated urinary tract infections.

For most included pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* complex, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and coagulase-negative *Staphylococcus* spp. including *Staphylococcus epidermidis*) at least 80% of the reported test values in each year were reinterpretable according to EUCAST clinical breakpoints version 8.1. When reinterpretation was not possible, this was because of missing crude data or test values that were not compatible with EUCAST breakpoints version 8.1. For *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* less than 80% of test values could be reinterpreted. Therefore, for these pathogens we calculated resistance percentages based on S/I/R interpretations as reported by laboratories.

Because data on inducible clindamycin resistance tests was often not available in ISIS-AR, we calculated resistance levels for clindamycin including inducible resistance based on laboratory S/I/R interpretation, for which we assumed that results of inducible resistance tests are taken into account.
Because not all laboratories used cefoxitin disks to screen for MRSA, or reported flucloxacillin results based on cefoxitin screening methods, we estimated resistance to flucloxacillin in *S. aureus* and coagulase-negative *Staphylococcus* spp. based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin interpretation was available, for oxacillin/flucloxacillin.

As some laboratories did not report (benzyl)penicillin results for *S. pneumoniae* if the isolate was susceptible to oxacillin, we estimated resistance and non-susceptibility percentages based on laboratory screening results for oxacillin, or, if the isolate was screen-positive, on laboratory S/I/R interpretation for (benzyl)penicillin.

For some antibiotic agents presented in this report, comparable resistance mechanisms exist, namely benzylpenicillin/penicillin, amoxicillin/ampicillin, cefotaxime/ceftriaxone, meropenem/imipenem (except for *P. aeruginosa* because of different resistance mechanisms for meropenem and imipenem), and doxycycline/tetracycline. For these combinations, we calculated the percentage of isolates that was resistant to at least one of both agents. Additionally, for Gram-negative bacteria except *E. cloacae* complex and *Acinetobacter* spp., we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (gentamicin + amoxicillin/ampicillin, gentamicin + co-amoxiclav, gentamicin + cefuroxime, gentamicin + cefotaxime/ceftriaxone, gentamicin + ceftazidime, gentamicin + piperacillin-tazobactam, tobramycin + ceftazidime, and tobramycin + ciprofloxacin). For these combinations, we defined resistance as resistance to both agents.

For *S. aureus* and coagulase-negative *Staphylococcus* spp. we calculated resistance to ciprofloxacin as class indicator for resistance to fluoroquinolones. However, ciprofloxacin should not be considered a first choice for treatment of infections with these microorganisms.

To calculate the percentage of highly resistant microorganisms (HRMO) we used the definitions of the Working Group on Infection Prevention (WIP, http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP). We considered Enterobacteriaceae (except *Enterobacter cloacae* complex) an HRMO if they were 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem. We considered *E. cloacae* complex an HRMO if either one or both of the situations 2 and 3 as described for all Enterobacteriaceae was true. We considered *P. aeruginosa* an HRMO if resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin-tazobactam. Finally, for *Acinetobacter* spp. we defined HRMO as either one or both of the following: 1) carbapenemase producing, estimated by carbapenemase confirmatory tests, or if no data on confirmatory tests were available, by resistance to imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.

In addition, for Enterobacteriaceae isolates from general practices, outpatient departments, urology departments, and long-term care facilities, we calculated multidrug resistance, which we defined as resistance to the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole combined.
We compared resistance levels in general practitioners' patients within the regional cooperative networks with the resistance percentage in all regions combined. We considered a difference with a p-value of <0.05 statistically significant. We considered a difference that was larger than the square root of the resistance percentage in all regions combined as clinically relevant. Statistically significant and clinically relevant differences in resistance percentages are given in the figures by an asterisk.

Calculation of time trends

For chapters 4.2 through 4.3.5, we calculated in addition to resistance levels in 2018, time trends over the five most recent years (2014 to 2018), using logistic regression models. 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 (for criteria see 'Calculation of resistance levels'-section above). We made an exception for trends in resistance for flucloxacillin and clindamycin including inducible resistance in *S. aureus*, which we based in laboratory S/I/R interpretation. However, we do not expect spurious time trends in resistance for these two pathogen-antibiotic combinations, because EUCAST breakpoints for these combinations were not changed between 2014 and 2018. However, for coagulase-negative *Staphylococcus* spp. breakpoints for cefoxitin were changed in 2017. Therefore, we did not calculate a time trend for flucloxacillin resistance in this pathogen.

We considered two-sided p-values <0.05 statistically significant. When the absolute difference in predicted resistance from the logistic regression model between 2014 and 2018 was larger than the square root of the predicted resistance of 2014, we considered the trend clinically relevant. Statistically significant increasing trends that are considered clinically relevant are shown in the tables as a red coloured font, whereas decreasing trends that meet the same criteria are shown as a green coloured font. In addition, for each pathogen-agent combination for which the resistance levels were between 0.5% and 30% in at least three years the resistance levels from 2014 to 2018 are shown in bar charts.

4.1.2 Description of the ISIS-AR data

In this chapter a number of descriptive characteristics of the data from the ISIS-AR antimicrobial resistance surveillance system is 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 chapters 4.2 through 4.4, 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 chapter 4.2 through 4.4 (see methods section for inclusion criteria). In table 4.1.2.1 descriptive characteristics of included isolates are listed by pathogen.

Figure 4.1.2.1 Smoothed geographical distribution of isolates, based on percentage of inhabitants for whom at least one isolate was included in the analyses in chapters 4.2 through 4.4, by 4-digit postal code area, together with geographical distribution of laboratories by status of connection to ISIS-AR and inclusion in the analyses in chapter 4.2 to 4.4, with regional cooperative network borders.

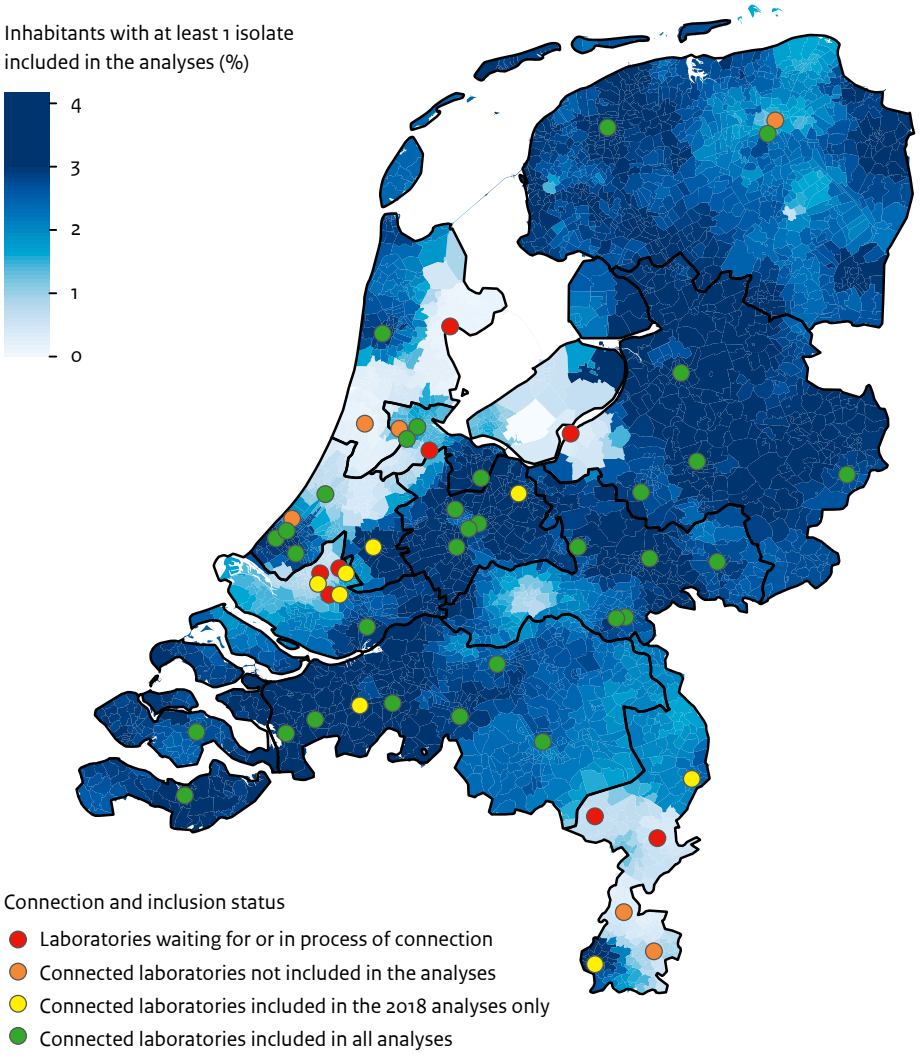


Table 4.1.2.1 Characteristics of 416,825 isolates included in the analyses in chapters 4.2 through 4.4, by pathogen.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*	CNS	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
Total number of isolates	178,288	29,945	23,806	10,914	24,981	4,585	29,991	6,491	61,277	25,573	6,031	11,614	3,329
Sex of patient (%)													
Male	28	34	42	55	54	52	53	53	53	52	55	53	51
Female	72	66	58	45	46	48	47	47	47	48	45	47	49
Type of care (%)													
General practitioners	56	44	44	27	29	43	38	8	24	18	6	10	11
Outpatient departments	16	21	20	25	31	26	24	11	40	13	27	42	38
Inpatient departments (excl. Intensive Care Units)	21	27	24	38	31	25	30	60	30	56	58	41	43
Intensive Care Units	2	3	2	6	4	4	3	19	4	13	9	6	6
Long-term care facilities	4	6	10	3	5	3	4	3	2	1	0	1	2
Age category of patient in years (%)													
0-4	3	1	3	4	2	4	4	1	5	5	6	8	9
5-18	5	2	2	2	6	4	2	1	7	5	3	4	3
19-64	34	28	22	30	29	32	29	31	44	42	37	35	29
>65	57	69	73	63	62	60	65	67	44	48	54	53	59
Isolate source (%)													
Blood	4	4	2	4	2	3	3	12	5	43	28	2	1
Lower respiratory tract	1	5	3	10	17	9	0	2	10	0	55	83	86
Urine	88	82	80	52	41	58	82	51	12	26	1	0	0
Wound/Pus/Skin	5	7	14	29	36	26	13	31	59	25	10	8	7
Other	3	3	2	4	4	4	2	4	15	6	6	7	6
Type of hospital (hospital isolates only, %)													
General	38	34	39	31	32	31	34	27	32	29	36	33	32
Top clinical	48	48	46	47	46	46	50	49	49	44	50	51	53
University hospital	15	18	15	22	22	23	17	24	19	27	14	16	15

CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis
The first isolate per patient, per microorganism, per type of care was selected.

Key results

- Included laboratories were well distributed throughout the country, although the proportion of laboratories of which the data could be included in the analyses was relatively low in the regions 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' (Figure 4.1.2.1).
- The distribution of included laboratories was reflected in the geographical distribution of isolates (Figure 4.1.2.1). The coverage was relatively high in the regions 'Noord Nederland', 'Euregio-Zwolle', 'Gelders Antibioticaresistentie & Infectiepreventie Netwerk' (GAIN)', 'Utrecht', and 'Noord-Brabant'. In the other regions the coverage was lower and less evenly distributed.
- *E. coli* (72%), *K. pneumoniae* (66%), and *P. mirabilis* (58%) were more often isolated from female patients, likely because women are more prone to urinary tract infections. For the other pathogens, the percentage of male and female patients was similar.
- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *S. aureus* were most often isolated from patients from general practitioners and outpatient departments (combined 62%-72%, depending on the pathogen), whereas a large part of *E. faecium* (79%), coagulase-negative *Staphylococcus* spp. (69%), and *S. pneumoniae* (67%) was isolated from inpatients.
- Most isolates originated from patients of 65 years and older (44-73%, depending on the pathogen).
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *E. faecium* were mainly isolated from urine (41-88%, depending on the pathogen), whereas *S. aureus* was mainly isolated from wound, pus, or skin (59%), coagulase-negative *Staphylococcus* spp. from blood (43%), and *H. influenzae*, *S. pneumoniae*, *M. catarrhalis* from the lower respiratory tract (55-86%).

4.2 Primary care

The distribution of pathogens in diagnostic urine and wound, pus, or skin samples from general practitioners' (GP) patients is presented in table 4.2.1. The resistance levels in 2018 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urinary samples are presented in table 4.2.2 and for *S. aureus* isolates from wound, pus, or skin samples in table 4.2.3. 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 urinary isolates are calculated separately for patients aged ≤12 years and patients aged >12 years. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and figure 4.2.4 (*S. aureus*). Finally, the smoothed geographical distribution of diagnostic isolates, and resistance levels for a selection of antibiotics in *E. coli*, *K. pneumoniae*, and *S. aureus* are shown by regional cooperative network in figures 4.2.2a and 4.2.2b (*E. coli*), 4.2.3a and 4.2.3b (*K. pneumoniae*), and 4.2.5 (*S. aureus*).

GPs usually send urinary, wound, pus, and skin samples for culture and susceptibility testing in case of antimicrobial therapy failure or (with regard to urinary 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 Enterobacteriaceae or *P. aeruginosa* or wound, pus, or skin infections caused by *S. aureus* presenting at the GP. Therefore, the patients from whom samples were taken are further referred to as 'selected general practitioners' patients'.

Table 4.2.1 Distribution of isolated pathogens in diagnostic urinary samples (by patient age category), and diagnostic wound, pus, and skin samples from selected general practitioners' patients, ISIS-AR 2018.

	Urine		Wound, pus, or skin
	Age≤12	Age>12	
Pathogen	N (%)	N (%)	N (%)
E. coli	8,495 (72)	84,909 (56)	649 (4)
K. pneumoniae	205 (2)	11,703 (8)	214 (1)
P. mirabilis	601 (5)	8,650 (6)	473 (3)
Other Enterobacteriaceae[1]	545 (5)	14,746 (10)	1,498 (8)
P. aeruginosa	193 (2)	3,781 (3)	2,558 (14)
Other non-fermenters[2]	145 (1)	2,347 (2)	682 (4)
Other Gram-negatives[3]	11 (0)	7 (0)	295 (2)
S. aureus	141 (1)	2,803 (2)	8,630 (48)
Other Gram-positives[4]	1,432 (12)	21,451 (14)	2,862 (16)

The first isolate per patient, per microorganism, per category (urine, age≤12; urine, age>12; wound, pus, or skin) was selected.

[1] *Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Morganella spp., Serratia spp., Proteus spp. (non-mirabilis), Providencia spp., Pantoea spp., Escherichia spp. (non-coli), Salmonella spp., Hafnia spp., Cronobacter spp., Shigella spp., Yersinia spp.*

[2] *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *H. influenzae, B. fragilis complex n.n.g., B. fragilis, H. pylori, C. jejuni, N. meningitidis.*

[4] *Enterococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Staphylococcus spp. (non-aureus), M. tuberculosis complex, M. tuberculosis.*

Table 4.2.2 Resistance levels (%) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients, by age category, ISIS-AR 2018.

	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	6	67	5	74	3	75	4	79
Antibiotic								
amoxicillin/ampicillin	33	38	-	-	18	21	-	-
co-amoxiclav[1]- non-uuti	27	30	27	18	5	6	-	-
cefuroxime	4	8	6	15	1	1	-	-
cefotaxime/ceftriaxone	2	4	1	5	0	1	-	-
ceftazidime	2	3	3	5	0	0	1	2
ciprofloxacin	5	11	5	14	7	10	1	10
gentamicin	3	4	1	2	4	5	0	3
tobramycin	3	4	1	3	2	3	0	0
fosfomycin	1	1	14	28	8	16	-	-
trimethoprim	21	23	10	22	24	33	-	-
co-trimoxazole	18	21	7	11	19	26	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
HRMO[2]	3	5	3	6	2	3	-	-
multidrug resistance[3] - non-uuti	1	4	1	3	1	1	-	-

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated

non-uuti = according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category.

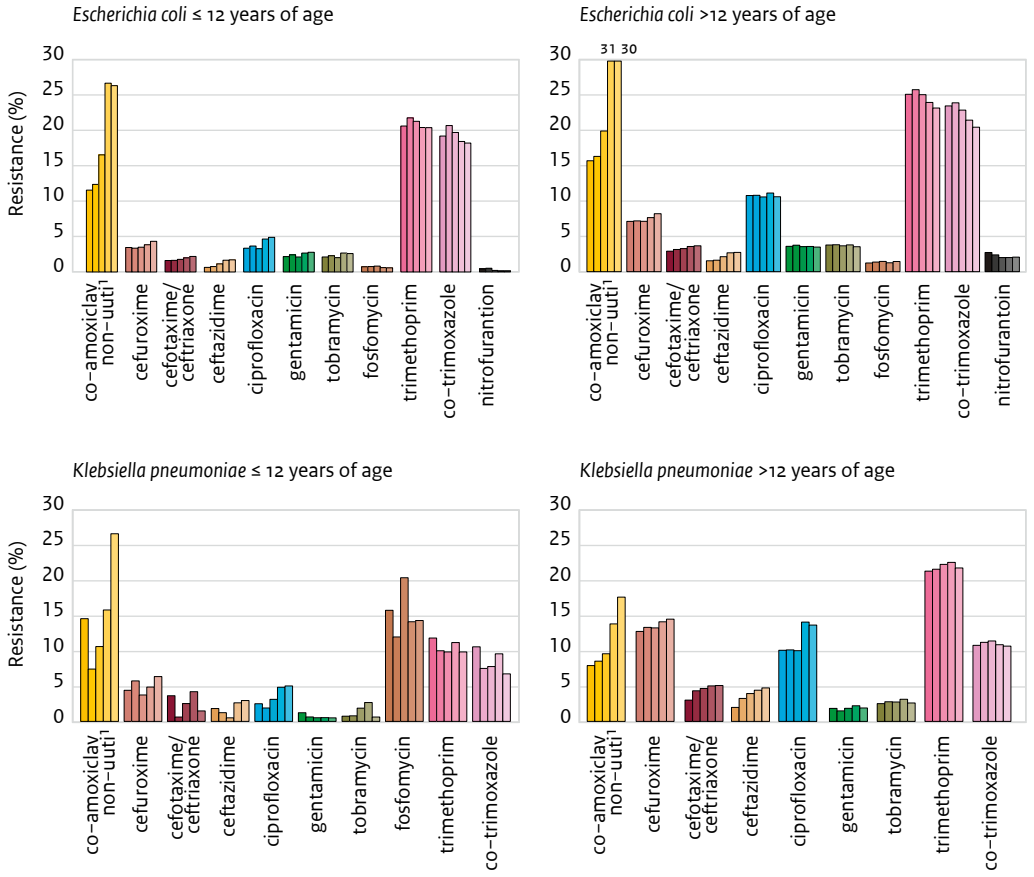
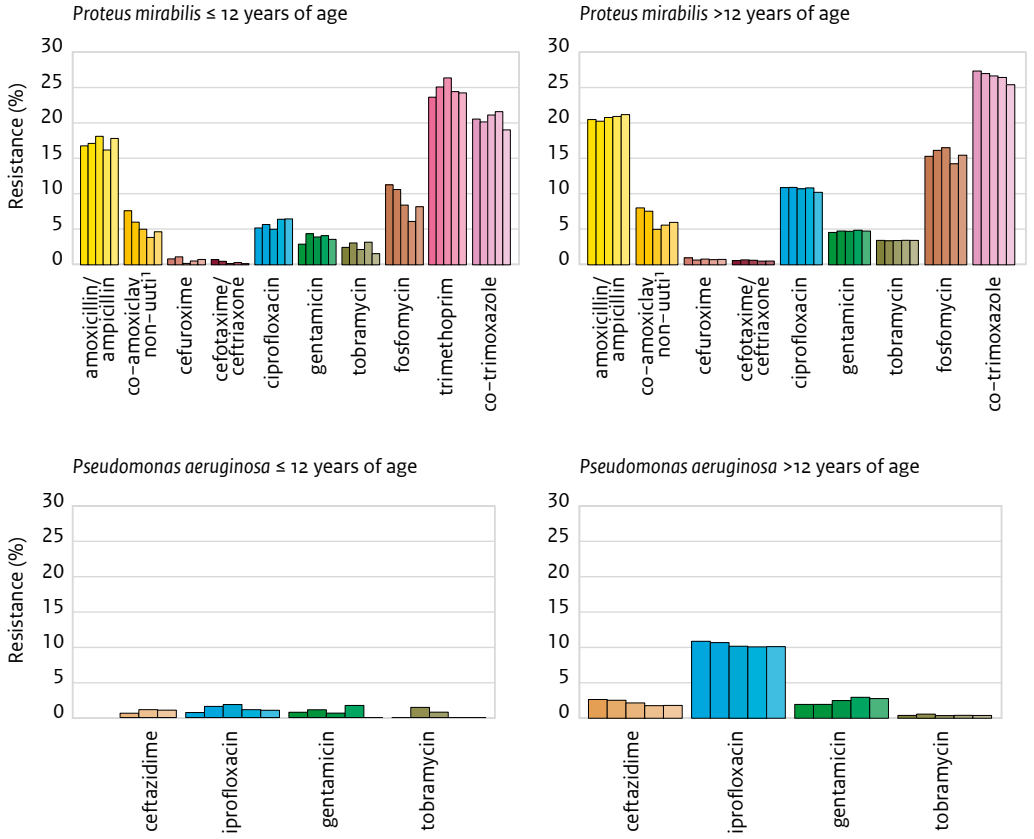


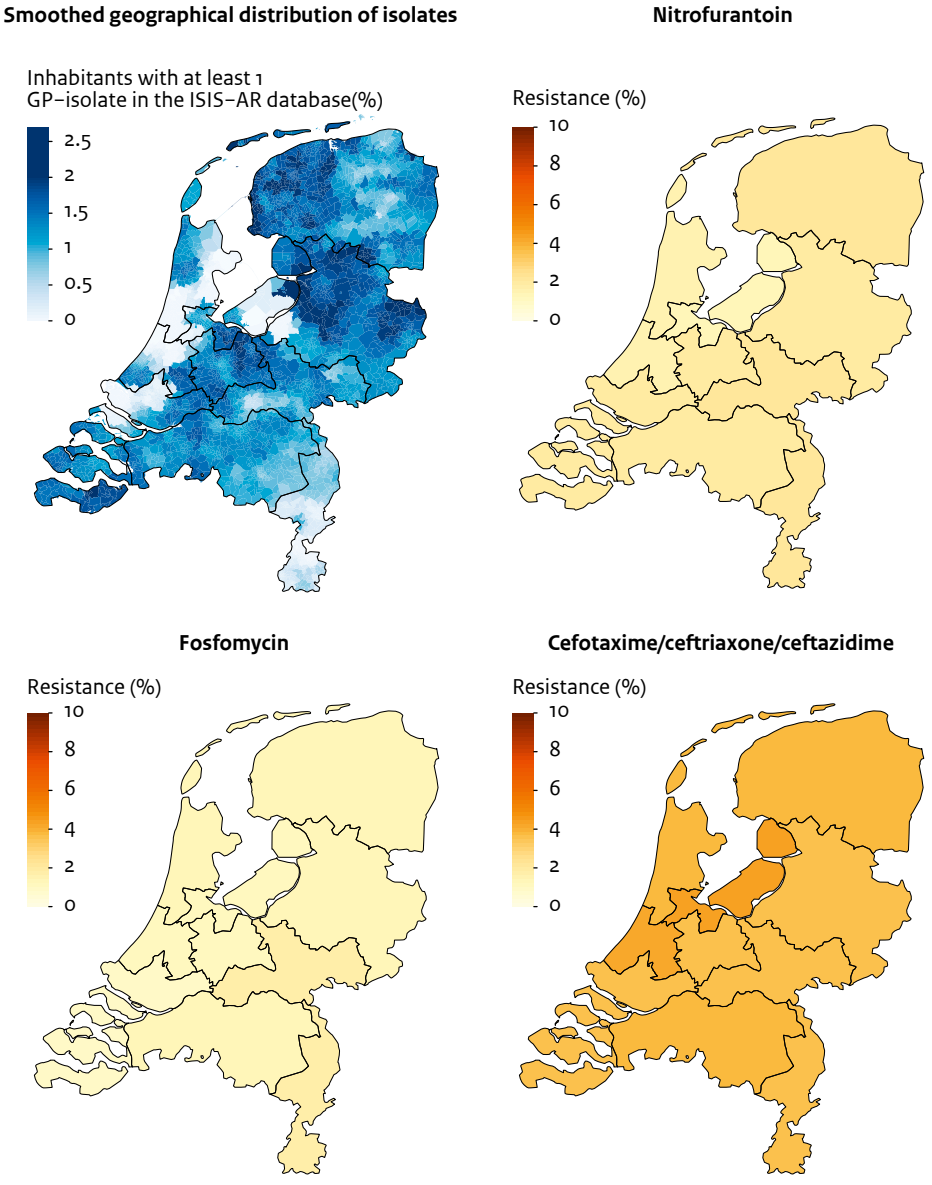
Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

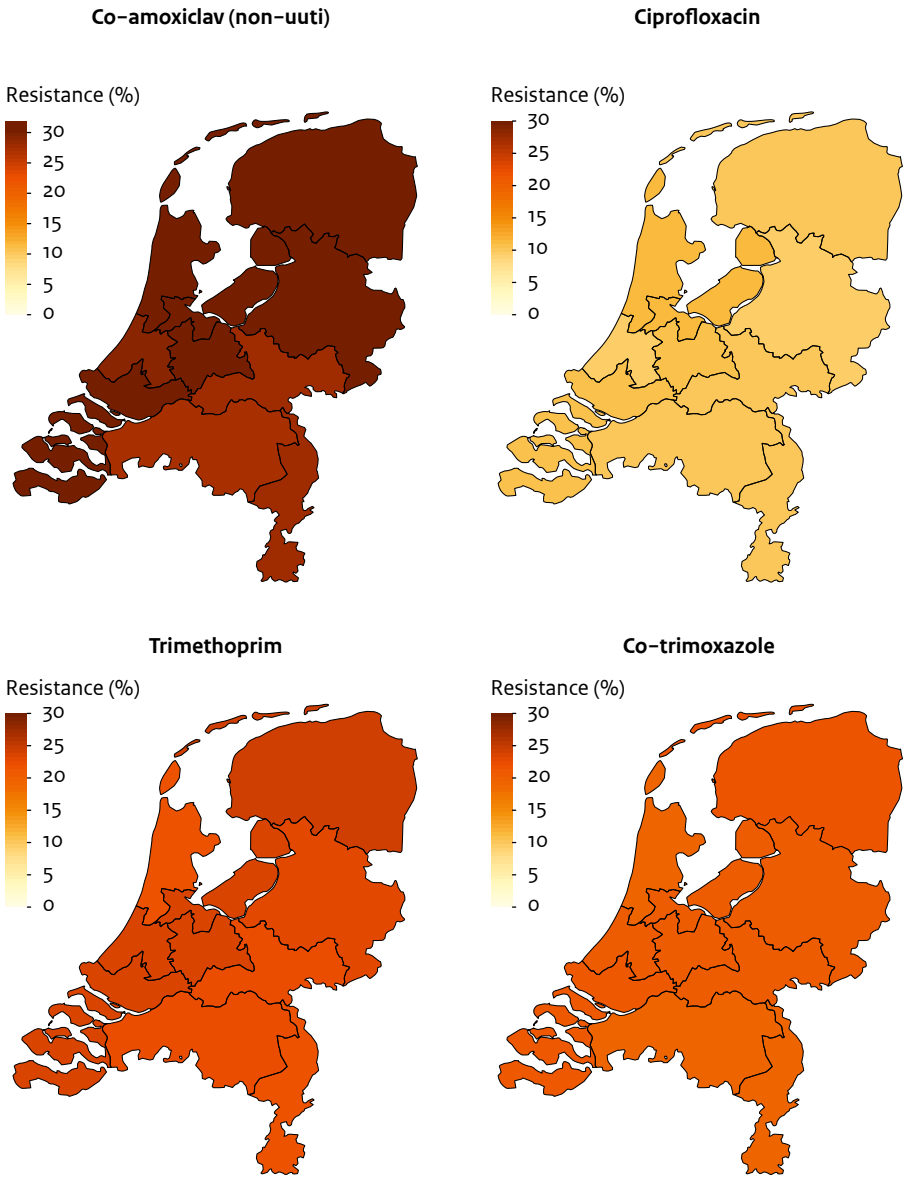
[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

Figure 4.2.2a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic urinary *E. coli* isolates on a gradient scale between 0 and 10% for nitrofurantoin, fosfomycin, and cefotaxime/ceftriaxone/ceftazidime by regional cooperative network, ISIS-AR 2018.



** Statistically significant and clinically relevant difference between resistance in the regional cooperative network and in all regions combined (for details see the methods section).*

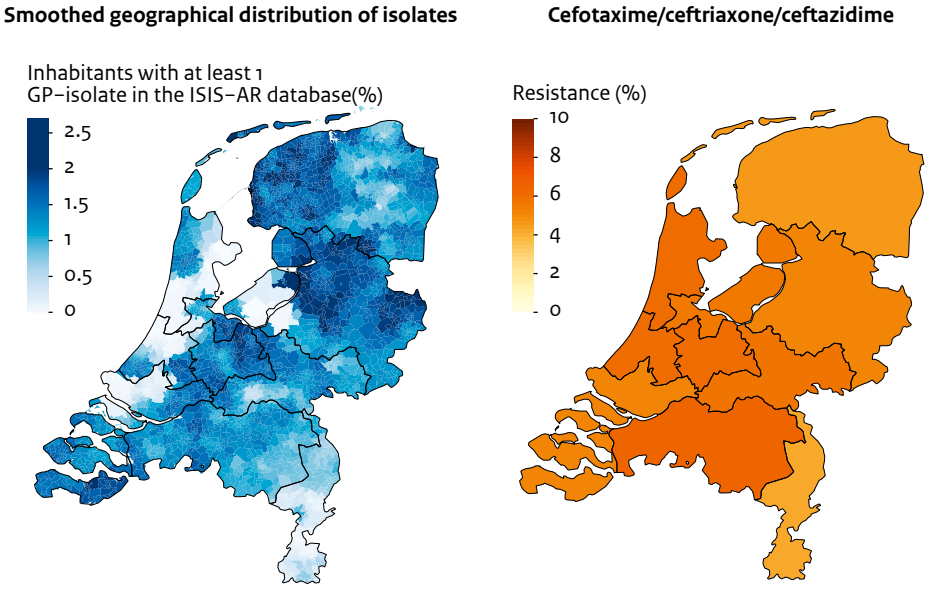
Figure 4.2.2b Resistance levels in diagnostic urinary *E. coli* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2018



** Statistically significant and clinically relevant difference between resistance in the regional cooperative network and in all regions combined (for details see the methods section).*

non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

Figure 4.2.3a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 10% for cefotaxime/ceftriaxone/ceftazidime by regional cooperative network, ISIS-AR 2018.



* *Statistically significant and clinically relevant difference between resistance in the regional cooperative network and in all regions combined (for details see the methods section).*

Figure 4.2.3b Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2018.

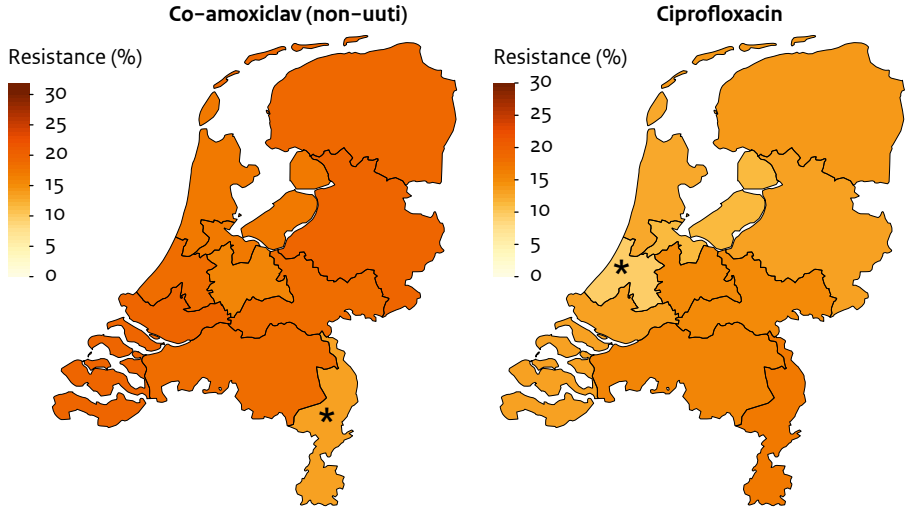
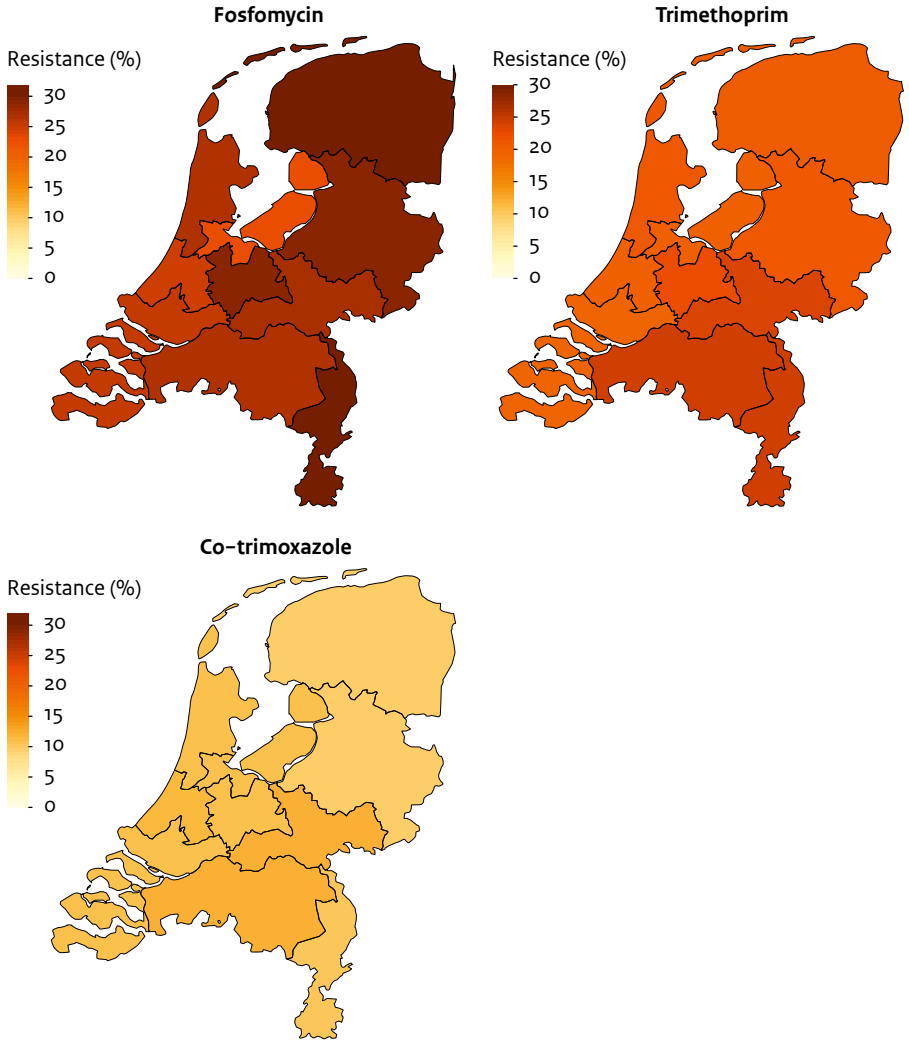


Figure 4.2.3b (continued) Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2018.



** Statistically significant and clinically relevant difference between resistance in the regional cooperative network and in all regions combined (for details see the methods section).*

non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

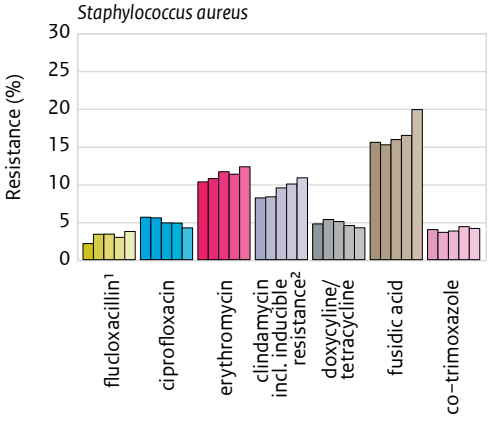
Table 4.2.3 Resistance levels (%) among diagnostic wound, pus, and skin isolates of *S. aureus* from selected general practitioners' patients, ISIS-AR 2018

	S. aureus
Antibiotic	
flucloxacillin[1]	4
ciprofloxacin[2]	4
erythromycin	12
clindamycin including inducible resistance[3]	11
doxycycline/tetracycline	4
fusidic acid	20
co-trimoxazole	4

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

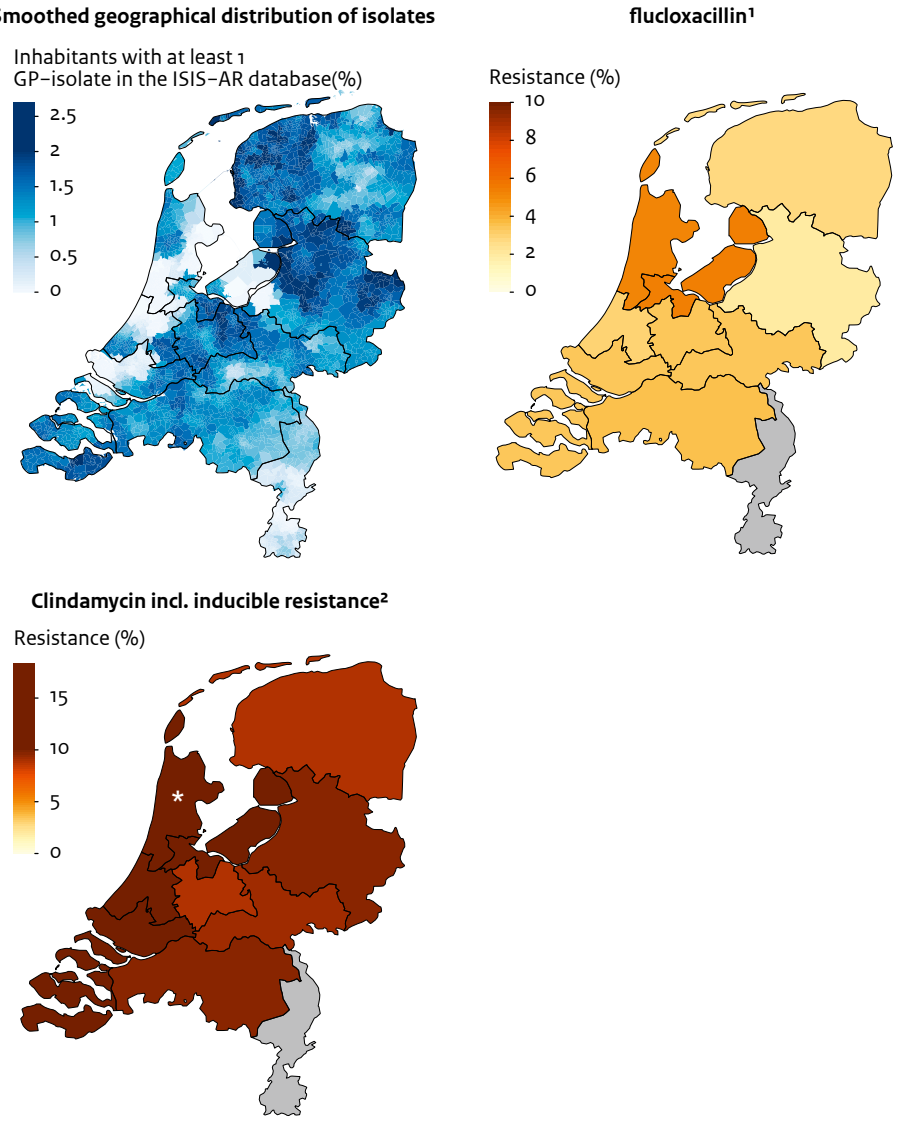
[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*
[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*
[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.2.4 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic wound, pus, and skin isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR.



[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*
[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.2.5 Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic wound, pus, or skin *S. aureus* isolates on a gradient scale between 0 and 10% for flucloxacillin and clindamycin including inducible resistance by regional cooperative network, ISIS-AR 2018.



* *Statistically significant and clinically relevant difference between resistance in the regional cooperative network and in all regions combined (for details see the methods section).*

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information)*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information)*

Key results

The coverage of isolates from GP patients in the regional cooperative networks 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' was low compared to other regional networks and regional resistance levels may be influenced by suboptimal representativeness.

Enterobacteriaceae

- Resistance levels in selected GP patients aged >12 years were generally higher than in patients aged ≤12 years.
- For all Enterobacteriaceae resistance levels of 10% or lower were observed for cefuroxime (≤8%), cefotaxime/ceftriaxone (≤5%), ceftazidime (≤5%), gentamicin (≤5%), and tobramycin (≤4%), except for cefuroxime in *K. pneumoniae* from patients aged >12 years (15%). Resistance levels ≤10% were also found for ciprofloxacin in *E. coli* and *K. pneumoniae* from patients aged ≤12 years only (5% for both pathogens) and for *P. mirabilis* in both age groups (≤10%). Additionally, resistance levels ≤10% were found for fosfomycin (1%) and nitrofurantoin (≤2%) in *E. coli*, trimethoprim (10%) and co-trimoxazole (7%) in *K. pneumoniae* from patients aged ≤12 years, and for co-amoxiclav (≤6%) and fosfomycin (patients aged ≤12 years only, 8%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥33%), co-amoxiclav (≥27%) trimethoprim (≥21%), and co-trimoxazole (patients aged >12 years only, 21%) in *E. coli*, for co-amoxiclav (27%) in *K. pneumoniae* from patients aged ≤12 years, for fosfomycin (28%) and trimethoprim (22%) in *K. pneumoniae* from patients aged >12 years, and for amoxicillin/ ampicillin (patients >12 years only, 21%), trimethoprim (≥24%) and co-trimoxazole (patients >12 years only, 26%) in *P. mirabilis*.
- There was a statistically significant and clinically relevant increase in resistance to co-amoxiclav in *E. coli* and *K. pneumoniae* in both age groups (In *E. coli* from 12% in 2014 to 27% in 2018 for patients aged ≤12 years, and from 16% to 30% for patients aged >12 years; In *K. pneumoniae* from 15% to 27% and from 8% to 18% in the respective age groups), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). Statistically significant and clinically relevant increases in resistance were also found for ceftazidime in *E. coli* in both age groups (from 1% in 2014 to 2% in 2018 for patients aged ≤12 years, and from 2% to 3% for patients aged >12 years) and in *K. pneumoniae* from patients aged >12 years (from 2% to 5%), and for ciprofloxacin in patients aged >12 years (from 10% to 14%). In *P. mirabilis* isolates from patients aged ≤12 years, statistically significant and clinically relevant decreasing trends in resistance between 2014 and 2018 were observed for co-amoxiclav (from 8% to 5%) and fosfomycin (from 11% to 8%).

- The percentage of HRMO and multidrug resistance was ≤6% in all Enterobacteriaceae, with significant and clinically relevant increasing trends in multidrug resistance for *E. coli* isolates from patients aged ≤12 years (from 0.6% to 1.5%) and *K. pneumoniae* isolates from patients aged >12 years (from 2% to 3%).
- For none of the selected agents statistically significant and clinically relevant differences of regional resistance levels in *E. coli* with the resistance in all regions combined were found.
- *For K. pneumoniae* a statistically significant and clinically relevant lower resistance percentage was found for co-amoxiclav in the regional cooperative network 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' (13% in the region versus 18% in all regions combined), and for ciprofloxacin in 'Holland West' (10% versus 14%).

P. aeruginosa

- Resistance levels ≤10% were found for each of the selected agents in both age groups**.**

S. aureus

- Resistance levels of 10% or lower were found for each of the selected agents, except for erythromycin (12%), clindamycin including inducible resistance (11%) and fusidic acid (20%).
- There was a significant and clinically relevant increase in resistance to fusidic acid (from 16% in 2014 to 20% in 2018).
- For clindamycin including inducible resistance a statistically significant and clinically relevant higher resistance percentage was found in the cooperative network 'Noord-Holland West' (18% in the region versus 11% in all regions combined).

4.3 Hospital departments

In this chapter resistance levels among isolates from patients in outpatient departments (chapter 4.3.1), inpatient departments (excluding intensive care units, chapter 4.3.2), and intensive care units (chapter 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 chapter 4.3.4, and for urinary isolates from patients in urology departments (outpatient and inpatient departments) in chapter 4.3.5.

4.3.1 Outpatient departments

The distribution of pathogens isolated from diagnostic samples (lower respiratory tract, urine, and wound, pus, or skin) from patients attending outpatient departments is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2018 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*).

Among patients attending outpatient departments, the rate of sampling is higher than among GP patients. Therefore, bias due to selective sampling will be lower than in GP patients and resistance percentages in this chapter are considered representative for resistance in outpatient departments.

Table 4.3.1.1 Distribution of isolated pathogens in diagnostic samples from patients attending outpatient departments, ISIS-AR 2018.

	Lower respiratory tract	Urine	Wound, pus, or skin
Pathogen	N (%)	N (%)	N (%)
E. coli	514 (5)	21,314 (44)	1,874 (6)
K. pneumoniae	250 (2)	4,385 (9)	472 (1)
P. mirabilis	156 (1)	2,512 (5)	1,172 (4)
Other Enterobacteriaceae[1]	938 (9)	6,260 (13)	3,207 (10)
P. aeruginosa	1,390 (13)	1,770 (4)	3,023 (9)
Other non-fermenters[2]	1,465 (13)	744 (2)	845 (3)
Other Gram-negatives[3]	3,431 (31)	15 (0)	828 (3)
S. aureus	1,603 (15)	1,730 (4)	13,919 (43)
Other Gram-positives[4]	1,236 (11)	9,595 (20)	6,741 (21)

The first isolate per patient, per microorganism, per category (lower respiratory tract; urine; wound, pus, or skin) was selected.

[1] *Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Serratia spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Pantoea spp., Hafnia spp., Escherichia spp. (non-coli), Salmonella spp., Cronobacter spp., Yersinia spp.*

[2] *M. catarrhalis, Acinetobacter spp., S. maltophilia, Pseudomonas spp. (non-aeruginosa).*

[3] *H. influenzae, B. fragilis complex n.n.g., B. fragilis, H. pylori, N. meningitidis.*

[4] *S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp., Staphylococcus spp. (non-aureus), M. tuberculosis complex, M. tuberculosis, 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 2018.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	44	-	24	-
co-amoxiclav[1] - non-uuti	36	22	8	-
piperacillin-tazobactam	4	9	0	5
cefuroxime	13	17	1	-
cefotaxime/ceftriaxone	6	9	1	-
ceftazidime	4	9	0	2
meropenem/imipenem	0	0	0	-
meropenem	-	-	-	1
imipenem	-	-	-	4
ciprofloxacin	18	15	12	13
gentamicin	5	4	6	5
tobramycin	5	5	5	2
fosfomycin	2	25	14*	-
trimethoprim	28	24	33	-
co-trimoxazole	25	16	27	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
gentamicin + amoxicillin/ampicillin	5	-	5	-
gentamicin + co-amoxiclav - non-uuti	4	3	2	-
gentamicin + cefuroxime	2	3	0	-
gentamicin + cefotaxime/ceftriaxone	1	3	0	-
gentamicin + ceftazidime	1	3	0	0
Multidrug resistance				
HRMO[2]	8	10	4	3
multidrug resistance[3] - non-uuti	7	6	2	-

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.

non-uuti = according to breakpoint for non-uncomplicated urinary tract infection

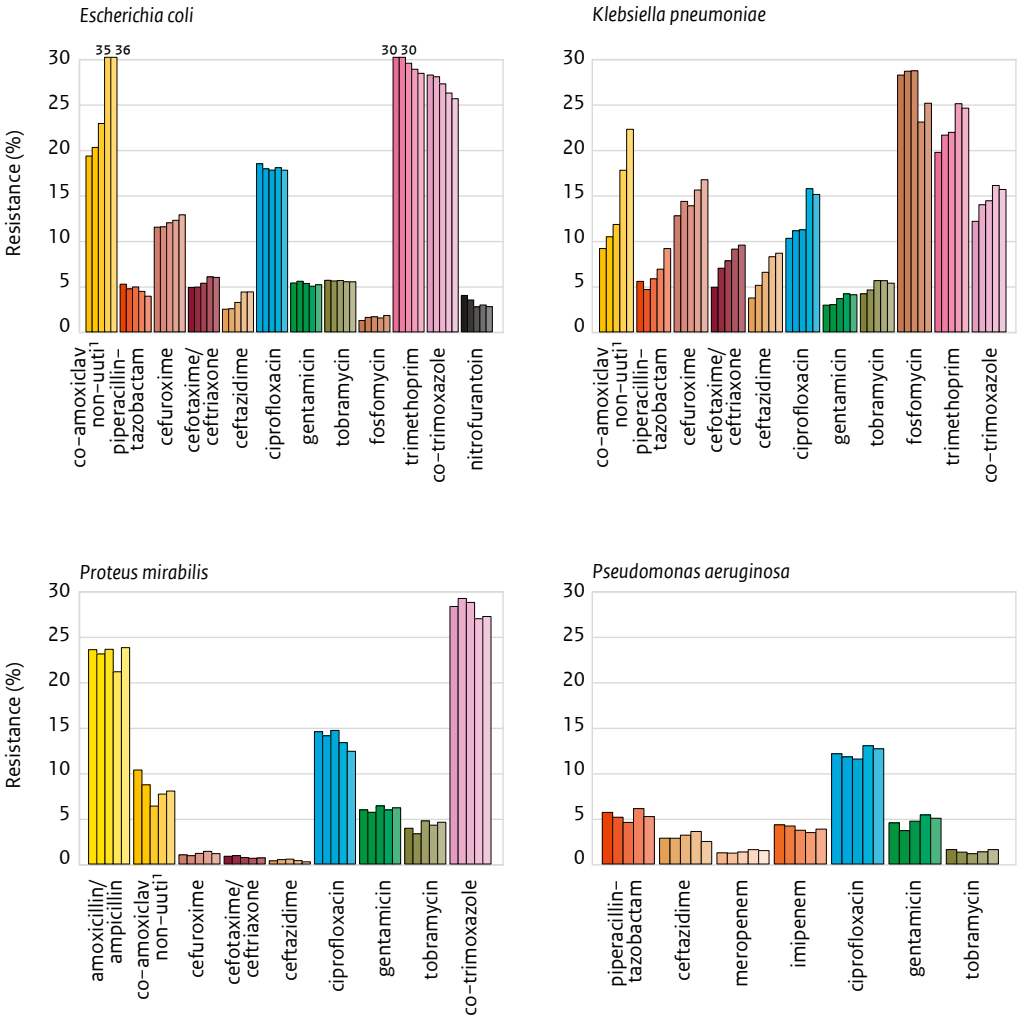
**Trend not calculated, because of a low number of tests in the years before 2018*

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments in ISIS-AR.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

Table 4.3.1.3 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2018.

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	7
gentamicin	1
erythromycin	14
clindamycin including inducible resistance[3]	12
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0
co-trimoxazole	3
rifampicin	0

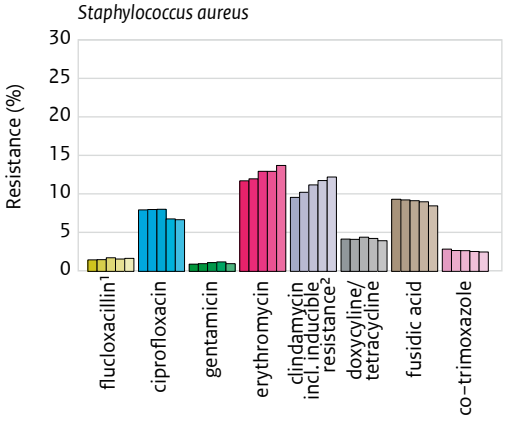
10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.3.1.2 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR.



[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Key results

Enterobacteriaceae

- For all Enterobacteriaceae, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤9%), ceftazidime (≤9%), meropenem/imipenem (0%), gentamicin (≤6%), and tobramycin (≤5%). Resistance levels ≤10% were also found for fosfomycin (2%) and nitrofurantoin (3%) in *E. coli* and co-amoxiclav (8%) and cefuroxime (1%) in *P. mirabilis*.
- Resistance of 20% or higher was found for trimethoprim (≥24%) in all Enterobacteriaceae, for amoxicillin/ampicillin (≥24%) and co-trimoxazole (≥25%) in *E. coli* and *P. mirabilis*, for co-amoxiclav (≥22%) in *E. coli* and *K. pneumoniae*, and fosfomycin in *K. pneumoniae* (25%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 19% in 2014 to 36% in 2018) and in *K. pneumoniae* (from 9% to 22%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). In *E. coli, resistance to* ceftazidime also increased in the last five years (from 2% to 4%). Furthermore, in *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed for piperacillin-tazobactam (from 6% in 2014 to 9% in 2018), cefuroxime (from 13% to 17%), cefotaxime/ceftriaxone (from 5% to 9%), ceftazidime (from 4% to 9%), ciprofloxacin (from 10% to 15%), and trimethoprim (from 20% to 24%).
- Resistance to empiric therapy combinations was ≤5% for all Enterobacteriaceae. In *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed for gentamicin + co-amoxiclav (from 2% in 2014 to 3% in 2018), gentamicin + cefotaxime/ceftriaxone (from 2% to 3%) and gentamicin + ceftazidime (from 1% to 3%).
- For all Enterobacteriaceae, the percentage HRMO was ≤10% and the percentage of multidrug resistance was ≤7%. In *E. coli*, the percentage of multidrug resistance increased to a statistically significant and clinically relevant extent (from 5% in 2014 to 7% in 2018). In *K. pneumoniae*, significant and clinically relevant increasing trends were observed for HRMO (from 6% in 2014 to 10% in 2018) and multidrug resistance (from 3% to 6%).

P. aeruginosa

- Resistance levels of 10% or lower were observed for each of the selected agents (≤5%), except for ciprofloxacin (13%).

S. aureus

- Resistance levels of 10% or lower were observed for each of the selected agents (≤8%), except for erythromycin (14%) and clindamycin including inducible resistance (12%).

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, pus, or skin) from patients admitted to inpatient hospital departments (excl. ICU) is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2018 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*), and 4.3.2.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). 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. faecium*) and 4.3.2.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

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 2018.

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound, pus, or skin
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	5,863 (25)	1,310 (9)	22,427 (44)	4,474 (13)
K. pneumoniae	1,076 (5)	650 (4)	4,430 (9)	979 (3)
P. mirabilis	406 (2)	236 (2)	3,318 (7)	1,060 (3)
E. cloacae complex	379 (2)	505 (3)	1,294 (3)	1,292 (4)
Other Enterobacteriaceae[1]	1,163 (5)	1,519 (10)	4,824 (10)	2,881 (9)
P. aeruginosa	529 (2)	1,589 (10)	2,681 (5)	1,982 (6)
Acinetobacter spp.	99 (0)	135 (1)	325 (1)	377 (1)
Other non-fermenters[2]	87 (0)	1,654 (11)	218 (0)	393 (1)
Other Gram-negatives[3]	596 (2)	3,790 (25)	22 (0)	862 (3)
E. faecalis	696 (3)	21 (0)	5,174 (10)	1,887 (6)
E. faecium	459 (2)	19 (0)	1,477 (3)	1,207 (4)
S. aureus	2,503 (10)	2,111 (14)	1,587 (3)	9,141 (27)
CNS	7,133 (30)	27 (0)	852 (2)	3,346 (10)
Other Gram-positives[4]	2,899 (12)	1,690 (11)	1,788 (4)	3,569 (11)

The first isolate per patient, per microorganism, per category (blood or cerebrospinal fluid; lower respiratory tract; urine; wound, pus, or skin) was selected.

CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

[2] *M. catarrhalis, S. maltophilia, Pseudomonas spp. (non-aeruginosa).*

[3] *H. influenzae, B. fragilis complex n.n.g., B. fragilis, N. meningitidis, C. jejuni, C. lari, H. pylori.*

[4] *S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp. (non-faecalis, non-faecium), M. tuberculosis complex, M. tuberculosis, L. monocytogenes, Staphylococcus spp. (non-aureus, non-CNS).*

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 2018.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter spp.*
Antibiotic						
amoxicillin/ampicillin	44	-	24	-	-	-
co-amoxiclav[1] - non-uuti	36	22	8	-	-	-
piperacillin-tazobactam	5	9	1	-	6	-
cefuroxime	13	16	1	-	-	-
cefotaxime/ceftriaxone	6	10	1	-	-	-
ceftazidime	5	9	0	-	3	-
meropenem/imipenem	0	0	0	0	-	4
meropenem	-	-	-	-	1	-
imipenem	-	-	-	-	3	-
ciprofloxacin	14	12	11	5	11	6
gentamicin	4	4	5	2	3	5
tobramycin	5	6	4	3	1	3
fosfomycin	1*	19*	12*	39*	-	-
trimethoprim	25	19	32	7	-	-
co-trimoxazole	23	15	26	6	-	5
nitrofurantoin	2	-	-	-	-	-
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	4	-	4	-	-	-
gentamicin + co-amoxiclav - non-uuti	4	4	2	-	-	-
gentamicin + piperacillin-tazobactam	1	1	0	-	1	-
gentamicin + cefuroxime	2	4	0	-	-	-
gentamicin + cefotaxime/ceftriaxone	2	4	0	-	-	-
gentamicin + ceftazidime	1	3	0	-	0	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	0	-
Multidrug resistance						
HRMO[2]	8	11	3	2	2	4

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection
**Trend not calculated, because of a low number of tests in the years before 2018*

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/imipenem; for E. cloacae complex as as either one or both of the situations 2 and 3 as described for the other Enterobacteriaceae; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as either one or both of the following: 1) carbapenemase producing, estimated by carbapenemase confirmatory tests, or if no data on confirmatory tests were available, by resistance to imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.*

Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2014 to 2018) 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.

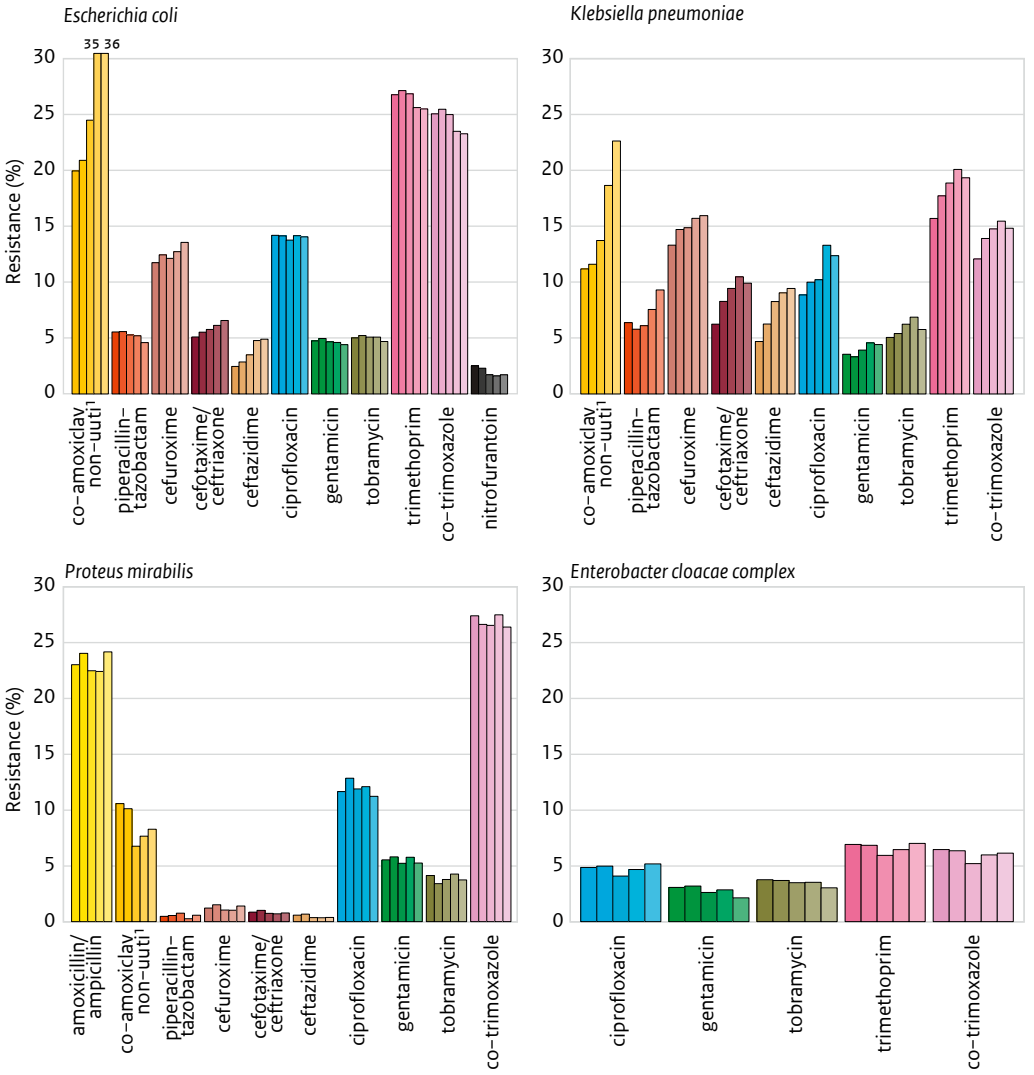
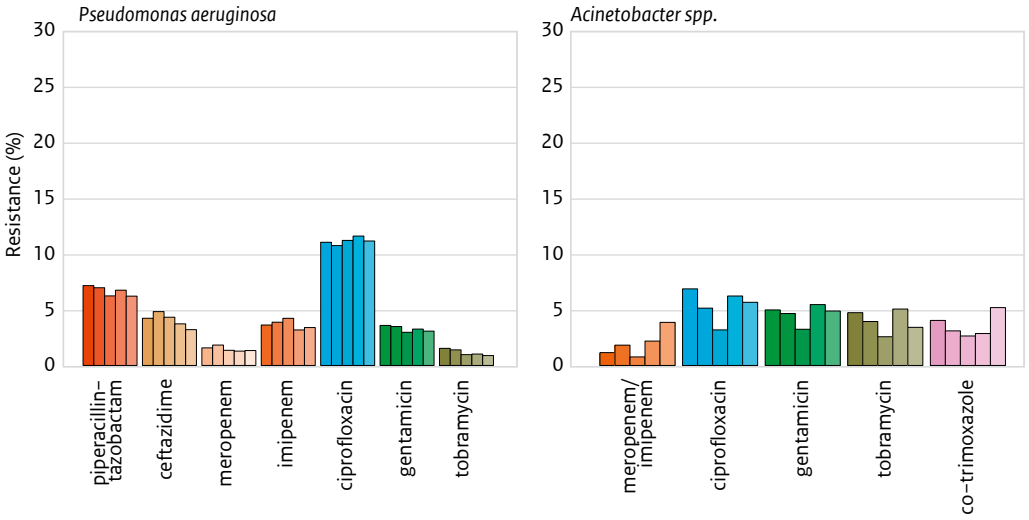


Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

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 2018.

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	86
vancomycin	0	0
nitrofurantoin	0	-

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *E. faecium* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.

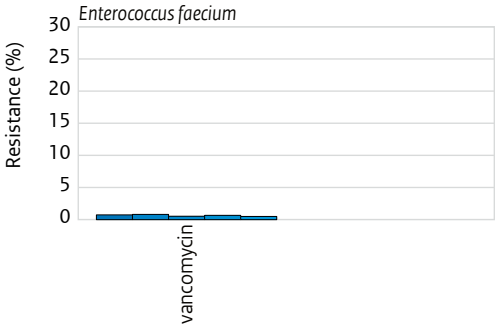


Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2018.

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	40
ciprofloxacin[2]	7	29
gentamicin	1	25
erythromycin	13	43
clindamycin including inducible resistance[3]	12	29
doxycycline/tetracycline	4	17
fusidic acid	7	44
linezolid	0	0
co-trimoxazole	2	17
rifampicin	0	3

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

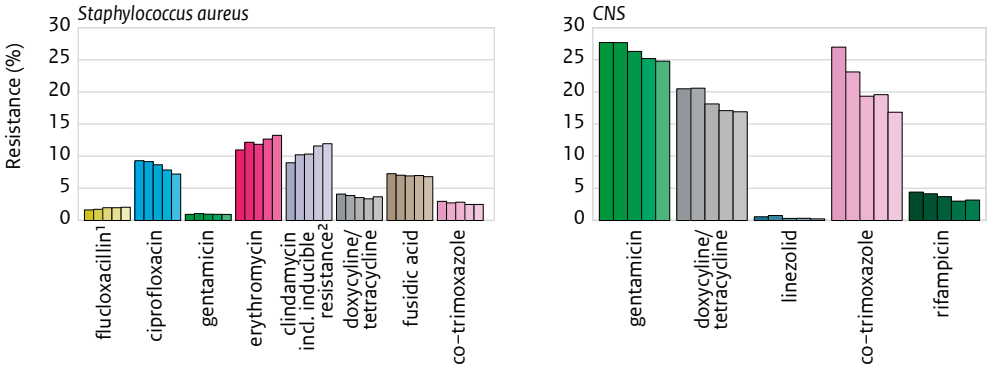
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017 no test for trend could be conducted for CNS (see methods section for more detailed information).*

[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Key results

Enterobacteriaceae

- For all Enterobacteriaceae, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤10%), ceftazidime (≤9%), meropenem/imipenem (0%), gentamicin (≤5%), and tobramycin (≤6%). Resistance levels ≤10% were also found for fosfomycin (1%) and nitrofurantoin (2%) in *E. coli*, co-amoxiclav (8%) and cefuroxime (1%) in *P. mirabilis, and* ciprofloxacin (5%), trimethoprim (7%), and co-trimoxazole (6%) in *E. cloacae* complex.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥24%), trimethoprim (≥25%) and co-trimoxazole (≥23%) in *E. coli* and *P. mirabilis*, *for* co-amoxiclav in *E. coli* and *K. pneumoniae* (≥22%), and for fosfomycin in *E. cloacae* complex (39%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2014 to 36% in 2018) and *K. pneumoniae* (from 11% to 22%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). In *E. coli*, resistance to ceftazidime also increased to a statistically significant and clinically relevant extent in the last five years (from 2% to 5%). Furthermore, in *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed for piperacillin-tazobactam (from 6% in 2014 to 9% in 2018), cefotaxime/ceftriaxone (from 6% to 10%), ceftazidime (from 5% to 9%) and ciprofloxacin (from 9% to 12%).

- For empiric therapy combinations, resistance was ≤4% in all Enterobacteriaceae. In *K. pneumoniae*, a statistically significant and clinically relevant increase in resistance was observed for gentamicin + ceftazidime (from 2% in 2014 to 3% in 2018).
- The percentage of HRMO was ≤8%, except for *K. pneumoniae* (11%). In *K. pneumoniae*, the percentage of HRMO increased to a statistically significant and clinically relevant extent (from 8% in 2014 to 11% in 2018).

P. aeruginosa

- Resistance to each of the selected agents, empiric therapy combinations, and the percentage HRMO, was ≤10% in 2018, except for ciprofloxacin (11%).

Acinetobacter spp.

- Resistance to each of the selected agents, and the percentage HRMO, was ≤6% in 2018.
- A statistically significant and clinically relevant increase in resistance was observed for meropenem/imipenem (from 1% in 2014 to 4% in 2018).

E. faecalis and E. faecium

- Vancomycin resistance (0%), and nitrofurantoin resistance (1%, calculated for *E. faecalis* only) was rare in 2018.
- *In E. faecium*, resistance to amoxicillin/ampicillin was 86%.

S. aureus

- Resistance of 10% or lower was observed for each of the selected agents (≤7%), except for erythromycin (13%) and clindamycin including inducible resistance (12%).

Coagulase-negative Staphylococcus spp.

- Apart from doxycycline/tetracycline (17%), linezolid (0%), co-trimoxazole (17%) and rifampicin (3%), resistance to each of the selected agents was ≥20%.
- A statistically significant and clinically relevant decreasing trend in resistance was observed for co-trimoxazole (from 27% in 2014 to 17% in 2018).

4.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound, pus, or skin) from patients admitted to intensive care units is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2018 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*), and 4.3.3.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). 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. faecium*) and 4.3.3.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

In intensive care units in the Netherlands, a sample is 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 2018.

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound, pus, or skin
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	390 (11)	596 (11)	838 (38)	609 (16)
K. pneumoniae	76 (2)	249 (5)	162 (7)	130 (3)
P. mirabilis	32 (1)	115 (2)	149 (7)	100 (3)
E. cloacae complex	54 (2)	289 (6)	55 (2)	171 (5)
Other Enterobacteriaceae[1]	121 (3)	854 (16)	210 (9)	374 (10)
P. aeruginosa	57 (2)	390 (7)	124 (6)	248 (7)
Acinetobacter spp.	16 (0)	73 (1)	13 (1)	34 (1)
Other non-fermenters[2]	14 (0)	377 (7)	8 (0)	66 (2)
Other Gram-negatives[3]	53 (1)	559 (11)	2 (0)	79 (2)
E. faecalis	120 (3)	42 (1)	249 (11)	337 (9)
E. faecium	261 (7)	114 (2)	218 (10)	467 (13)
S. aureus	323 (9)	1,068 (20)	89 (4)	422 (11)
CNS	1,801 (51)	32 (1)	67 (3)	434 (12)
Other Gram-positives[4]	242 (7)	477 (9)	49 (2)	256 (7)

The first isolate per patient, per microorganism, per category (blood or cerebrospinal fluid; lower respiratory tract; urine; wound, pus, or skin) was selected.

CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

[2] *S. maltophilia, M. catarrhalis, Pseudomonas spp. (non-aeruginosa).*

[3] *H. influenzae, B. fragilis complex n.n.g., B. fragilis, N. meningitidis.*

[4] *S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp. (non-faecalis, non-faecium), Staphylococcus spp. (non-aureus, non-CNS), L. monocytogenes, M. tuberculosis complex.*

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 2018.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter spp.*
Antibiotic						
amoxicillin/ampicillin	47	-	26	-	-	-
co-amoxiclav[1] - non-uuti	39	23	6	-	-	-
piperacillin-tazobactam	6	11	0	-	12	-
cefuroxime	17	19	1	-	-	-
cefotaxime/ceftriaxone	9	14	1	-	-	-
ceftazidime	6	12	0	-	6	-
meropenem/imipenem	0	1	0	0	-	9
meropenem	-	-	-	-	3	-
imipenem	-	-	-	-	5	-
ciprofloxacin	14	13	12	5	13	14
gentamicin	5	7	6	5	5	11
tobramycin	6	8	4	7	2	7
co-trimoxazole	24	15	27	7	-	6
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	5	-	5	-	-	-
gentamicin + co-amoxiclav - non-uuti	4	7	2	-	-	-
gentamicin + piperacillin-tazobactam	1	3	0	-	2	-
gentamicin + cefuroxime	2	6	0	-	-	-
gentamicin + cefotaxime/ceftriaxone	2	6	0	-	-	-
gentamicin + ceftazidime	2	5	0	-	1	-
tobramycin + ceftazidime	-	-	-	-	1	-
tobramycin + ciprofloxacin	-	-	-	-	2	-
Multidrug resistance						
HRMO[2]	10	15	3	4	5	9

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem; for E. cloacae complex as either one or both of the situations 2 and 3 as described for the other Enterobacteriaceae; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as either one or both of the following: 1) carbapenemase producing, estimated by carbapenemase confirmatory tests, or if no data on confirmatory tests were available, by resistance to imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2014 to 2018) 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.

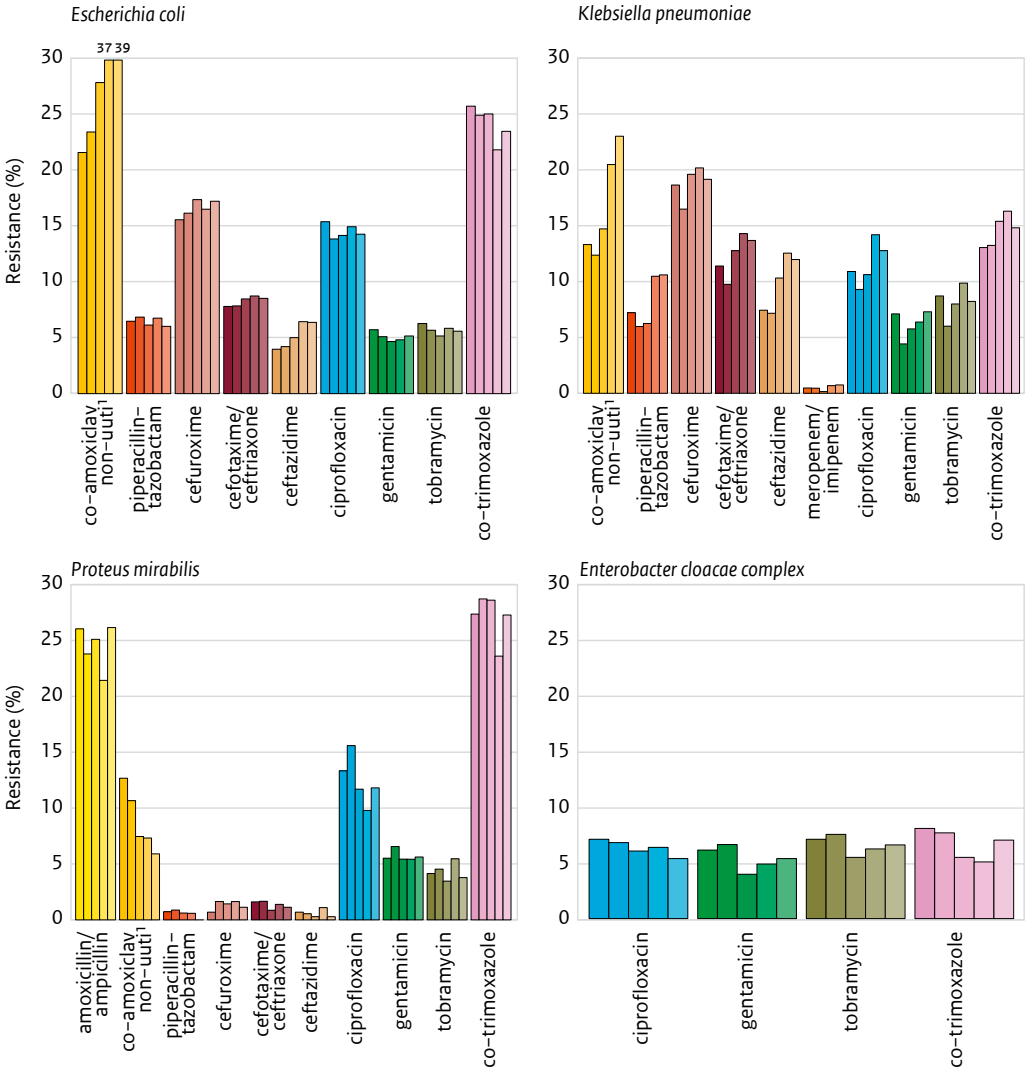
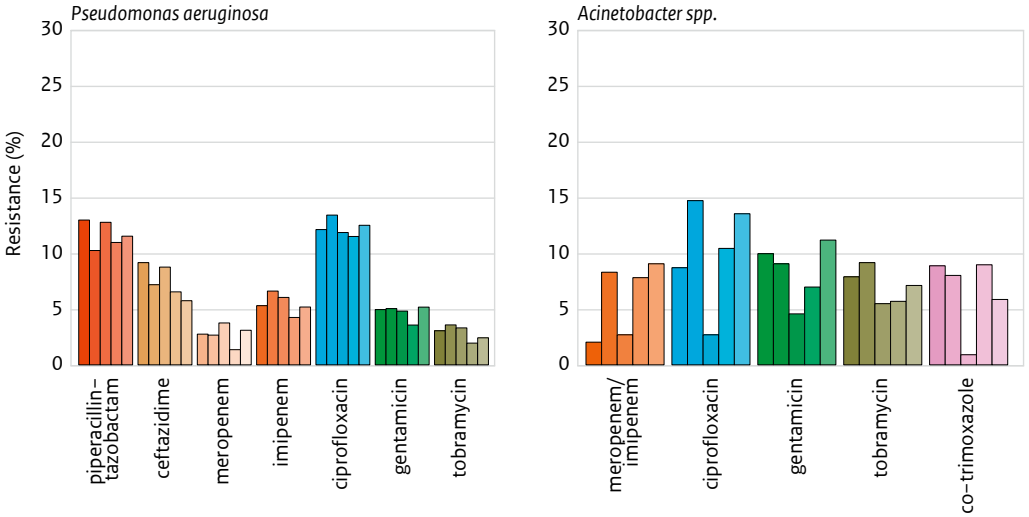


Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

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 2018.

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

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *E. faecium* from patients admitted to intensive care units in ISIS-AR.

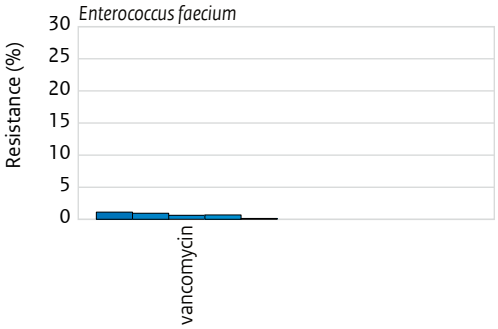


Table 4.3.3.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units, ISIS-AR 2018.

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	69
ciprofloxacin[2]	5	56
gentamicin	1	52
erythromycin	12	62
clindamycin including inducible resistance[3]	12	53
doxycycline/tetracycline	3	20
linezolid	0	0
co-trimoxazole	3	29
rifampicin	0	8

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

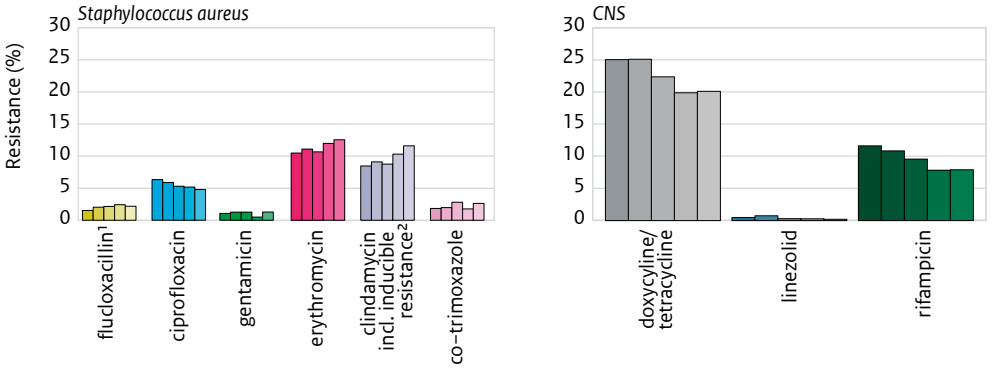
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017 no test for trend could be conducted for CNS (see methods section for more detailed information).*

[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.3.3.3 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units in ISIS-AR.



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Key results

Enterobacteriaceae

- For all Enterobacteriaceae, resistance levels ≤10% were found for meropenem/imipenem (≤1%), gentamicin (≤7%), and tobramycin (≤8%). Resistance levels ≤10% were also found for piperacillin-tazobactam (≤6%), cefotaxime/ceftriaxone (≤9%) and ceftazidime (≤6%) in *E. coli* and *P. mirabilis*, for co-amoxiclav (6%) and cefuroxime (1%) in *P. mirabilis*, and for ciprofloxacin (5%) and co-trimoxazole (7%) in *E. cloacae* complex.
- Resistance levels ≥20% were found for co-amoxiclav (≥23%) in *E. coli* and *K. pneumoniae*, and for amoxicillin/ampicillin (≥26%) and co-trimoxazole (≥24%) in *E. coli* and *P. mirabilis*.
- A statistically significant and clinically relevant increasing trend in resistance was observed for co-amoxiclav in *E. coli* (from 22% in 2014 to 39% in 2018) and *K. pneumoniae* (from 13% to 23%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). In *E. coli*, resistance to ceftazidime also increased to a statistically significant and clinically relevant extent in the last five years (from 4% to 6%). Furthermore, in *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed for piperacillin-tazobactam (from 7% in 2014 to 11% in 2018), cefotaxime/ceftriaxone (from 11% to 14%), ceftazidime (from 7% to 12%) and ciprofloxacin (from 11% to 13%). In *P. mirabilis*, a significant and clinically relevant decrease in resistance between 2014 and 2018 was found for co-amoxiclav (from 13% to 6%).

- Resistance to the empiric therapy combinations was ≤7% for all Enterobacteriaceae.
- The percentage HRMO was ≤10%, except for *K. pneumoniae* (15%). In *K. pneumoniae,* the percentage of HRMO increased significantly and to a clinically relevant extent (from 12% in 2014 to 15% in 2018).

P. aeruginosa

- Resistance levels for each of the selected agents, the empiric therapy combinations, and the percentage HRMO, were ≤10%, except for resistance to piperacillin-tazobactam (12%) and ciprofloxacin (13%).

Acinetobacter spp.

- Resistance levels for each of the selected agents and the percentage HRMO were ≤10%, except for ciprofloxacin (14%) and gentamicin (11%).

E. faecalis and E. faecium

- Resistance to vancomycin was rare (0%).
- In *E. faecium*, resistance to amoxicillin/ampicillin was 88%.

S. aureus

- Resistance of 10% or lower was observed for each of the selected agents (≤5%), except for erythromycin and clindamycin including inducible resistance (both 12%).
- A statistically significant and clinically relevant increasing trend in resistance was observed for clindamycin including inducible resistance (from 8% in 2014 to 12% in 2018).

Coagulase-negative Staphylococcus spp.

- Apart from linezolid (0%) and rifampicin (8%), resistance to each of the selected agents was ≥20%.
- Significant and clinically relevant decreases in resistance were found for doxycycline/tetracycline (from 25% in 2014 to 20% in 2018), co-trimoxazole (from 48% to 29%), and rifampicin (from 12% to 8%).

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) is presented in table 4.3.4.1. The resistance levels for a selection of pathogens isolated from these patients in 2018 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 4.3.4.3 (*E. faecalis* and *E. faecium*), and 4.3.4.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). Five-year trends in resistance are presented in figures 4.3.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 4.3.4.2 (*E. faecium*) and 4.3.4.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

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. However, particularly for coagulase-negative *Staphylococcus* spp., a substantial part of isolates is likely to be contamination rather than cause of infection.

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 2018.

Pathogen	Non-ICU N (%)	ICU N (%)
E. coli	5,858 (25)	387 (11)
K. pneumoniae	1,073 (5)	75 (2)
P. mirabilis	405 (2)	32 (1)
E. cloacae complex	376 (2)	52 (1)
Other Enterobacteriaceae[1]	1,155 (5)	120 (3)
P. aeruginosa	522 (2)	56 (2)
Acinetobacter spp.	96 (0)	14 (0)
Other non-fermenters[2]	86 (0)	14 (0)
Other Gram-negatives[3]	581 (2)	49 (1)
E. faecalis	685 (3)	120 (3)
E. faecium	454 (2)	255 (7)
S. aureus	2,483 (10)	319 (9)
CNS	7,017 (30)	1,771 (51)
Other Gram-positives[4]	2,862 (12)	215 (6)

The first isolate per patient, per microorganism, per category (non-ICU; ICU) was selected.
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

[2] *Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *B. fragilis complex n.n.g., B. fragilis, H. influenzae, N. meningitidis, C. jejuni, C. lari.*

[4] *S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp. (non-faecalis, non-faecium), L. monocytogenes, Staphylococcus spp. (non-aureus, non-CNS).*

Table 4.3.4.2 Resistance levels (%) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2018.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	45	-	23	-	-
co-amoxiclav[1] - non-uuti	37	21	8	-	-
piperacillin-tazobactam	4	7	0	-	6
cefuroxime	12	14	1	-	-
cefotaxime/ceftriaxone	7	10	1	-	-
ceftazidime	5	9	0	-	3
meropenem/imipenem	0	0	0	0	-
meropenem	-	-	-	-	2
imipenem	-	-	-	-	3
ciprofloxacin	15	13	12	6	9
gentamicin	5	6	5	3	3
tobramycin	5	7	4	4	1
co-trimoxazole	24	15	24	8	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	5	-	-
gentamicin + co-amoxiclav - non-uuti	4	5	3	-	-
gentamicin + piperacillin-tazobactam	1	2	0	-	1
gentamicin + cefuroxime	2	5	1	-	-
gentamicin + cefotaxime/ceftriaxone	2	5	1	-	-
gentamicin + ceftazidime	2	4	0	-	1
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	1
Multidrug resistance					
HRMO[2]	8	12	3	2	2

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection
[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*
[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem; for E. cloacae complex as either one or both of the situations 2 and 3 as described for the other Enterobacteriaceae; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.

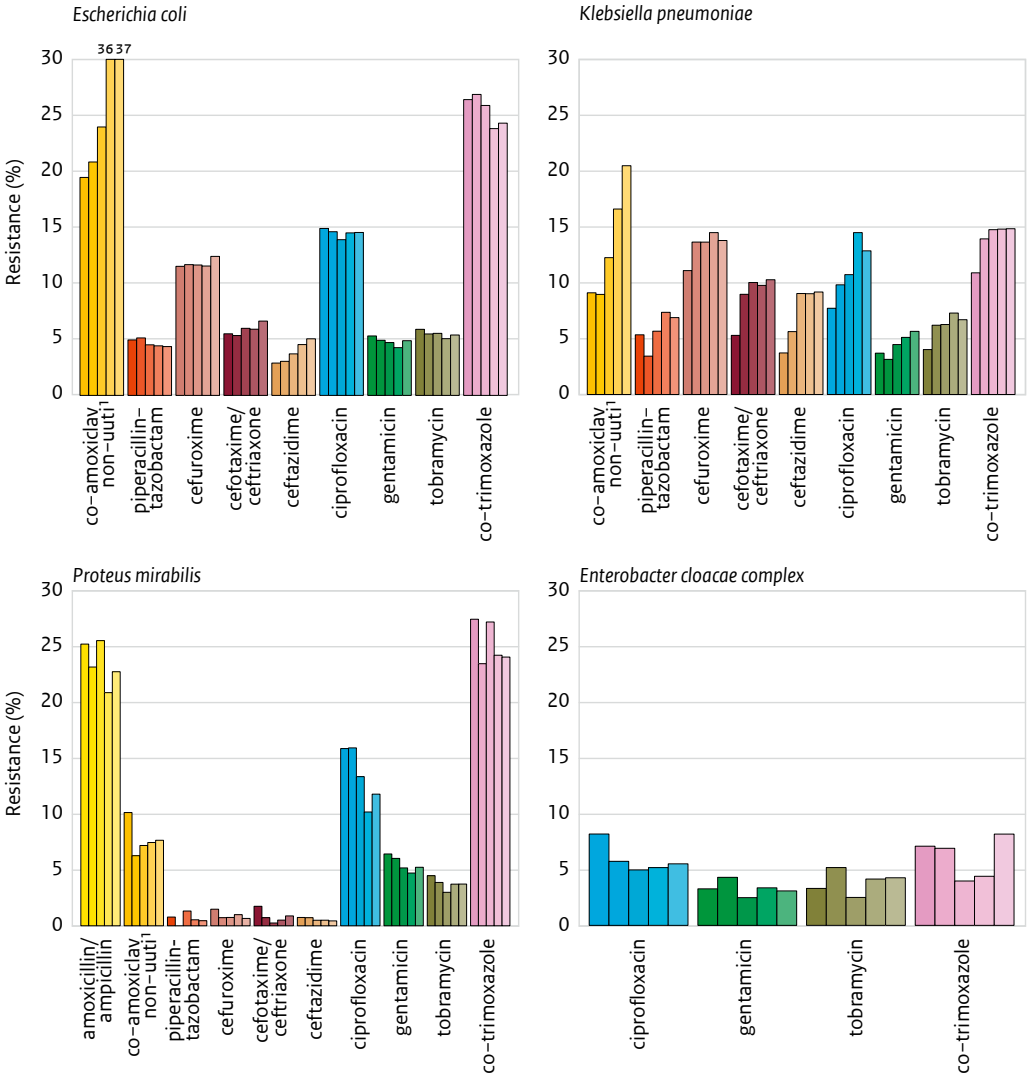
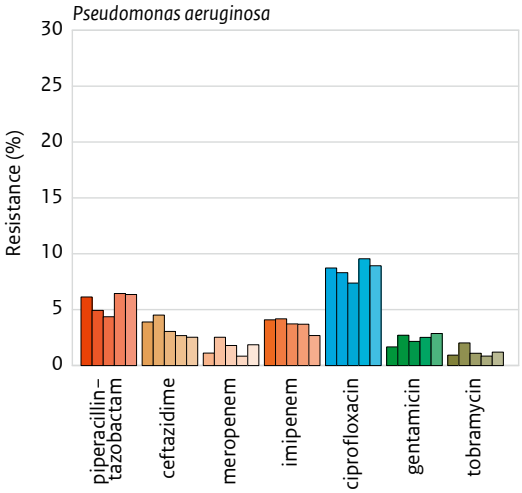


Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

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 2018.

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

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.

Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic blood isolates of *E. faecium* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.

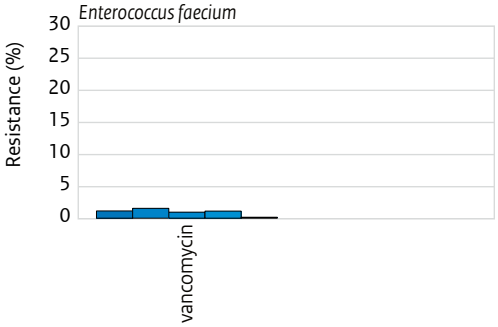


Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2018.

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	1	44
ciprofloxacin[2]	6	31
gentamicin	1	26
erythromycin	10	46
clindamycin including inducible resistance[3]	10	31
doxycycline/tetracycline	3	19
linezolid	0	0
co-trimoxazole	2	16
rifampicin	0	3

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

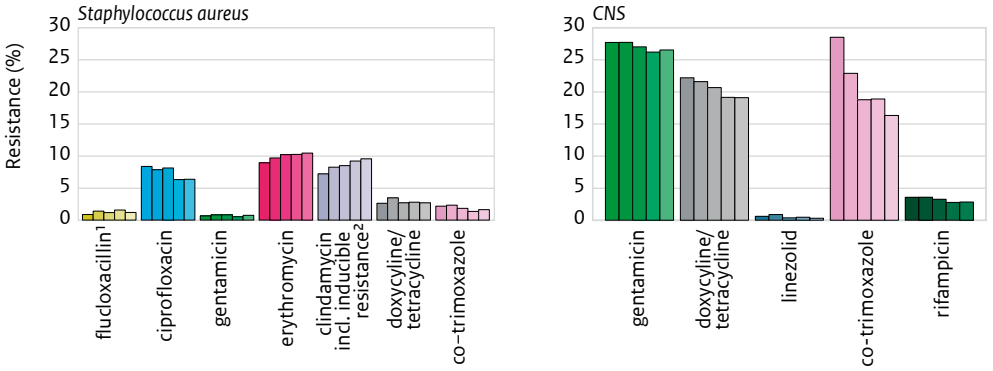
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017 no test for trend could be conducted for CNS (see methods section for more detailed information).*

[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Key results

- The majority (87%) of inpatient blood isolates originated from non-ICU departments.
- Resistance was similar to resistance in non-ICU departments in all diagnostic samples combined (chapter 4.3.2).
- Statistically significant and clinically relevant trends in resistance were also similar to trends in non-ICU departments in all diagnostic samples combined. In addition, in blood isolates from non-ICU and ICU departments specifically, statistically significant and clinically relevant increasing trends in resistance were observed in *K. pneumoniae*, for gentamicin (from 4% in 2014 to 6% in 2018), tobramycin (from 4% to 7%), and the empiric therapy combinations gentamicin + co-amoxiclav, gentamicin + cefuroxime, and gentamicin + cefotaxime/ ceftriaxone (all from 3% to 5%). Furthermore, in *P. mirabilis*, resistance to ciprofloxacin decreased significantly and to a clinically relevant extent from 16% in 2014 to 12% in 2018.

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) is presented in table 4.3.5.1. The resistance levels for a selection of pathogens isolated from these patients in 2018 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*).

Table 4.3.5.1 Distribution of isolated pathogens in diagnostic urinary samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2018.

	OPD	IPD
Pathogen	**N (%)**	**N (%)**
E. coli	11,978 (41)	2,180 (35)
K. pneumoniae	2,730 (9)	500 (8)
P. mirabilis	1,488 (5)	346 (5)
Other Enterobacteriaceae[1]	4,329 (15)	1,069 (17)
P. aeruginosa	1,098 (4)	387 (6)
Other non-fermenters[2]	543 (2)	169 (3)
Other Gram-negatives[3]	6 (0)	6 (0)
E. faecalis	3,385 (12)	795 (13)
E. faecium	202 (1)	135 (2)
Other Gram-positives[4]	3,574 (12)	716 (11)

The first isolate per patient, per microorganism, per category (OPD; IPD) was selected.

[1] *Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Morganella spp., Serratia spp., Proteus spp. (non-mirabilis), Providencia spp., Pantoea spp., Hafnia spp., Salmonella spp., Escherichia spp. (non-coli), Cronobacter spp.*

[2] *Acinetobacter spp., S. maltophilia, Pseudomonas spp. (non-aeruginosa).*

[3] *B. fragilis, H. influenzae.*

[4] *Staphylococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp. (non-faecalis, non-faecium), M. tuberculosis.*

Table 4.3.5.2 Resistance levels (%) among diagnostic urinary 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 2018.

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	46	50	-	-	24	23	-	-
co-amoxiclav[1] - non-uuti	37	40	21	25	8	7	-	-
piperacillin-tazobactam	4	4	9	12	0	1	4	6
cefuroxime	14	17	17	20	1	1	-	-
cefotaxime/ceftriaxone	6	9	9	13	1	1	-	-
ceftazidime	5	6	9	12	0	0	1	2
meropenem/imipenem	0	0	0	0	0	0	-	-
meropenem	-	-	-	-	-	-	0	0
imipenem	-	-	-	-	-	-	2*	3*
ciprofloxacin	22	27	17	17	15	17	12	16
gentamicin	6	7	4	6	7	4	2	3
tobramycin	6	7	5	8	5	3	0	1
fosfomycin	2	1	28*	22	16	18	-	-
trimethoprim	31	32	27	25	34	34	-	-
co-trimoxazole	28	29	16	18	28	28	-	-
nitrofurantoin	4	3	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ampicillin	5	6	-	-	6	3	-	-
gentamicin + co-amoxiclav - non-uuti	5	6	3	5	3	1	-	-
gentamicin + piperacillin-tazobactam	-	1	-	2	-	1	1	2
gentamicin + cefuroxime	2	3	3	4	0	1	-	-
gentamicin + cefotaxime/ceftriaxone	2	2	3	4	0	1	-	-
gentamicin + ceftazidime	1	2	3	4	0	0	0	0
tobramycin + ceftazidime	-	-	-	-	-	-	0	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	0	1
Multidrug resistance								
HRMO[2]	9	13	10	14	5	4	1	3
multidrug resistance[3] - non-uuti	9	11	6	8	3	1	-	-

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection
**Trend not calculated, because of a low number of tests in the years before 2018*
[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*
[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam.*
[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.

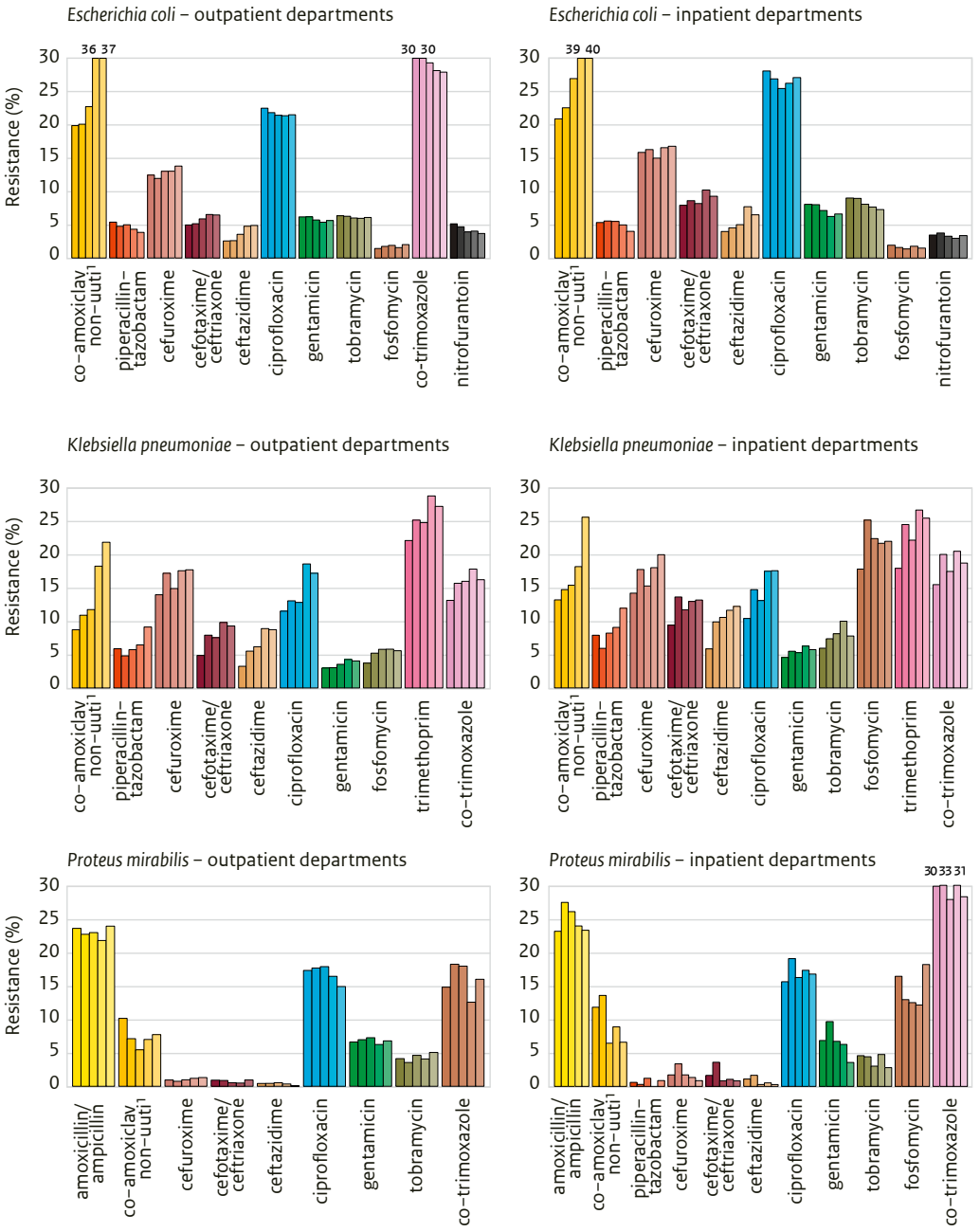
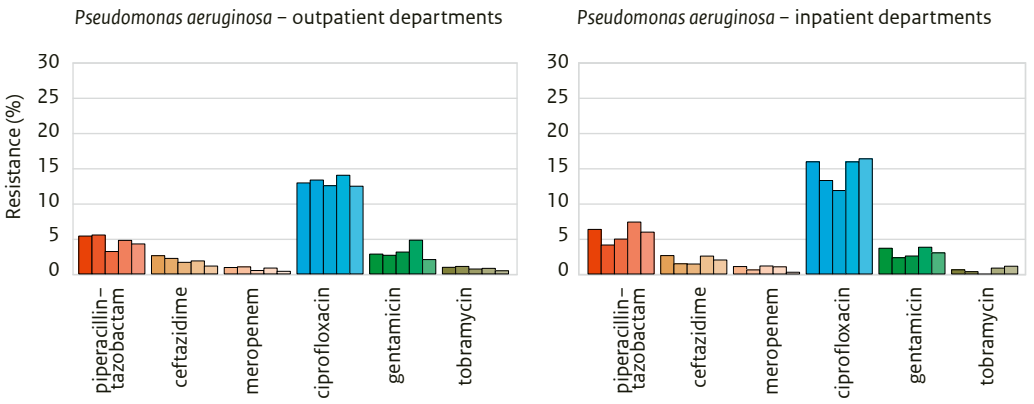


Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection

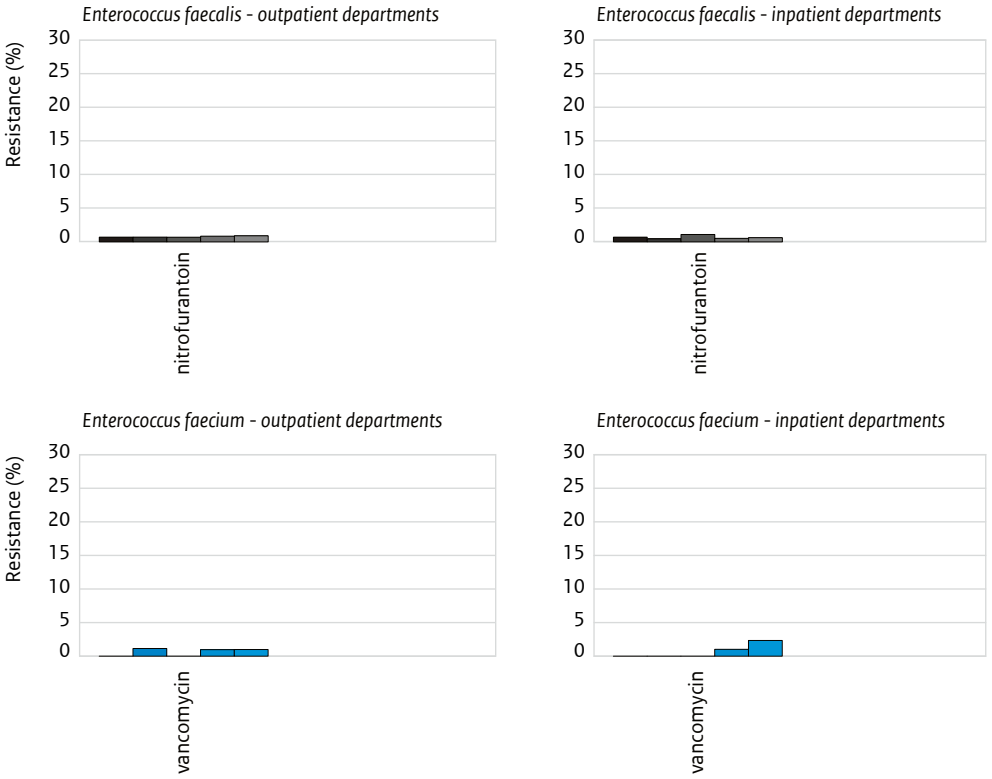
[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

Table 4.3.5.3 Resistance levels (%) among diagnostic urinary isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2018.

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	78	94
vancomycin	0	0	1	2
nitrofurantoin	1	1	-	-

10	Significant and clinically relevant increasing trend since 2014
10	Significant and clinically relevant decreasing trend since 2014
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2014 to 2018) among diagnostic urinary isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.



Key results

Enterobacteriaceae

- In all Enterobacteriaceae, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%, except in *K. pneumoniae* from IPD patients: 12%), cefotaxime/ceftriaxone (≤9%, except in *K. pneumoniae* from IPD patients: 13%), ceftazidime (≤9%, except in *K. pneumoniae* from IPD patients: 12%), meropenem/imipenem (0%), gentamicin (≤7%), and tobramycin (≤8%). In addition, levels of 10% or lower were found for fosfomycin (≤2%) and nitrofurantoin (≤4%) in *E. coli* and for co-amoxiclav (≤8%) and cefuroxime (1%) in *P. mirabilis*.
- In all Enterobacteriaceae, resistance of 20% or higher was observed for trimethoprim (≥25%). Furthermore, resistance of 20% or higher was found for co-amoxiclav in *E. coli* (≥37%) and *K. pneumoniae* (≥21%), for ciprofloxacin (≥22%) in *E. coli*, for cefuroxime (IPD only; 20%) and fosfomycin (≥22%) in *K. pneumoniae*, and for amoxicillin/ampicillin (≥23%) and co-trimoxazole (≥28) in *E. coli* and *P. mirabilis*.

- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2014 to 37% in 2018 in OPD, from 21% to 40% in IPD) and *K. pneumoniae* (from 9% to 21% in OPD, from 13% to 25% in IPD), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). Also for ceftazidime a statistically significant and clinically relevant increasing trend was observed for both *E. coli* (from 3% to 5% in OPD, and from 4% to 6% in IPD) and *K. pneumoniae* (from 3% to 9% in OPD, and from 6% to 12% in IPD). Furthermore, in *K. pneumoniae*, resistance increased to a statistically significant and clinically relevant extent for piperacillin-tazobactam (from 6% to 9% in OPD, from 8% to 12% in IPD), cefuroxime (IPD patients only; from 14% to 20%), cefotaxime/ceftriaxone (OPD patients only; from 5% to 9%), meropenem/imipenem (OPD patients only; from 0.0% to 0.2%), ciprofloxacin (from 11% to 17% in OPD, from 10% to 17% in IPD), and trimethoprim (from 22% to 27% in OPD, from 18% to 25% in IPD). A statistically significant and clinically relevant decrease in resistance was observed for co-amoxiclav (from 12% to 7%), cefotaxime/ceftriaxone (from 2% to 1%), and gentamicin (from 7% to 4%) in *P. mirabilis* from IPD patients.
- Resistance to empiric therapy combinations was ≤6%. In *K. pneumoniae*, a significant and clinically relevant increase was observed for gentamicin + co-amoxiclav (OPD patients only; from 2% in 2014 to 3% in 2018), and gentamicin + ceftazidime (from 1% to 3% in OPD patients and from 2% to 4% in IPD patients). A significant and clinically relevant decrease was observed for gentamicin + amoxicillin/ampicillin in *P. mirabilis* from IPD patients (from 5% to 3%).
- In all Enterobacteriaceae the percentage of HRMO was ≤10% for OPD patients and in *P. mirabilis* also for IPD patients (4%). A statistically significant and clinically relevant increase in HRMO was observed in *K. pneumoniae* from OPD patients (from 7% in 2014 to 10% in 2018). The percentage of multidrug resistance was ≤9%, except for *E. coli* in IPD patients (11%). Multidrug resistance increased to a statistically significant and clinically relevant extent in *E. coli* (from 6% in 2014 to 9% in 2018 in OPD, from 9% to 11% in IPD), and in *K. pneumoniae* in OPD patients (from 3% to 6%).

P. aeruginosa

- Resistance levels of 10% or lower were found for each of the selected agents, except for ciprofloxacin (OPD: 12%, IPD: 16%).
- Resistance to empiric therapy combinations and the percentage HRMO was ≤3%.

E. faecalis and E. faecium

- Resistance to vancomycin (≤2%) and nitrofurantoin (1%, presented for *E. faecalis* only) were both ≤10%.
- *In* E. *faecium*, resistance to amoxicillin/ampicillin was ≥78%.

4.3.6 Respiratory pathogens

In the current chapter the distribution of pathogens isolated from diagnostic lower and upper respiratory tract samples and resistance levels of respiratory pathogens (*S. pneumoniae*, *H. influenza*, and *M. catarrhalis*) are presented separately for general practitioners' (GP) patients and hospital patients (outpatient and inpatient departments, incl. intensive care units, combined). For GP patients the pathogen distribution is presented in table 4.3.6.1, and the resistance levels in table 4.3.6.2. For hospital patients the results are displayed in tables 4.3.6.3 and 4.3.6.4, respectively.

Although patients from general practitioners are assumed to be representative for the community with respect to resistance levels of pathogens, 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, a sample is taken for routine diagnostic purposes when a lower respiratory tract infection is suspected and therefore selective sampling bias is expected to be smaller compared with the GP setting. However, 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 former treatment failure, chronic obstructive pulmonary diseases (COPD) and cystic fibrosis (CF) may be overrepresented.

Table 4.3.6.1 Distribution of isolated pathogens in diagnostic respiratory samples from general practitioners' patients, ISIS-AR 2018.

Pathogen	Lower respiratory tract N (%)	Upper respiratory tract N (%)
S. pneumoniae	195 (7)	14 (1)
Other Gram-positives[1]	371 (14)	1,519 (79)
H. influenzae	860 (32)	50 (3)
M. catarrhalis	267 (10)	20 (1)
Other non-fermenters[2]	429 (16)	119 (6)
Enterobacteriaceae[3]	517 (20)	190 (10)
Other Gram-negatives[4]	10 (0)	1 (0)

The first isolate per patient, per microorganism, per category (lower respiratory tract; upper respiratory tract) was selected.

[1] *Staphylococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, M. tuberculosis, Enterococcus spp..*

[2] *Pseudomonas spp., S. maltophilia, Acinetobacter spp.*

[3] *Klebsiella spp., Escherichia spp., Serratia spp., Enterobacter spp., Citrobacter spp., Proteus spp., Morganella spp., Hafnia spp., Pantoea spp., Providencia spp.*

[4] *N. meningitidis.*

Table 4.3.6.2 Resistance levels (%) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients, ISIS-AR 2018.

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)[1]	0	-	-
(benzyl)penicillin (I+R)[1]	4	-	-
amoxicillin/ampicillin	-	22	-
co-amoxiclav	-	10	1
erythromycin	13	-	4
doxycycline/tetracycline	11	1	2
co-trimoxazole	12	20	3

- = Resistance not calculated.

[1] *Resistance and non-susceptibility to (benzyl)penicillin were estimated based on laboratory S/I/R interpretation for oxacillin, or, if the result for oxacillin was I or R, for (benzyl)penicillin (see methods section for more detailed information).*

Table 4.3.6.3 Distribution of isolated pathogens in diagnostic blood or cerebrospinal fluid and respiratory samples from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2018.

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Upper respiratory tract N (%)
S. pneumoniae	1,832 (5)	2,081 (9)	67 (2)
Other Gram-positives[1]	18,817 (54)	4,629 (19)	2,140 (54)
H. influenzae	205 (1)	4,973 (20)	130 (3)
M. catarrhalis	25 (0)	1,537 (6)	46 (1)
Other non-fermenters[2]	1,018 (3)	3,572 (15)	352 (9)
Enterobacteriaceae[3]	12,065 (35)	7,454 (31)	1,255 (31)
Other Gram-negatives[4]	616 (2)	87 (0)	2 (0)

[1] *Staphylococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Enterococcus spp., M. tuberculosis complex, M. tuberculosis, L. monocytogenes.*

[2] *Pseudomonas spp., S. maltophilia, Acinetobacter spp.*

[3] *Escherichia spp., Klebsiella spp., Enterobacter spp., Serratia spp., Proteus spp., Citrobacter spp., Morganella spp., Salmonella spp., Hafnia spp., Pantoea spp., Providencia spp., Cronobacter spp., Yersinia spp.*

[4] *B. fragilis complex n.n.g., B. fragilis, N. meningitidis, C. jejuni, C. lari.*

Table 4.3.6.4 Resistance levels (%) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2018.

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)[1]	1	-	-
(benzyl)penicillin (I+R)[1]	5	-	-
amoxicillin/ampicillin	-	23	-
co-amoxiclav	-	9	1
erythromycin	10	-	3
doxycycline/tetracycline	9	1	1
co-trimoxazole	8	23	5

- = Resistance not calculated.

[1] *Resistance and non-susceptibility to (benzyl)penicillin were estimated based on laboratory S/I/R interpretation for oxacillin, or, if the result for oxacillin was I or R, for (benzyl)penicillin (see methods section for more detailed information).*

Key results

S. pneumoniae

- For (benzyl)penicillin, resistance (0% in GP patients, 1% in hospital patients) and nonsusceptibility (4% and 5% in the respective patient groups) was ≤10%. Furthermore, resistance levels of 10% or lower were found for erythromycin (10%), doxycycline/tetracycline (9%) and co-trimoxazole (8%) in hospital patients.

H. influenzae

- Resistance of 10% or lower was found for co-amoxiclav (10% in GP patients and 9% in hospital patients) and for doxycycline/tetracycline in both patient groups (1%).
- Resistance levels of 20% or higher were found for amoxicillin/ampicillin (22% in GP patients and 23% in hospital patients) and for co-trimoxazole (20% and 23% in the respective patient groups).

M. catarrhalis

- Resistance to each of the selected agents was ≤5% in both patient groups.

4.4 Long-term care facilities

The distribution of pathogens in diagnostic urine and wound, pus, or skin samples from residents of long-term care facilities (LTCF) is presented in table 4.4.1. The resistance levels in 2018 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urinary samples are presented in table 4.4.2 and for *S. aureus* isolates from wound, pus, or skin samples in table 4.4.3.

LTCFs usually send urinary, wound, pus, and skin samples for culture and susceptibility testing in case of antimicrobial therapy failure or (with regard to urinary samples) complicated urinary tract infection. As a result, the presented resistance levels are likely to be higher than those for all residents with urinary tract infections caused by Enterobacteriaceae or *P. aeruginosa* or wound, pus, and skin infections caused by *S. aureus* presenting in LTCFs. Therefore, residents from whom samples were taken are further referred to as 'selected residents of long-term care facilities'.
Sampling policies in LTCFs are currently subject to change. Because the degree of restrictive sampling influences the magnitude of overestimation of resistance percentages this may result in spurious time trends. Therefore, time trends were not calculated for this chapter.

Table 4.4.1 Distribution of isolated pathogens in diagnostic urine and wound, pus, or skin samples from selected residents of long-term care facilities, ISIS-AR 2018.

Pathogen	Urine N (%)	Wound, pus, or skin N (%)
E. coli	7,684 (43)	178 (10)
K. pneumoniae	1,809 (10)	54 (3)
P. mirabilis	2,396 (14)	178 (10)
Other Enterobacteriaceae[1]	1,753 (10)	141 (8)
P. aeruginosa	996 (6)	211 (11)
Other non-fermenters[2]	160 (1)	34 (2)
Other Gram-negatives[3]	0 (0)	25 (1)
S. aureus	735 (4)	756 (41)
Other Gram-positives[4]	2,164 (12)	276 (15)

The first isolate per patient, per microorganism, per category (urine; wound, pus, or skin) was selected.

[1] *Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Serratia spp., Pantoea spp., Salmonella spp., Cronobacter spp., Escherichia spp. (non-coli), Hafnia spp.*

[2] *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *B. fragilis.*

[4] *Enterococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pneumoniae, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Staphylococcus spp. (non-aureus).*

Table 4.4.2 Resistance levels (%) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected residents of long-term care facilities, ISIS-AR 2018.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	47	-	22	-
co-amoxiclav[1] - non-uuti	39	25	7	-
piperacillin-tazobactam	6	14	1	5
cefuroxime	16	16	1	-
cefotaxime/ceftriaxone	7	8	0	-
ceftazidime	6	7	0	2
meropenem/imipenem	0	0	0	-
meropenem	-	-	-	1
imipenem	-	-	-	3
ciprofloxacin	21	15	16	11
gentamicin	6	3	4	3
tobramycin	7	4	3	1
fosfomycin	2	26	17	-
trimethoprim	27	21	37	-
co-trimoxazole	24	11	28	-
nitrofurantoin	4	-	-	-
Multidrug resistance				
HRMO[2]	11	9	4	1
multidrug resistance[3] - non-uuti	7	5	1	-

- = Resistance not calculated

non-uuti = according to breakpoint for non-uncomplicated urinary tract infection

[1] *During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see methods section for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidim, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by carbapenemase confirmatory tests, or, if no data on confirmatory tests were available, by resistance to meropenem/ imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem/ imipenem, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole*

Table 4.4.3 Resistance levels (%) among diagnostic wound, pus, and skin isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2018.

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	23
erythromycin	13
clindamycin including inducible resistance[3]	13
doxycycline/tetracycline	2
fusidic acid	10
co-trimoxazole	3

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see methods section for more detailed information).*

[2] *Resistance to ciprofloxacin is meant as class indicator for resistance to fluoroquinolones.*

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information).*

Key results

Enterobacteriaceae

- For all Enterobacteriaceae resistance levels of 10% or lower were found for cefotaxime/ceftriaxone (≤8%), ceftazidime (≤7%), meropenem/imipenem (0%), gentamicin (≤6%), and tobramycin (≤7%). In addition, resistance to piperacillin-tazobactam in *E. coli* (6%) and *P. mirabilis* (1%), to co-amoxiclav (7%) and cefuroxime (1%) in *P. mirabilis*, and to fosfomycin (2%) and nitrofurantoin (4%) in *E. coli* were also ≤10%.
- For all Enterobacteriaceae, a resistance level ≥20% was found for trimethoprim (≥21%). Additionally, resistance levels for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥25%)), for amoxicillin/ampicillin (≥22%) and co-trimoxazole in *E. coli* and *P. mirabilis* (≥24%), for ciprofloxacin in *E. coli* (21%), and for fosfomycin in *K. pneumoniae* (26%), were ≥20%.
- The percentage of HRMO in all Enterobacteriaceae was ≤9%, except for *E. coli* (11%). In all Enterobacteriaceae the percentage of multidrug resistance was ≤7%.

P. aeruginosa

- Resistance levels for each of the selected agents were ≤5%, except for ciprofloxacin (11%).

S. aureus

- Resistance of 10% or lower was found for flucloxacillin (2%), doxycycline/tetracycline (2%), fusidic acid (10%) and co-trimoxazole (3%).
- Resistance of 20% or higher was found for ciprofloxacin (23%).

4.5 Highly resistant microorganisms

4.5.1 Carbapenem-resistant and carbapenemase-producing *Enterobacterales*

Introduction

Carbapenem-resistant *Enterobacterales* (CRE) and carbapenemase-producing *Enterobacterales* (CPE), particularly *Klebsiella pneumoniae* and *Escherichia coli*, have been reported all over the world. Because carbapenems represent a drug of last resort for treatment of many enterobacterial infections, they pose significant challenges to clinicians and negatively impact patient care.[1] CRE were first described in Europe in the early 2000s and their prevalence has increased since.[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 health care settings.[3] So far, CRE are mainly a problem in hospitals, but community-spread has been described. CRE are therefore considered a growing public health threat.[4]

Information on CRE is obtained from ISIS-AR data and the Type-Ned database.

Prevalence and confirmatory testing of CRE in the Netherlands

Methods

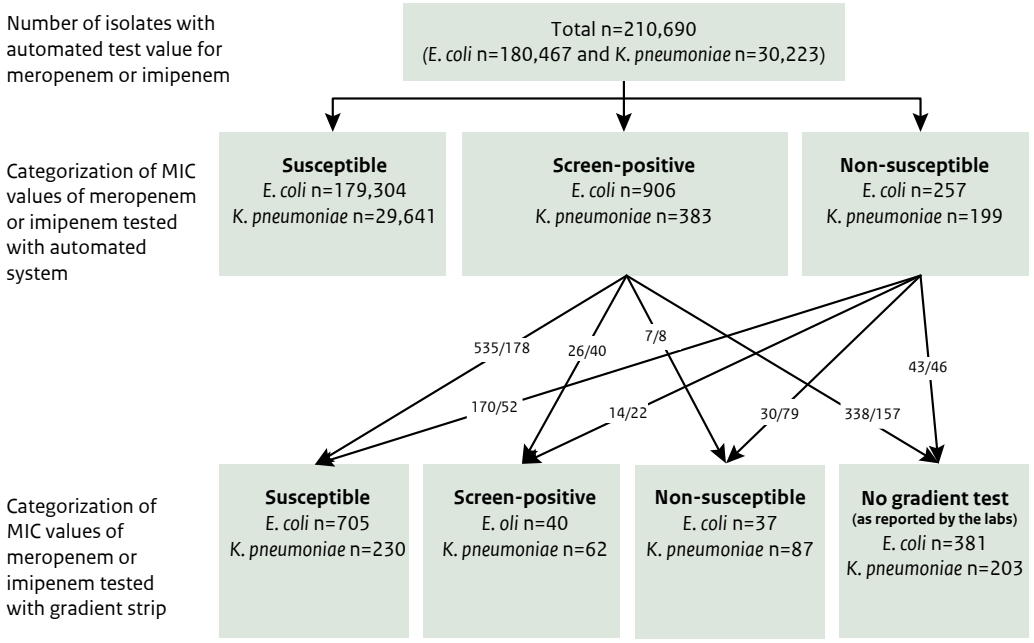
We searched the ISIS-AR database (years 2014-2018) for *E. coli* and *K. pneumoniae* isolates that were tested for meropenem and/or imipenem by automated system. Based on the crude automated test values, we categorized them as either i) non-susceptible to meropenem and/or imipenem according to EUCAST clinical breakpoints (MIC >2 mg/L), ii) screen-positive for meropenem (MIC >0.25 mg/L) and/or imipenem (MIC >1 mg/L), or iii) fully susceptible, as defined by the NVMM (NVMM Guideline Laboratory detection of highly resistant microorganisms, version 2.0, 2012). According to this guideline, elevated MICs (i.e. non-susceptible or screen-positive isolates) should be confirmed with gradient tests. Subsequently, we searched the database for data on confirmatory tests for these isolates, i.e. gradient tests and tests for carbapenemase production (phenotypical) or carbapenemase genes (genotypical). If these test results were not available in ISIS-AR, we searched the Type-Ned database for additional information. We included both diagnostic and non-diagnostic isolates, but only one isolate per patient per species: we prioritized an isolate with a gradient test over an isolate with an automated test only. Within those categories, we prioritized the most resistant isolate.
Based on data on isolates from 40 laboratories, we calculated numbers of non-susceptible, screen-positive and susceptible isolates in 2018 based on automated testing. We subsequently categorized these isolates by gradient test results. Based on data from 28 laboratories that continuously submitted data to ISIS-AR from 2014 to 2018, we calculated the percentage of isolates with elevated MIC (screen-positive and non-susceptible isolates combined) in the last five years. Additionally, we calculated the percentage of those isolates that underwent further testing.

Results

Results of sequential testing of carbapenem susceptibility in 2018, as prescribed by the NVMM, are presented in Figure 4.5.1.1. Of a total number of 210,690 isolates with an automated test value for meropenem or imipenem (180,467 *E. coli* and 30,223 *K. pneumoniae*), an elevated MIC on automated testing was found in 0.8% of isolates (1,745). Confirmatory testing using a gradient strip method (performed in 66.5% of eligible isolates) confirmed elevated carbapenem MIC values (meropenem >0.25 mg/L and/or imipenem >1 mg/L) in 19% (226/1,161) of tested isolates (10% (77/782) of *E. coli* and 39% (149/379) of *K. pneumoniae*). Among isolates with elevated MICs on automated testing, 124 gradient test confirmed carbapenem resistant isolates (MIC meropenem and/or imipenem >2 mg/L) were found (37 *E. coli*, 87 *K. pneumoniae*).

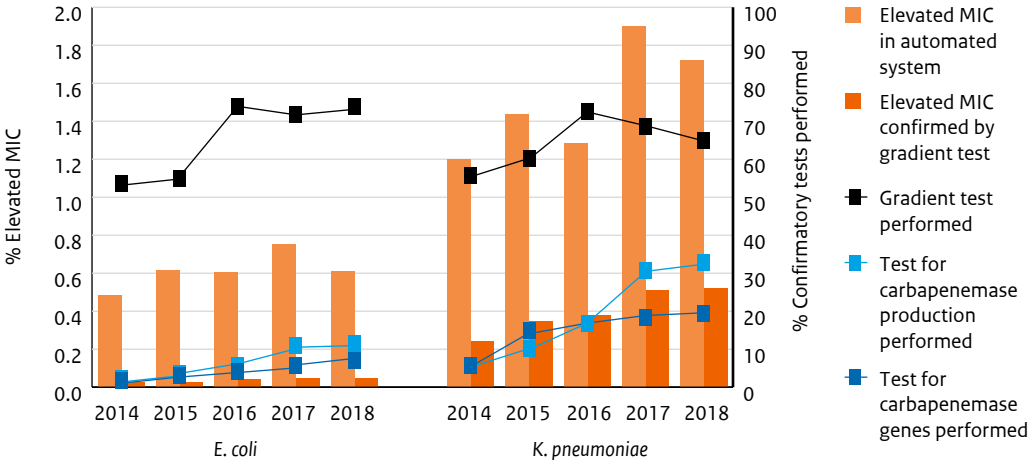
The overall prevalence of gradient test confirmed *E. coli* and *K. pneumoniae* has increased slightly over the past five years (from 0.02% in 2014 to 0.05% in 2018 in *E. coli*, and from 0.25% to 0.52% in *K. pneumoniae*, Figure 4.5.1.2), which is worrying although it is still low. The use of gradient tests to confirm elevated automated carbapenem MIC values increased between 2014 and 2016 but decreased thereafter, to 73% in *E. coli* and 64% in *K. pneumoniae* in 2018. There was an increase in tests for carbapenemase production (from 2% in 2014 to 10% in 2018 in *E. coli* and from 5% to 32% in *K. pneumoniae*) and carbapenemase genes (from 1% to 7% in *E. coli* and from 5% to 19% in *K. pneumoniae*) in the past five years.

Figure 4.5.1.1 Results of (sequential) testing of carbapenem susceptibility in *E. coli* and *K. pneumoniae* in 2018, according to NVMM guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012) in 40 laboratories participating in ISIS-AR.



Susceptible: meropenem ≤0.25 mg/L or imipenem ≤ 1 mg/L
Screen-positive: meropenem >0.25 and ≤2 mg/L or imipenem >1 and ≤2 mg/L
Non-susceptible: meropenem or imipenem >2 mg/L

Figure 4.5.1.2 (Confirmation of) elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* between 2014 and 2018, in 28 laboratories participating in ISIS-AR.



Elevated carbapenem MIC: meropenem >0.25 mg/L or imipenem >1 mg/L
The percentages of gradient tests and tests for carbapenemase production and carbapenemase genes performed were calculated for isolates with elevated MIC on automated testing

Discussion

An elevated carbapenem MIC on automated testing was found in 0.8% of isolates in 2018. This is comparable with previous years. The actual percentage of gradient test confirmed elevated MIC is much lower and is also influenced by the specificity of the automated systems and possibly by the sensitivity of the gradient tests. The percentage of isolates with a gradient test performed has not increased further since 2016. This is probably partly compensated by an increase in additional tests for carbapenemase production or carbapenemase genes in the past five years. This means that the vast majority of the suspected isolates are investigated further with one or more confirmatory tests, phenotypically and/or genotypically. It is important that confirmatory testing on both levels is performed, since phenotypic resistance does not always correlate with genotypic test results.

Molecular epidemiology

Methods

For the enhanced surveillance of CPE, Dutch laboratories submit isolates with an MIC for meropenem >0.25 mg/L and/or MIC for imipenem >1 mg/L using the Type-Ned system, with the restriction that they only send the first isolate from a person within a year. The RIVM allows consecutive isolates from the same person if these are other *Enterobacterales* species- carbapenemase combinations. The RIVM confirms the species by MALDI-ToF, MIC for meropenem, carbapenemase production by carbapenemase inactivation method (CIM), and assesses the presence of carbapenemase-coding genes by PCR (carba-PCR). Since August 2016, next-generation sequencing (NGS) is added to the enhanced CPE surveillance for all isolates that are CIM positive.

The data described in this chapter are based on the first unique CIM positive species-gene combination per person per year (gene is based on carba-PCR). Samples without a person ID were excluded from further analysis. In previous years, all *Enterobacter* species were included as separate unique carbapenemase-producing species. This has now been adapted to only the first isolate per person belonging to the *Enterobacter cloacae* complex comprising *E. cloacae, E. kobei, E. asburiae, E. ludwigii, E. hormaechi* and *E. nimipressuralis.*

Results

A total of 578 *Enterobacterales* isolates obtained in 2018, were submitted to the RIVM by 52 Dutch laboratories. Among these were 306 unique carbapenemase-producing *Enterobacterales* isolates, obtained from 266 persons (mean age 60 years and 53% male). In 2017, this was 233 CPE isolates from 201 persons.
In 2018, four outbreaks with carbapenemase-producing *Enterobacterales* were reported to the Early warning and response meeting for Hospital-acquired Infections and Antimicrobial Resistance (SO-ZI/AMR). In 2017 this were three outbreaks, see Table 4.5.1.1.

Table 4.5.1.1 Outbreaks reported in 2018 to the Early warning and response meeting of Hospital-acquired Infections and Antimicrobial Resistance (SO-ZI/AMR).

Region	Main organism	*Gene*	*No of patients*
West	*K. pneumoniae*	NDM + OXA-48	5
South	*K. pneumoniae*	OXA-48	2
West	*K. pneumoniae*	NDM + OXA-48	4
West	*C. freundii*	NDM	26

The majority (n=212; 69%) of the 306 isolates, was identified from throat, nose, perineum or rectum swabs, followed by urine (45/306; 15%) and wound material or pus (17/306; 6%), which is a similar distribution to 2017.

In 238 of the 266 persons, a single carbapenemase-producing species was found, whereas multiple unique carbapenemase-producing species (68 isolates) were isolated from 28 persons. The most frequently identified genes were the genes coding for OXA-048, NDM, VIM and KPC. In 18 persons both OXA-048 and NDM coding genes were detected in the same isolate. Of the 28 persons with multiple unique carbapenemase-producing species, this involved the same gene in different species, up to four species, in 18 persons. In seven persons, a different gene in different species was detected, including two cases with a mixture of the same gene in different species. In the remaining three, different combinations of single and double carbapenemase genes in different species were present.
A different gene in the same species was not observed in 2018. Twelve unique isolates from 12 patients did not yield a PCR product in the carba-PCR and no carbapenemase gene was identified with NGS. Eleven of these isolates were *Enterobacter cloacae* complex species. NGS analysis was performed for 303 isolates originating from 264 persons (Figure 4.5.1.3). Meropenem susceptibility for the major carbapenemases in *E. coli* and *K. pneumoniae* is shown in Figure 4.5.1.4.

Figure 4.5.1.3 Distribution of carbapenemase-encoding genes in carbapenemase producing isolates submitted in 2018.

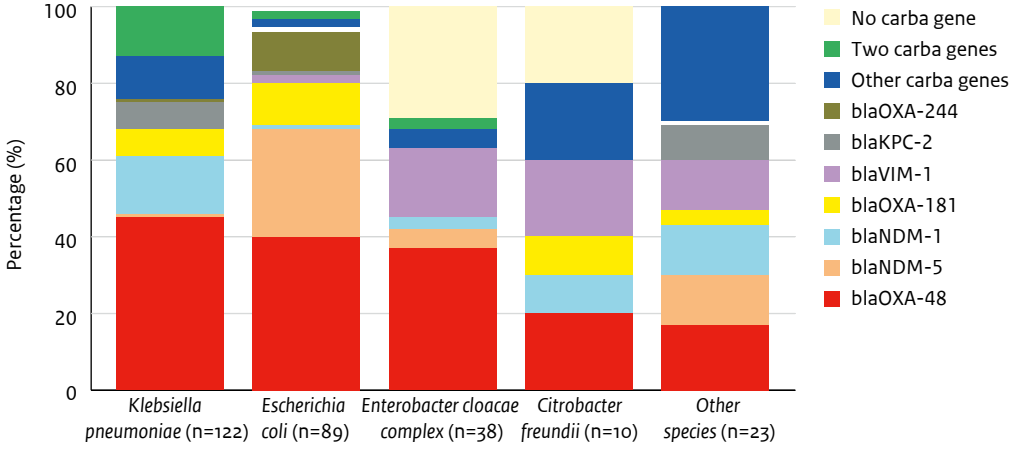
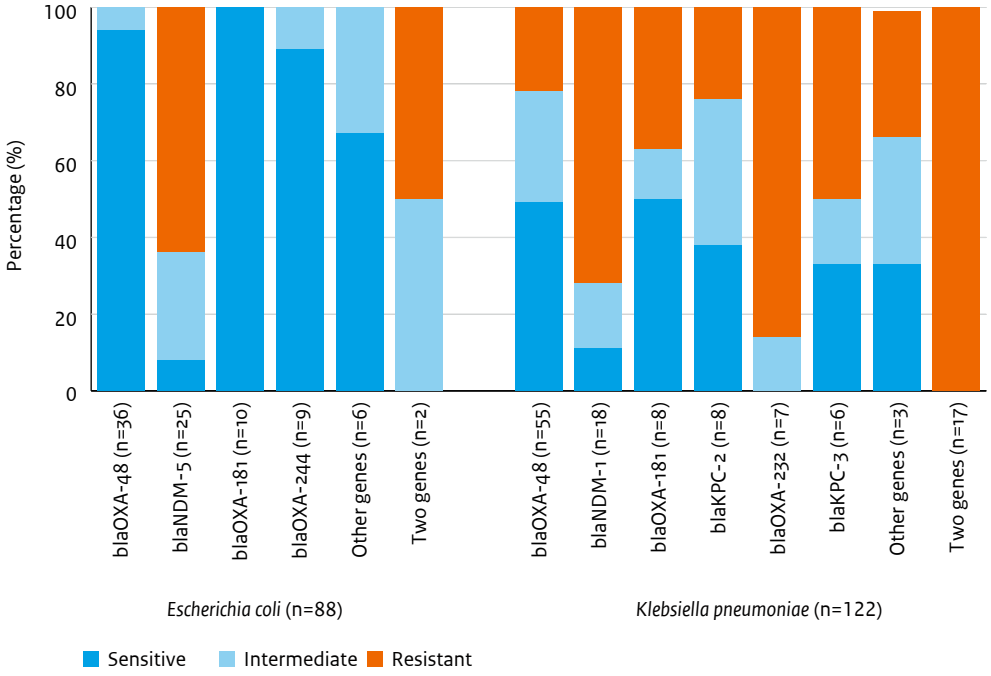


Figure 4.5.1.4 Relationship between MIC for meropenem and carbapenemase-coding genes in *E. coli* and *K. pneumoniae* isolates submitted in 2018.



Clinical breakpoints based on 2018 EUCAST criteria.

The blaOXA-48 gene was the most frequently found carbapenemase-encoding gene in carbapenemase-producing isolates submitted in 2018 and was present in approximately 40% of the major species *E. coli*, *K. pneumoniae* and *Enterobacter cloacae* complex (Figure 4.5.1.3). The second most frequently found carbapenemase-encoding genes found in *K. pneumoniae* and *E. coli* were blaNDM-1 and blaNDM-5, respectively. Although all *Enterobacter cloacae* complex isolates produced carbapenemase, no carbapenemase-encoding gene could be identified in 29% (n=11) of the isolates. In *Citrobacter freundii* blaNDM-5 was the most predominant carbapenemase-encoding gene. However, all isolates of this species/gene-combination (n=23) were obtained during a large outbreak in 2018 in a Dutch hospital and therefore have not been included in the figure. In *K. pneumoniae* 14% (n=17) of the isolates carried two different carbapenemase-encoding genes simultaneously.

There was a strong correlation between MIC for meropenem and the presence of particular species/gene combinations. Ninety-four percent of all *E. coli* carrying blaOXA-48 were sensitive (MIC ≤2 mg/L) and none of the isolates had MICs above the clinical breakpoint for meropenem resistance (MIC >8 mg/L; Figure 4.5.1.4). In *K. pneumoniae*, this was slightly better with 22% of the blaOXA-48 carrying isolates above the clinical breakpoint for meropenem resistance. All blaOXA-181 *E. coli* were sensitive, but this was different for blaOXA-181 *K. pneumoniae* of which only 50% was sensitive and 38% was resistant.

The majority (64%) of the blaNDM-5 carrying *E. coli* did have MICs above the clinical breakpoint and only 8% was sensitive. All *K. pneumoniae* isolates (n=17) carrying two different carbapenemase-encoding genes simultaneously, were resistant.

Additional epidemiological questionnaire data was available for 183 isolates (60%) originating from 161 persons (61%) with a confirmed CPE isolate (Table 4.5.1.2). Questionnaire data was analyzed on person level and not on isolate level.

Screening was the reason for taking the sample in 71% of the isolates, which is comparable to 2017. Hospitalization abroad for at least 24 hours during the previous two months was the most common risk factor for the presence of CPE (58%), with Turkey (n=20) and Morocco (n=14) leading the list of countries reported. This was 48% in 2017. No risk factor was identified in 50 patients (31%), which was 34% in 2017. When risk factors are assessed for diagnostic isolates solely, hospitalization abroad for at least 24 hours during the previous two months is reported less often (18%) and the majority has no risk factor (64%). Among screening isolates, 74% had been hospitalized abroad for at least 24 hours during the previous two months.
Over the past period, a considerable number of CPE was obtained from cultures requested by general practitioners (GPs). Between January 2016 and October 2018, this concerned 102 (14%) of 751 CPE isolates. Only a small proportion (26%) was from cultures for clinical indication, such as a possible urinary tract infection and 68% was for screening. Often, the screening was performed upon request from the hospital, or in expectation that the patient would visit a hospital soon.

Table 4.5.1.2 CPE epidemiological questionnaire data from the CPE enhanced surveillance system in 2018.

Characteristic	CPE positive persons, n (%)*
Any questionnaire data available	161/266 (61); isolates: 183/306 (60)
Sample taking location	
Outpatient departments	37 (23)
Inpatient departments (excluding Intensive Care Units)	68 (42)
Intensive Care Units	22 (14)
At home	6 (4)
Other (mainly general practitioners)	27 (17)
Unknown	1 (1)
Reason for culturing	
Diagnostic	45 (28)
Screening	115 (71)
In the context of research	1 (1)
Residence	
Living independently	142 (88)
Nursing or elderly home	5 (3)

Characteristic	CPE positive persons, n (%)*
Rehabilitation centre	3 (2)
Other	11 (7)
Underlying illness	
No underlying illness	89 (55)
Malignancy/leukaemia	19 (12)
Dialysis	5 (3)
Other	48 (30)
Risk factors	
No risk factor known	50 (31)
Hospitalization abroad >24 hours during the previous two months	93 (58)
Hospitalized in a country in:	
West Asia (including Turkey)	21/93 (23)
North Africa	20/93 (22)
South Europe	17/93 (18)
South Asia	12/93 (13)
Southeast Asia	11/93 (12)
Other region of the world/unknown	12/93 (13)
Already known carrier of CPE	6 (4)
Received care in a department of another healthcare facility with an ongoing outbreak of CPE in the previous two months	6 (4)
Contact with a hospital abroad in the last year in a different way than >24 hours during the previous two months	4 (2)
Travelling abroad in the past six months without hospitalization or visiting a hospital	2 (1)
Known CPE outbreak in own healthcare facility	3 (2)
Work-related exposure to livestock animals	1 (1)

* *Numbers and percentages are reported on person level with available questionnaire data for the particular characteristic (N=161 as denominator) unless otherwise indicated*

Discussion

In 2018, more *Enterobacterales* isolates were submitted to the RIVM than in 2017, and as a result more CIM positive isolates were detected (n=306 in 2018 vs. n=233 in 2017). It is unknown to what extent the increase in the number of CPEs submitted to Type-Ned is reflecting the increased awareness among laboratories to test and submit samples for the national surveillance, how large the influence is of more attention for surveillance in general (i.e. letter from the Minister), and what part may reflect an actual increase of the occurrence of CPE in the Netherlands.

Performing WGS on all isolates will allow identification of clusters involving multiple hospitals or other health care institutes.

Conclusions

- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing has remained stable (around 0.8%) over the past five years.
- The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* has increased slightly over the past five years, but was still low overall (0.05% and 0.52% in 2018, respectively).
- Confirmatory testing of elevated MIC values with a gradient strip method has not increased further since 2016, but the use of additional tests for carbapenemase production or carbapenemase genes has increased over the past five years.
- The number of CPE submitted to the RIVM increased in 2018 compared to 2017.
- The most frequently identified carbapenemase encoding genes in *Enterobacterales* were genes encoding for OXA-048, NDM, VIM and KPC and *E. coli*, *K. pneumoniae* and *E. cloacae* complex were the species most frequently involved.
- MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli*. Strains harboring OXA-048 and VIM-1 were mostly sensitive (MIC ≤2 mg/L), whereas strains harboring NDM-5 or two carbapenemase genes were mostly resistant (MIC >8 mg/L).
- Hospitalization abroad for more than 24 hours during the last two months is the main risk factor for CPE in the Netherlands (58%).

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[2] Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P; European Network on Carbapenemases. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect. 2012 May;18(5):413-31.

[3] Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H; European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) Working Group. Carbapenemase producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill. 2013 Jul 11;18(28). pii: 20525. Erratum in: EuroSurveill. 2013;18. pii: 20575. Euro Surveill. 2014;19(47): pii=20972.

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4.5.2 Vancomycin-resistant Enterococci

Introduction

In the last few years, a growing number of Dutch hospitals have been confronted with outbreaks of vancomycin-resistant *Enterococcus faecium* (VRE). From 2012 onwards, in-depth analysis of the evolutionary relatedness of *E. faecium* genotypes on a population level using Multi Locus Sequence Typing (MLST) was performed by the UMC Utrecht. Unfortunately, since 2018, centrally collected and aggregated national data on molecular typing of VRE are no longer available.

Methods

VRE outbreaks are reported through the Early warning and response meeting for Hospital-acquired Infections and Antimicrobial Resistance (SO-ZI/AMR, see section 4.5.6).
In the national surveillance system of antimicrobial resistance, ISIS-AR, the proportion of VRE in *E. faecium* isolates among patients in various healthcare settings in the Netherlands was determined. Only diagnostic isolates (i.e. infection-related and thus non-screening samples) from routine practice were included. Numbers are based on data from 32 laboratories that continuously reported to the ISIS-AR database in the previous five years. The first *E. faecium* isolate per patient was selected.

Results

In 2018, 15 outbreaks with VRE have been reported in the Netherlands in SO-ZI/AMR, of which 13 in hospitals and two in long-term care facilities. The number of outbreaks over the last few years remains stable. In total, since the start of SO-ZI/AMR in April 2012, 87 outbreaks with VRE have been reported in the Netherlands. The contribution of VRE outbreaks is substantial, with a proportion varying between 20 and 25% of all reported outbreaks in SO-ZI/AMR yearly.

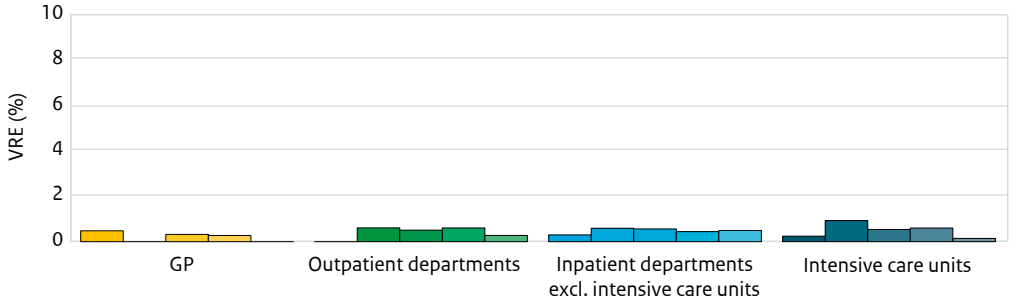
The percentage of VRE isolates in general practitioner patients and outpatient and inpatient hospital departments in 2018 in the Netherlands based on ISIS-AR is shown in table 4.5.2.1. Figure 4.5.2.1 shows the trends in vancomycin-resistance over the years. The number of diagnostic isolates with VRE was continuously low over the years.

Table 4.5.2.1 Vancomycin-resistant *E. faecium* (VRE) in the Netherlands in 2018 in diagnostic samples, based on ISIS-AR data.

Type of department	Tested isolates, N	VRE, N (%)
GP	310	0 (0)
Outpatient departments	327	1 (0)
Inpatient departments excluding intensive care units	2,019	11 (1)
Intensive care units	623	1 (0)

Numbers are based on a selection of 32 laboratories.
The first diagnostic E. faecium isolate per patient was selected.
Based on re-interpretation according to EUCAST 2018.
The prevalence of VRE isolates was based on positivity of confirmation tests, or, if these tests were lacking, on laboratory S/I/R interpretation for amoxicillin/ampicillin and vancomycin, with VRE being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 4.5.2.1 Trends in vancomycin-resistant *E. faecium* (VRE) in the Netherlands (from left to right 2014 to 2018), based on ISIS-AR data.



Numbers are based on a selection of 32 laboratories.
The first diagnostic E. faecium isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2018
The prevalence of VRE isolates was based on positivity of confirmation tests, or, if these tests were lacking, on laboratory S/I/R interpretation for amoxicillin/ampicillin and vancomycin, with VRE being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

Since there are no centrally collected data on molecular typing of VRE isolates, there are no longer reliable data available on the molecular epidemiology of VRE in Dutch hospitals since 2018. The number of reported VRE outbreaks seems to be stable in the last few years, just as the low proportion of infection-related isolates with VRE in various healthcare settings.

Infection prevention and control of a VRE outbreak are expensive and cause a high financial burden for a hospital. It is not clear if VRE infections impose an extra burden on morbidity and mortality compared to ampicillin-resistant, vancomycin-susceptible *E. faecium* (ARE). A recent retrospective matched cohort study investigated the fraction of mortality in VRE bacteremia superimposed by vancomycin resistance in both the Netherlands and Denmark. The study showed that VRE bacteremia was associated with a higher risk for 30-day mortality compared to ARE bacteremia. The increased risk could not be explained by a delay in appropriate antibiotic therapy, but might be related with unmeasured confounding.

An alternative explanation would be a higher virulence of VRE compared to ARE, in spite of a high resemblance of the core genomes of ARE and VRE.[1] In a recent study from Germany, where they used adjustment for various underlying diseases, vancomycin resistance in patients with *E. faecium* bacteremia was not associated with higher in-hospital mortality compared to vancomycin susceptibility in the same species.[2] A study by Woudt et al (based on ISIS-AR data) showed that the absolute number of VRE bacteremias yearly in the Netherlands is very low. Furthermore, in their analyses, a primary VRE bacteremia only lead to a marginally elevated risk of a recurrent VRE bacteremia, compared to a primary vancomycin- and amoxicillin-susceptible bacteremia (relative risk of 1.6 (95% CI 0.6-4.2)). Thus, the attribution of vancomycin-resistance expressed as the number of primary infections leading to recurrent resistant infections was limited.[3]

Conclusions

- The number of hospital outbreaks with VRE remains stable over the last few years
- The proportion of VRE in infection-related isolates with *E. faecium* in various healthcare settings varies marginally below 1% and has not changed in the previous five years
- There are no longer reliable data available on the molecular epidemiology of VRE in Dutch hospitals, which is a cause for great concern.
- Vancomycin resistance in *E. faecium* does possibly not impose an extra burden on morbidity and mortality compared to vancomycin-susceptible *E. faecium*, if accounting for underlying diseases.

References

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2 Kramer TS, Remschmidt C, Werner S, et al. The importance of adjusting for enterococcus species when assessing the burden of vancomycin resistance: a cohort study including over 1000 cases of enterococcal bloodstream infections. Antimicrob Resist Infect Control 2018; 7:133

3 Woudt SHS, de Greeff SC, Schoffelen AF, et al. Antibiotic resistance and the risk of recurrent bacteremia. Clin Infect Dis 2018; 66(11):1651-1657.

4.5.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Introduction

The Netherlands is a country with a low MRSA prevalence. This is most probably explained by the strict "search and destroy" MRSA policy and the low use of antibiotics. The ISIS-AR database contains information regarding MRSA test results from routine diagnostics in medical microbiology laboratories. To monitor the occurrence of MRSA and the molecular characteristics of circulating MRSA types more in-depth at a national level enhanced MRSA surveillance was started in 1989 by the RIVM.

Methods

From the ISIS-AR database, *S. aureus* isolates including MRSA isolates were identified for unique patients in 2018. Numbers are based on data from 32 laboratories that continuously reported complete data to the ISIS-AR database during the five most recent years (2014 to 2018). The first *S. aureus* isolate per patient was selected.

For the enhanced MRSA surveillance, Dutch laboratories are requested to submit identified MRSA isolates using the Type-Ned system for molecular typing using multiple-locus variable number of tandem repeat analysis (MLVA). Isolates in the database were categorized as either diagnostic (isolated from samples of infection-related materials, i.e. blood, cerebrospinal fluid, sputum, pus, urine or wound) or screening (isolated from screening-related materials). Livestock-associated MRSA (LA-MRSA) was defined for the MLVA-complex MC0398.

From November 2016 on, next-generation sequencing (NGS) has been added to the enhanced MRSA surveillance for diagnostic isolates only. A risk factor questionnaire is requested to be completed as part of the enhanced surveillance. Late November 2018, a new version of the epidemiological questionnaire was launched.

The data used in this chapter were based on the first MRSA isolate per person in 2018, with the exception that the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person. In addition, samples from non-human origin, *S. aureus* negative for the *mecA* and *mecC* gene, samples that could not be typed with MLVA, and isolates without a person ID were also excluded from further analysis.

Results

Prevalence

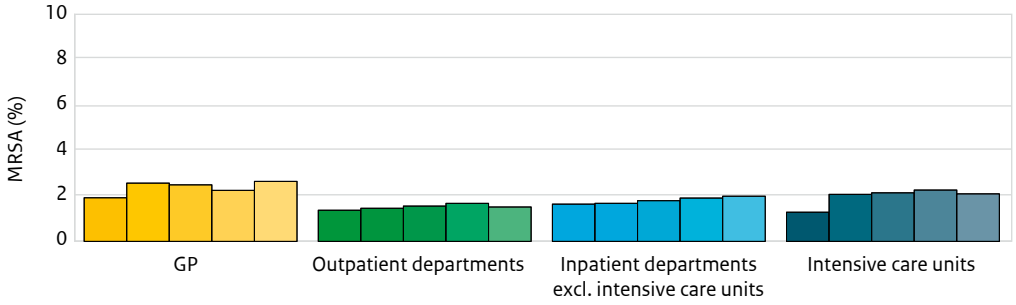
The proportion of *S. aureus* that is MRSA in diagnostic isolates (including blood samples) based on ISIS-AR was 2% (634/31,266) and it was comparable in all types of departments (Table 4.5.3.1).

Figure 4.5.3.1 shows the trends in MRSA from 2014 to 2018 in all diagnostic isolates, which seems to be quite stable. However, screening using selective culture media will strongly favor the isolation of MRSA over methicillin susceptible *S. aureus*. Therefore, the MRSA prevalence in the population may be overestimated if based on all samples. In blood isolates, expected to be most unbiased, MRSA prevalence was 1.2% (35/2,818).

Table 4.5.3.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2018, based on ISIS-AR data.

Type of department	Tested isolates, N	MRSA, N (%)
GP	7,626	206 (3)
Outpatient departments	11,172	173 (2)
Inpatient departments excluding Intensive Care Units	11,113	226 (2)
Intensive Care Units	1,355	29 (2)
Total	**31,266**	**634 (2)**

Figure 4.5.3.1 Trends in methicillin-resistant *S. aureus* (MRSA) in the Netherlands (from left to right 2014 to 2018), based on ISIS-AR data.



The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2), or, if these tests were lacking, on laboratory S/I/R interpretation for cefoxitin. If no data on a cefoxitin test was available, the prevalence was based on laboratory S/I/R interpretation of flucloxacillin/oxacillin.
Numbers are based on a selection of 32 laboratories.
The first diagnostic S. aureus isolate per patient was selected.
Based on laboratory S/I/R interpretation.

Molecular results and epidemiology

A total of 3,525 genotyped isolates obtained in 2018 from 3,296 persons (mean age 46 years and 1,782 (54%) male) submitted by 54 laboratories fulfilled the inclusion criteria (*S. aureus mecA* or *mecC* gene positive, from human origin with a known person ID). Thus, 3,296 isolates from single persons were used for further analysis.

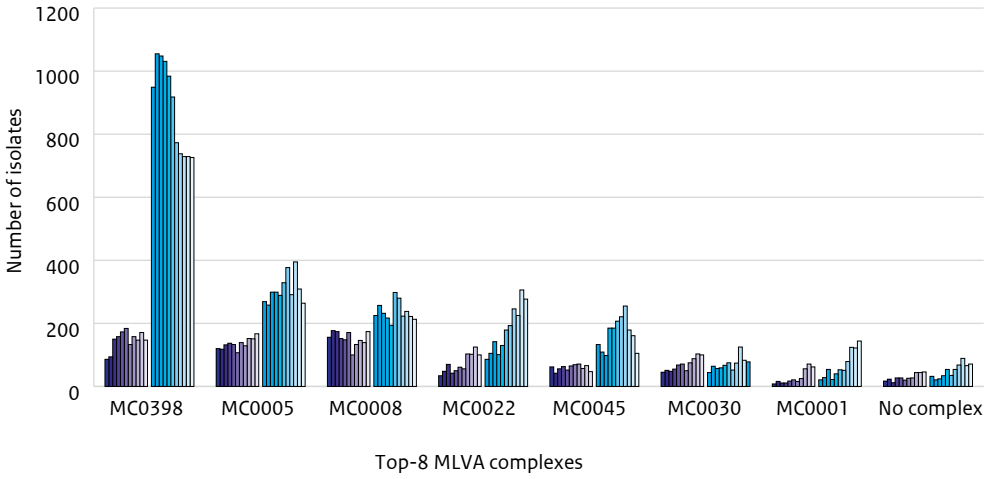
As in previous years, the majority of isolates were cultured from samples submitted to the MML by hospitals (2,059/3,296; 62%), followed by GPs (975/3,296; 30%) and nursing or elderly homes (169/3,296; 5%). Based on culture methods and origin of the samples, 68% (2,240/3,296) of the isolates were identified as screening samples (mainly swabs of nose, throat and perineum) (Figure 4.5.3.2). A total of 1,036 samples (31%) were identified as diagnostic sample, with the majority being wound material or pus (749/1,036; 72%) and 34 blood samples (3%). For 20 samples (1%), the origin of the sample was unknown. All these proportions are similar to data from 2017.

For 2018, the MRSA population could be divided into 689 MLVA-types, which were grouped into 24 MLVA-complexes (MCs). The most common MLVA-complex in 2018 was MC0398, also known as LA-MRSA, which was detected in 879/3,296 (27%) of the isolates. Of the LA-MRSA isolates, 17% were diagnostic isolates (based on culture methods and origin of the samples), 83% were obtained from targeted screening, and for 1% it was unknown, comparable to previous years. The number of LA-MRSA screening isolates decreased over time, while this was not seen for diagnostic isolates (Figure 4.5.3.2).

The number and proportion of diagnostic isolates was higher among the non-LA-MRSA (889/2,417; 37%) than among the LA-MRSA isolates and seems to be increasing over time: from 26% (n=573) in 2014 to 37% (n=889) in 2018. Among the diagnostic isolates, MC0005, MC0008 and MC0022 were the most prevalent non-LA-MRSA MLVA complexes (Figure 4.5.3.2). There has been a considerable increase in the prevalence of MC0022 and MC0001 isolates. The prevalence of MC0045 MRSA isolated from screening samples has dropped, but the prevalence of MC0045 isolates from diagnostic samples remained unchanged over time.

Presence of *mecA* was confirmed in 3,287/3,296 isolates, while 9 isolates contained *mecC* (all non-LA-MRSA, 7/9 (78%) belonging to MC0429). Panton-Valentine Leukocidin (PVL) positivity increased from 18% in 2014 to 24% (806/3,296) in 2018, of which 7% (58/806) were LA-MRSA, compared to 3% in 2017. Most of the PVL-positive LA-MRSA isolates were of MLVA type MT0569 (48/58; 83%) and non-LA-MRSA was often MC0008 (247/748; 33%).

Figure 4.5.3.2 Temporal trends of the most frequently identified MLVA complexes of MRSA in the Netherlands (2008 to 2018), based on the enhanced MRSA surveillance data.



Only the first MRSA isolate per person was selected
The purple bars represent the diagnostic isolates the lighter blue bars denote screening isolates
Diagnostic indicates that the material originates from blood, cerebrospinal fluid, sputum, pus, urine or wound; screening indicates swabs of nose, throat, perineum, rectum or insertion site

Risk groups

Epidemiological questionnaire data were available for 2,632 unique persons (80%) (Table 4.5.3.2).

Hospitalization abroad for at least 24 hours during the previous two months was recorded for 143/2,603 persons (5%), which is similar to data from 2016 and 2017. Turkey was most often mentioned as country of hospitalization (21% of all countries listed). Work-related exposure to livestock animals was reported for 253 persons (10%), all except 14 of them (95%) had LA-MRSA (98% in 2017).

Of the patients with MRSA in diagnostic isolates, the large majority was previously not suspected for MRSA carriage. The data presented in Table 4.5.3.2 for diagnostic isolates only is also similar to 2016 and 2017.

Table 4.5.3.2 MRSA questionnaire data and risk groups from the enhanced MRSA surveillance in 2018.

Characteristic	MRSA positive persons, n/N (%)
Questionnaire	
Any data available	2,632/3,296 (80); isolates: 2,808/3,525 (80)
The person is a(n)	
Patient	2,480/2,632 (94)
Employee	152/2,632 (6)
Sample taking location	
Outpatient departments	847/2,594 (33)
Inpatient departments (excluding Intensive Care Units)	578/2,594 (22)
Intensive Care Units	59/2,594 (2)
Other/unknown	1,110/2,594 (43)
Reason for culturing	
Diagnostic	963/2,632 (37)
Screening	1,669/2,632 (63)
Risk factors	
Work-related exposure to livestock animals	253/2,603 (10)
Pigs	197/253 (78)
Cattle	32/253 (13)
Hospitalization abroad >24 hours during the previous two months	143/2,603 (5)
Hospitalized in a country in:	
Western Asia (including Turkey)	35/143 (24)
Southern Europe	25/143 (17)
Western Europe	22/143 (15)
Asylum seeker living in asylum centre	107/2,603 (4)
Profession in healthcare with direct patient contact	27/2,603 (1)
Meeting WIP[1] risk category 1, 2 or 3	1,445/2,245 (64)
Data for diagnostic isolates only	
Any questionnaire data available	807/1,036 (79)
Work-related exposure to livestock animals	7/795 (1)
Hospitalization abroad >24 hours during the previous two months	21/795 (3)
Hospitalized in a country in:	
Western Europe	6/21 (29)
Southern Europe	5/21 (24)
Western Asia (including Turkey)	4/21 (19)
Asylum seeker living in asylum centre	8/795 (1)
Profession in healthcare with direct patient contact	3/795 (0)
Meeting WIP[1] risk category 1, 2 or 3	124/603 (21)

WIP: Working Party in Infection Control
Numbers and percentages are reported on person level with available questionnaire data for the particular characteristic

Discussion

Screening isolates originate from selective cultures, from which methicillin sensitive *S. aureus* is not reported, and can therefore not be used to calculate the percentage of MRSA among all *S. aureus*. In the ISIS-AR database, screening samples could potentially be misclassified as diagnostic samples, thereby falsely increasing the proportion of MRSA in diagnostic isolates.
The distinction between screening and diagnostic isolates of the enhanced surveillance is solely based on the material and origin of the samples and not based on the reason for culturing since this information was missing for 20% of the isolates. Therefore, some misclassification of screening and diagnostic isolates will have occurred. The most common MLVA-complex found in the enhanced surveillance still is MC0398 (LA-MRSA). This is probably due to the search and destroy policy, where persons with exposure to livestock are actively screened for MRSA carriage.

Conclusions

- The proportion of *S. aureus* that was MRSA positive in unbiased blood-culture isolates was 1.2%. The overall prevalence in biased diagnostic samples of all materials (including blood samples) was around 2% (3% in general practices and 2% in outpatient departments, hospital departments, and Intensive Care Units. There is no increasing trend in the occurrence of MRSA in all diagnostic samples).
- LA-MRSA is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance.
- The proportion of diagnostic isolates among non-LA-MRSA subtypes is higher than among LA-MRSA isolates (37% vs. 17%).
- A large proportion (36%) of the persons positive for MRSA does not seem to have a risk factor as defined in the WIP risk categories. This is similar to 2016 and 2017.

References

[1] Dutch Working Party on Infection Control (WIP) MRSA guidelines. 2012; available from: www.wip.nl.

4.5.4 Carbapenemase-producing *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is one of the most common nosocomial pathogens that are intrinsically resistant to various antibiotics. The emergence of multidrug resistant (MDR) *P. aeruginosa* is a problem of global concern and in 2017, the World Health Organization classified carbapenem-resistant *P. aeruginosa* as 'priority 1: critical'.[1]

Methods

Data on MDR *P. aeruginosa* were extracted from the ISIS-AR database. Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.
In addition, *P. aeruginosa* isolates were sent by medical microbiology laboratories to the RIVM as part of the national surveillance on carbapenemase-producing *Enterobacterales* via Type-Ned, although not belonging to the group of *Enterobacterales*. Submitted isolates were analyzed to confirm the species by MALDI-ToF. Carbapenem resistance was determined by assessing minimal inhibitory concentrations (MIC) for meropenem by Etest. Carbapenemase production was evaluated by the carbapenemase inactivation method (CIM)[2] and the presence of carbapenemase-encoding genes by multiplex PCR.

Results

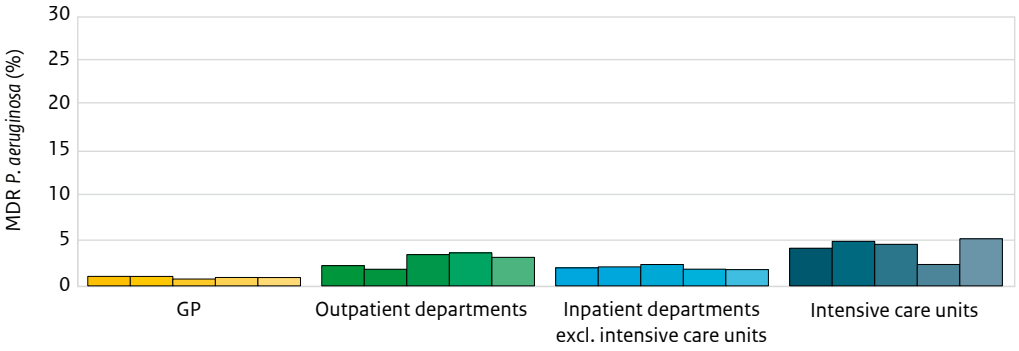
A search in the ISIS-AR database in 2018 revealed that 2% (269/13,151) of the diagnostic (infection-related) *P. aeruginosa* isolates were MDR. Approximately 50% (135/269) of the MDR isolates were phenotypically resistant to carbapenems (>8 mg/L). This fraction was highest in isolates from ICUs (18/26; 69%) and lowest for isolates obtained from patients attending the general practitioner (14/37; 38%) (Table 4.5.4.1). The observed distribution appears to be relatively stable over the 2014-2018 time period (Figure 4.5.4.1).

Table 4.5.4.1 Multidrug resistant MDR *P. aeruginosa* in the Netherlands in 2018, based on ISIS-AR data.

Type of department	Tested isolates, N	MDR P *aeruginosa* spp., N(%)	Carbapenem resistant MDR P *aeruginosa* spp., N(%)
GP	4,028	37 (1)	14 (38)
Outpatient departments	3,777	119 (3)	59 (50)
Inpatient departments excluding intensive care units	4,846	87 (2)	44 (51)
Intensive care units	500	26 (5)	18 (69)
Total	**13,151**	**269 (2)**	**135 (50)**

Numbers are based on a selection of 32 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Based on re-interpretation according to EUCAST 2018.
Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.

Figure 4.5.4.1 Trends in Multidrug resistant (MDR) *P. aeruginosa* in the Netherlands (from left to right 2014 to 2018), based on ISIS-AR data.



Numbers are based on a selection of 32 laboratories.
The first diagnostic P. aeruginosa isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2018.
Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.

The RIVM received 1873 *P. aeruginosa* isolates sampled between January 2014 and December 2018 (Table 4.5.4.2). Of these isolates, 237 (13%) produced carbapenemase (one isolate per person per year). PCR revealed that the majority of the carbapenemase-producing isolates (192/237; 81%) carried a blaVIM gene. The remaining isolates carried blaIMP (7%), blaNDM (2%) and blaKPC (0.4%) and 22 isolates (9%) did not yield a PCR product. Isolates incapable of producing carbapenemase as determined by the CIM test, did not yield a PCR product. Of the carbapenemase-producing *P. aeruginosa* (CPPA) isolates 67% (158/237) had MICs for meropenem above the clinical breakpoint.

Discussion

In 2018, 2% of *P. aeruginosa* in diagnostic isolates were MDR and approximately 50% of these MDR isolates were phenotypically resistant to carbapenems, similarly as in 2017.
The majority (81%) of the CPPA carried the blaVIM gene. Only 67% of the CPPA isolates had MICs for meropenem above the clinical breakpoint. The observed annual distribution was similar throughout 2014-2018.

Table 4.5.4.2 Distribution of carbapenemase-encoding genes based on PCR in carbapenemase-producing *P. aeruginosa* isolates received by the RIVM during the CPE surveillance 2014-2018.

	Carbapenemase gene					Total (%)
MIC meropenem	**VIM**	**IMP**	**NDM**	**KPC**	**PCR-negative**	
≤2 mg/L (S)	11					11 (5)
2-8 mg/L (I)	51	6			11	68 (29)
>8 mg/L (R)	130	11	5	1	11	158 (67)
Total	**192**	**17**	**5**	**1**	**22**	**237**

Numbers are based on isolates producing carbapenemase as indicated by the CIM test
The first isolate per person per year was selected.
Definitions for resistance (clinical breakpoints) are based on the EUCAST criteria of 2018.

Conclusions

- In 2018, 2% of the Dutch *P. aeruginosa* isolates was MDR and 50% of these MDR isolates were carbapenem-resistant and predominantly originated from ICUs.
- The most predominant (81%) carbapenemase-encoding gene in carbapenemase-producing *P. aeruginosa* was blaVIM.
- Only 67% of the carbapenemase-producing *P. aeruginosa* had MICs as measured by Etest interpreted as resistant according to the EUCAST clinical breakpoints.

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4.5.5 Extended spectrum beta-lactamases

Introduction

Extended spectrum beta-lactamase producing *Enterobacterales* (ESBL-E) have become a major concern worldwide. The prevalence of ESBL-E carriage has increased rapidly, even in countries known for prudent antibiotic use.[1] In addition higher carriage rates of ESBL-E are assumed to lead to higher proportions of ESBL-producing isolates in infections.[2] In the Netherlands several recent studies show carriage rates between 4.5-8.6 % in different populations.[3-5] Over the last years, the percentage of ESBLs in clinical isolates of *Enterobacterales* in the Netherlands was also estimated using the ISIS-AR database. We here 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, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime) based on EUCAST 2018 clinical breakpoints. Although screening isolates were excluded in the analysis (see also chapter 4.1.1 Methods), the prevalence of ESBLs is likely slightly overestimated because screening cultures might not always be labeled correctly.

Results and discussion

In table 4.5.5.1 and 4.5.5.2 the estimated percentages of ESBL carrying *E. coli* and *K. pneumoniae* are shown by site, i.e. general practice (GP), outpatient departments, inpatient departments and intensive care units, in 2018. Trends in ESBL percentages (from left to right 2014 to 2018) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown in figure 4.5.5.1. Overall, the percentages of ESBL have increased over the years (more clearly in *K. pneumoniae*) with ESBL percentages between 3 and 7% for *E. coli* and between 5 and 13% for *K. pneumoniae* depending on type of department in 2018. The data show an increase correlated with the complexity of care with highest ESBL percentages in the intensive care units. Despite this clinically relevant increase in ESBL-E prevalence in the Netherlands, percentages still remain low compared to many other countries in Europe.[1]

Antimicrobial use is one of the most important drivers of antibiotic resistance as its use in humans, animals and agriculture selects for resistant micro-organisms and resistance genes. An important source of ESBL acquisition in humans is international travel. High ESBL acquisition rates, between 37-75%, have been found in Dutch travellers to Asia and Northern Africa. Estimations are that each year 4.6 % (95% CI 3.0-7.1) of the Dutch population acquires an ESBL during travel to destinations outside Europe, Northern America, and Oceania.[6] Indeed, next to antibiotic use, travel to Asia or Africa was identified as one of the major risk factors for ESBL-E carriage in the Dutch community.[4,5] Livestock is hypothesized to be an important source of antibiotic resistance in humans. It has been suggested that successful ESBL-carrying plasmids facilitate transmission between different reservoirs. However, a recent study failed to demonstrate a close link between ESBL-carrying plasmid types from people in the general population and livestock or food-associated reservoirs.[7,8] In addition, a recent study comparing vegetarians with frequent meat consumers showed that vegetarians and pescatarians do not have a lower risk of ESBL-E/K carriage compared to non-vegetarians.[9] Also, the use of prescribed antacids has been identified as a risk factor for ESBL-E carriage in the Dutch population and at hospital admission.[4,10]

Conclusions

- The percentages of ESBL are between 3 and 7% for *E. coli* and between 5 and 13% for *K. pneumoniae* depending on type of department in 2018
- Antimicrobial use, international travel, and use of prescribed antacids have been identified as risk factors for ESBL-E carriage in the Dutch population

Table 4.5.5.1 Extended spectrum beta-lactamase (ESBL) producing *E. coli* in the Netherlands in 2018, based on ISIS-AR data.

Type of department	Tested isolates, N	ESBL, N (%)
GP	91,875	3,035 (3)
Outpatient departments	19,570	993 (5)
Inpatient departments excluding intensive care units	27,552	1,550 (6)
Intensive care units	1,568	107 (7)

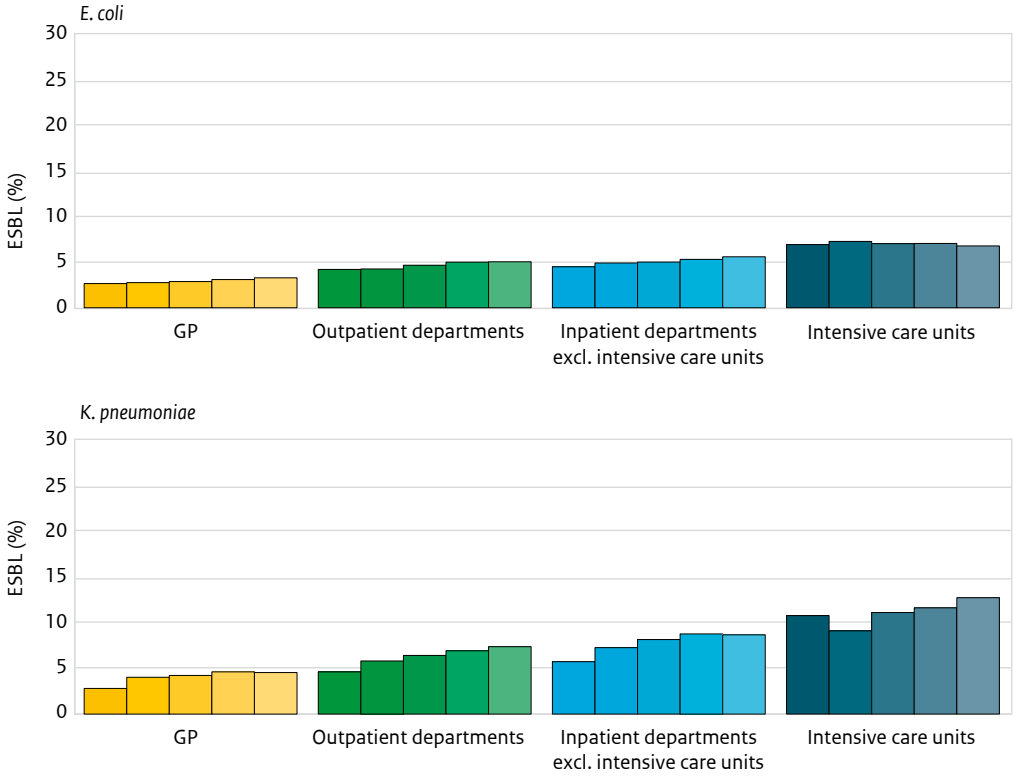
Numbers are based on a selection of 32 laboratories.
The first diagnostic E. coli isolate per patient was selected.
Based on re-interpretation according to EUCAST 2018
The percentage of ESBL producing E. coli was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime).

Table 4.5.5.2 Extended spectrum beta-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2018, based on ISIS-AR data.

Type of department	Tested isolates, N	ESBL, N (%)
GP	11,754	555 (5)
Outpatient departments	4,281	328 (8)
Inpatient departments excluding intensive care units	5,884	530 (9)
Intensive care units	446	59 (13)

Numbers are based on a selection of 32 laboratories.
The first diagnostic K. pneumoniae isolate per patient was selected.
Based on re-interpretation according to EUCAST 2018
The percentage of ESBL producing K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime).

Figure 4.5.5.1 Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* (a) and *K. pneumoniae* (b) in the Netherlands (from left to right 2014 to 2018), based on ISIS-AR data.



Numbers are based on a selection of 32 laboratories.
The first diagnostic isolate per organism per patient per year was selected.
Based on re-interpretation according to EUCAST 2018
The percentage of ESBL producing E. coli and K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime).

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4.5.6 Early warning and response meeting for Hospital-acquired 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 purpose of the SO-ZI/AMR is to mitigate large-scale outbreaks of AMR in hospitals and nursing homes and to prevent spread to other health care facilities through early warning and reporting. The SO-ZI/AMR consists of clinical microbiology, infection prevention, elderly care and public health experts 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 may advise a hospital to request external expertise. Based on this risk assessment (including updates based on follow-up), outbreaks are categorized in one of six phases, with 1 as lowest, 5 as highest risk. Once an outbreak is contained it is classified as phase 0. An outbreak (phase 1) that lasts more than 2 months is automatically categorized as phase 2. If a potential threat to the public health exists, the outbreak will be classified as phase 3; phase 4 and 5 describe potential management issues. An overview of active outbreaks is reported to professionals involved in infection prevention on a monthly basis. Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to participate in SO-ZI/AMR. Since 2015 long-term care facilities (LTCFs) are also invited to report outbreaks of highly-resistant microorganisms (HRMO).

Methods

Health care facilities send outbreak notifications using a standardized form to RIVM/NVMM, where the information is copied into an MS Access database. Monthly updates are provided by institutions until the outbreak is considered ended.

Table 4.5.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2018.

	Hospitals n=34 n (%)	LTCFs n=25 n (%)	Total 2018 n=59 n (%)
Microorganism (resistance mechanism)*			
Enterococcus faecium (VRE)	13 (38)	2 (8)	15 (25)
Staphylococcus aureus (MRSA)	7 (20)	13 (52)	20 (34)
Acinetobacter spp. *(CP)*	3 (9)	0 (0)	3 (5)
Citrobacter freundii (CP)	1 (3)	0 (0)	1 (2)
Escherichia coli (ESBL)	0 (0)	8 (32)	8 (13)
Klebsiella pneumoniae (CP)	3 (9)	0 (0)	3 (5)
Klebsiella pneumoniae (ESBL)	1 (3)	0 (0)	1 (2)
Pseudomonas aeruginosa (CP)	1 (3)	0 (0)	1 (2)
Norovirus	3 (9)	0 (0)	3 (5)
Other	2 (6)	2 (8)	4 (7)
Reason of reporting			
threatening of ward closure	26 (76)	5 (20)	31 (53)
ongoing transmission	2 (6)	2 (8)	4 (7)
combination of both	2 (6)	1 (4)	3 (5)
HRMO outbreak (not in a hospital)	0 (0)	15 (60)	15 (25)
unknown	4 (12)	2 (8)	6 (10)
Highest level phase			
phase 1	30 (88)	23 (92)	53 (89)
phase 2	2 (6)	2 (8)	4 (7)
phase 3	1 (3)	0 (0)	1 (2)
phase 4	1 (3)	0 (0)	1 (2)
phase 5	0 (0)	0 (0)	0 (0)
Median number of patients: (range)	12 (2-55)	3 (1-4)	6 (1-55)
Median duration outbreak in days from reporting date until end of the outbreak: (range)	48 (13-113)	69 (0-153)	49 (0-153)
Request for help	0 (0)	1 (4)	1 (2)

**MRSA=methicillin-resistant Staphylococcus aureus; VRE=vancomycin-resistant Enterococcus faecium; ESBL=extended-spectrum beta-lactamase; CP=carbapenemase-producing*

Results

Table 4.5.6.1 provides an overview of the fifty-nine outbreaks reported in 2018. These were reported by 52 healthcare institutions. These included 30 hospitals, 21 LTCFs and one ambulatory care organization. Most outbreaks (n=45) ended in 2018. As reported in the table, most frequent reasons for notification of an outbreak were the imminent closure of wards; a few were notified because transmission of outbreak strains was ongoing despite infection control measures. The median number of patients involved in outbreaks in LTCFs was lower compared to hospitals.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) were most often reported, comparable to the years before. A new finding in 2018, compared to 2017, was that eight outbreaks with ESBL-producing *E. coli* were reported. Five of these were detected in the national Point Prevalence Survey in LTCF conducted in 2018. Eight outbreaks were caused by carbapenemase-producing strains.

Six outbreaks included more than 10 patients. The outbreaks classified as phase 2 comprised one MRSA outbreak, one VRE outbreak, and one outbreak with ESBL-producing *E. coli*. One outbreak with VRE was evaluated as presenting a possible threat to public health (phase 3), due to the long duration of the outbreak. One outbreak with carbapenemase-producing *Citrobacter freundii* was classified as phase 4, which indicates a (potential) insufficient effect of outbreak management response and/or a request for help. Of the data available, the majority of the outbreaks appear to have been reported within a month after detection.

Discussion

In 2018, we noticed an increase in outbreaks due to multidrug-resistant *Enterobacterales*, and for the first time, an outbreak (*Citrobacter freundii*) was classified as phase 4. This outbreak was carefully monitored and support was offered to the relevant institution. The problems that led to this extensive outbreak (with 24 cases of carriage and/or infections with an NDM-producing strain), have been assessed and a plan of approach was set up.

Conclusions

- On average five outbreaks a month were reported to the SO-ZI/AMR.
- Most outbreaks were classified as phase 1 or phase 2, one as phase 3 and one as phase 4.
- Eight outbreaks by carbapenemase-producing strains were reported, caused by different organisms, all were hospital outbreaks.
- The majority of the outbreaks were reported to SO-ZI/AMR within a month after detection.
- Most outbreaks were due to MRSA and VRE.
- Most outbreaks were controlled quickly (within 2 months).
- The median number of patients involved in an outbreak was 6.

4.6 Resistance in specific pathogens

4.6.1 *Neisseria meningitidis*

Introduction

Neisseria meningitidis isolates cultured from CSF and/or blood in microbiological laboratories in the Netherlands are submitted to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) at the Amsterdam UMC, Location AMC, Amsterdam. In *N. meningitidis*, the interpretation of the phenotypic susceptibility testing might not be fully reliable, because the susceptible/moderately susceptible breakpoint is exactly at the peak of the wild-type susceptibility distribution (0.06 mg/L). Since any MIC assay is not 100% reproducible, this likely results in a considerable number of minor and major interpretation errors. Therefore, the *penA* gene of all isolates was sequenced.

Methods

From 2009- 2018, a total of 415 strains from cerebrospinal fluid (CSF) or CSF and blood and 795 strains from blood were included in the surveillance project of the NRLBM. The MIC for penicillin was determined by Etest using MHF plates, incubation 18-24 h at 37°C under 5% Co2.EUCAST criteria for resistance were applied (susceptible: MIC ≤0.06 mg/L; resistant: MIC >0.25 mg/L). In addition, the nucleotide sequence of *penA* coding for penicillin binding protein 2 was sequenced.1 In case of moderate susceptibility or resistance to penicillin, susceptibility to ceftriaxone was also assessed by E-test using MHF plates, incubation 18-24 h at 37°C under 5% Co_2

Results

In 2018 three isolates were resistant to penicillin (one non-groupable from CSF and two serogroup Y from blood), whereas 22% (31/139) of the isolates from blood and 28% (13/46) of the isolates from CSF were moderately susceptible to penicillin (MIC 0.06-0.25 mg/L) (tables 4.6.1.1 and 4.6.1.2). Of those 44 moderately susceptible isolates from blood and/or CSF, 17 belonged to serogroup B, two to serogroup C, 18 to serogroup W and 7 to serogroup Y. Resistance to ceftriaxone or rifampicin was absent in 2018.

Alterations in the *penA* gene, associated with non- susceptibility to penicillin[1], were detected in 9 (5%) of the 185 isolates. Of these isolates, one was phenotypically susceptible and 5 were moderately susceptible by Etest (table 4.6.1.3). Three isolates with alterations in PenA were also phenotypically resistant. *PenA* genotyping yields more isolates (4.9%) resistant to penicillin as compared to phenotypic testing withE-test using EUCAST criteria (1.6%). Furthermore, both methods do not agree completely.

Discussion

Alterations in *penA* associated with non-susceptibility to penicillin are present in 5% of all isolates compared to 2% with Etest and both methods do not agree completely. One or more of the following reasons may be involved: 1) other factors than *penA* gene alterations also confer non-susceptibility to penicillin; 2) a considerable number of minor interpretation errors occurs because the susceptible/ moderately susceptible breakpoint lies at the peak of the wild-type susceptibility distribution; 3) this EUCAST breakpoint is too low and should be repositioned at 0.25 mg/L.

Conclusions

- Penicillin resistance is sporadic (two strains in 2013, one strain in 2017 and three in 2018).
- In 2018 the proportion of moderately susceptible strains increased (from around 19% in 2017 to 24% in 2018).
- Alterations in *penA* associated with non-susceptibility to penicillin are present in 5% of all isolates.
- Resistance to rifampicin and ceftriaxone was not found in 2018.

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Table 4.6.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2009-2018.

	Penicillin[a]								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2009	51	**98.1**	1	**1.9**	0	**0**	0	**0**	**52**
2010	43	**81.1**	10	**18.9**	0	**0**	0	**0**	**53**
2011	29	**78.4**	8	**21.6**	0	**0**	0	**0**	**37**
2012	24	**58.5**	16	**39.0**	1	**2.4**	0	**0**	**41**
2013	35	**89.7**	3	**7.7**	1	**2.6**	0	**0**	**39**
2014	26	**83.9**	5	**16.1**	0	**0**	0	**0**	**31**
2015	31	**96.9**	1	**3.1**	0	**0**	0	**0**	**32**
2016	34	**89.5**	4	**10.5**	0	**0**	0	**0**	**38**
2017	37	**80.4**	9	**19.6**	0	**0**	0	**0**	**46**
2018	32	**69.6**	13	**28.3**	1	**2.2**	0	**0**	**46**

* *MIC values in mg/L*

Table 4.6.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2018.

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2009	77	**88.5**	10	**11.5**	0	**0**	0	**0**	**87**
2010	67	**84.8**	12	**15.2**	0	**0**	0	**0**	**79**
2011	34	**64.2**	19	**35.9**	0	**0**	0	**0**	**53**
2012	27	**67.5**	13	**32.5**	0	**0**	0	**0**	**40**
2013	53	**73.6**	18	**25.0**	1	**1.4**	0	**0**	**72**
2014	37	**88.1**	5	**11.9**	0	**0**	0	**0**	**42**
2015	46	**88.5**	6	**11.5**	0	**0**	0	**0**	**52**
2016	89	**87.3**	13	**12.7**	0	**0**	0	**0**	**102**
2017	104	**80.6**	24	**18.6**	1	**0.8**	0	**0**	**129**
2018	106	**76.3**	31	**22.3**	2	**1.4**	0	**0**	**139**

** MIC values in mg/L*

Table 4.6.1.3 Alterations in the penA gene and penicillin susceptibility in Neisseria meningitidis, 2018.

	Number of strains with penicillin MIC*:			
Alterations *penA* gene**	**MIC ≤ 0.06 sensitive**	**0.064 < MIC ≤ 0.25**	**0.25 < MIC ≤1.0**	**MIC >1.0**
Yes	1	5	3	0
No	137	39	0	0
Total	**138**	**44**	**3**	**0**

** MIC values in mg/L*

***Resulting in five amino acids substitutions in PenA associated with non-susceptibility to penicillin*[1]

4.6.2 *Neisseria gonorrhoeae*

Introduction

Neisseria gonorrhoeae is a species of Gram-negative bacteria responsible for the sexually transmitted infection (STI) gonorrhoea. Gonorrhoea is the second most common bacterial STI in the Netherlands. It can result in severe reproductive complications and can increase the transmission of HIV. Third generation cephalosporins, such as ceftriaxone and cefixime, are the current first-line treatment for gonorrhoea in most countries. In the Netherlands, cefotaxime became the first-line therapy for gonorrhoea in 2003, and ceftriaxone in 2006. However, the susceptibility of gonococci to these cephalosporins has been decreasing and *Neisseria gonorrhoeae* has developed antimicrobial resistance to most drugs used for treatment, including azithromycin, which is used as an alternative treatment in patients allergic for ceftriaxone.

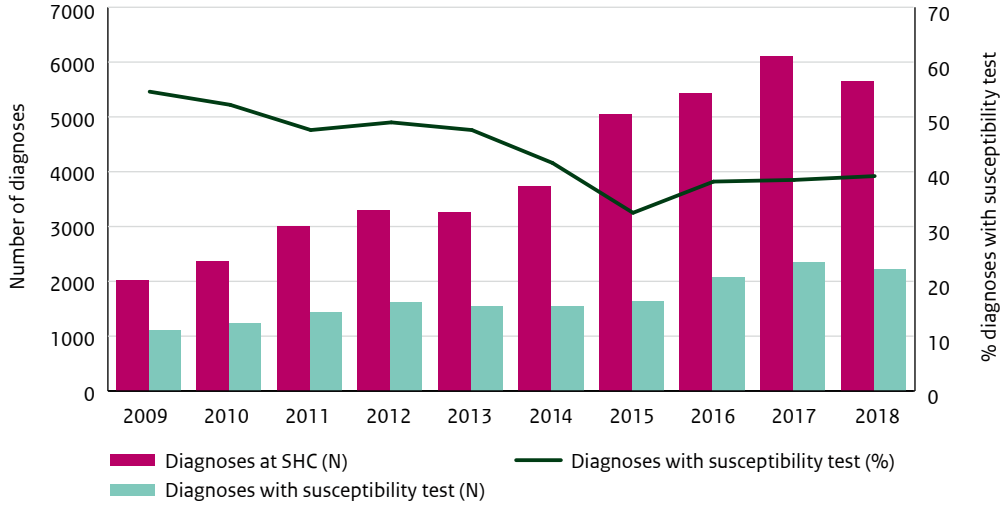
Methods

The national Gonococcal Resistance to Antimicrobials Surveillance (GRAS) started in 2006, collecting epidemiological data on gonorrhoea and resistance patterns of isolated strains from Sexual Health Centres (SHC) across the Netherlands. Eighteen out of 24 SHC participated in GRAS in 2018 and they performed 88% of gonorrhoea diagnoses among SHC attendees. Diagnosis of gonorrhoea is made by culture and/or PCR on patients' materials, and additional susceptibility testing is performed using Etest. From 2006, isolates were tested for penicillin, tetracycline, ciprofloxacin, and cefotaxime. In 2011, ceftriaxone, azithromycin and spectinomycin were added to the panel and testing for penicillin and tetracycline became optional. In 2014, testing for spectinomycin was also made optional. In 2015, penicillin and tetracycline were removed from the panel. Resistance levels were calculated using the EUCAST breakpoints for resistance[1]. In 2019, EUCAST altered the breakpoint for azithromycin resistance. The clinical breakpoint of MIC >0.5 mg/L was changed to an epidemiological cut-off value (ECOFF) of MIC >1.0 mg/L. Trends for azithromycin resistance have been altered retrospectively using the new ECOFF.

Results

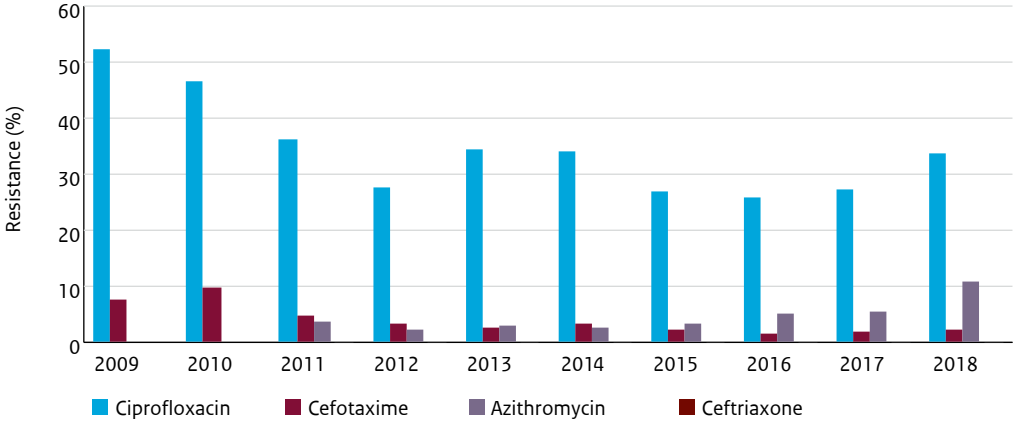
Since 2008, the number of gonorrhoea diagnoses at SHC participating in GRAS has increased until 2017 and slightly decreased in 2018 to 5,648 diagnoses. The percentage of diagnoses including a susceptibility test has been stable around 38% for the past years (39.2% in 2018) (Figure 4.6.2.1).

Figure 4.6.2.1 Number of gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test at Sexual Health Centres participating in GRAS, 2009-2018.



Gonococcal resistance for ciprofloxacin has decreased from 52.4% in 2009 to 25.6% in 2016, but increased again after 2016 to 33.7% in 2018. Resistance levels for cefotaxime have also decreased, and were stably around 1.5% in the last four years (1.9% in 2018). For azithromycin, resistance has steadily increased since 2012; from 2.1% to 10.8% in 2018. No resistance for ceftriaxone has been reported yet (Figure 4.6.2.2).

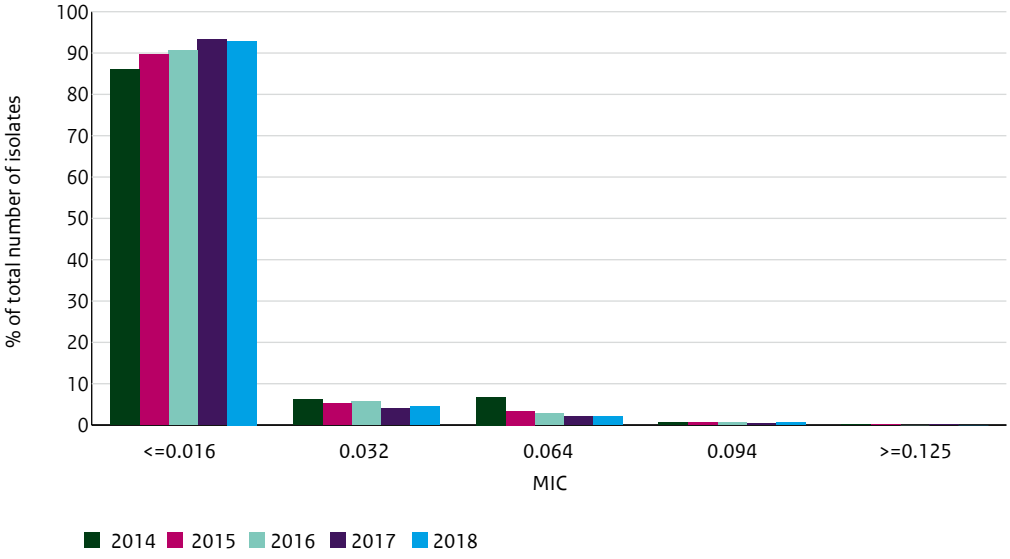
Figure 4.6.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2009-2018.



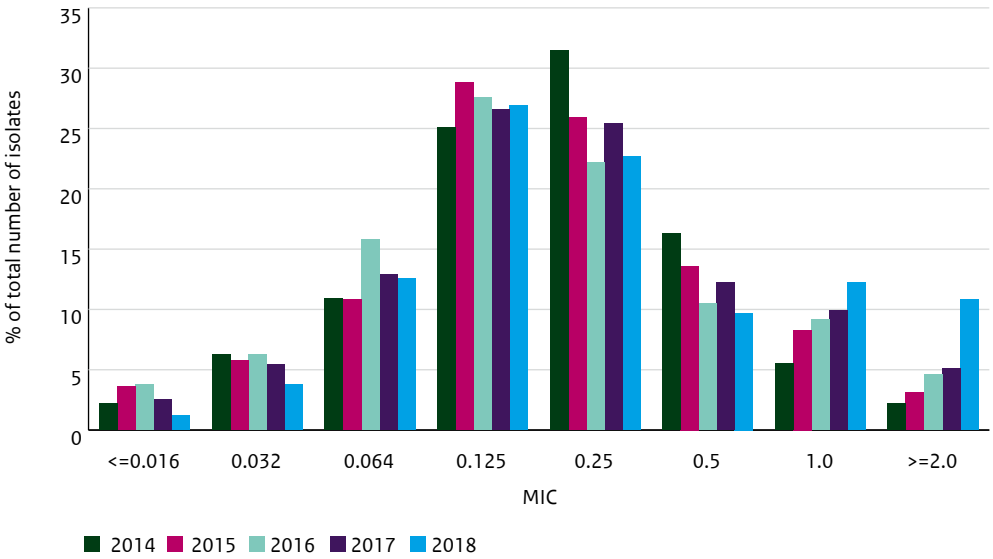
Ceftriaxone and azithromycin were added to the panel in 2011. No resistance for ceftriaxone has been reported.

Figure 4.6.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2014-2018.

a. MIC distribution for ceftriaxone



b. MIC distribution for azithromycin



The MIC distribution of ceftriaxone is highly skewed to the right, and shows a unimodal shape. In recent years, isolates seem to have become more susceptible for ceftriaxone, as the proportion of isolates with an MIC below or equal to 0.016 mg/L increased since 2013 (Figure 4.6.2.3a). In 2018 however, the proportion of highly susceptible isolates slightly decreased. The MIC distribution of azithromycin shows a more normal distribution, with the largest proportion of isolates having an MIC of 0.125 or 0.250 mg/L. Since 2013, the number of isolates with an MIC of 1 mg/L and the number of resistant isolates with an MIC of 2 mg/L and higher has been increasing (Figure 4.6.2.3b), especially in 2018.

Discussion

In 2018 in less than half (39.2%) of all gonorrhoea diagnoses at SHC participating in GRAS resistance levels were measured by additional susceptibility testing. This low number can partially be explained by a large proportion of cultures being negative, making susceptibility testing impossible. In addition, the STI surveillance data show that gonorrhoea diagnoses are sometimes only confirmed by PCR, not by culture.

In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500 mg). Thus far, no ceftriaxone resistance or clinical failure has been reported. Yet, a few isolates have reached the borderline MIC value of 0.125 mg/L in the last years (0 isolates in 2018). Many other countries have been recommending treatment of gonorrhoea with combination therapy of ceftriaxone and azithromycin. Since 2012, the percentage of isolates resistant to azithromycin has been steadily increasing. Therefore, the use of combination therapy in the Netherlands is not preferred. Also in the United Kingdom treatment guidelines were recently altered to recommend ceftriaxone monotherapy (1g) instead of combination therapy, due to the increasing levels of azithromycin resistance.

Conclusions

- The number of gonorrhoea diagnoses which include susceptibility testing at the SHC remains relatively low (39.2% in 2018).
- No resistance to ceftriaxone, the current first-line treatment, has been reported.
- Azithromycin resistance levels continue to increase; from 2.1% in 2012 to 10.8% in 2018.

References

[1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, 2019. Available from http://www.eucast.org/clinical_breakpoints/

4.6.3 *Mycobacterium tuberculosis*

Introduction

Of all infectious diseases, tuberculosis (TB) has the highest mortality worldwide. Although the incidence is slowly declining, it has been estimated that about one third of the global population is latently infected by its main causative agent; *Mycobacterium tuberculosis*. In the Netherlands we have reached the elimination phase in natives. Not less than 75% of the TB cases is currently diagnosed in foreign-born persons. Because of the increased influx of asylum seekers and immigrants, in 2016 there was an increase of about 3% in the notification of TB (889 cases). In 2017, the number of TB cases declined to 794 cases. Results reveal a slight increase to 806 cases in 2018.

Worldwide, there is a concern on the development of resistance, which hampers adequate treatment of tuberculosis. The majority of resistance testing of *M. tuberculosis* isolates in the Netherlands is performed at the RIVM and the results are used both for direct therapy guidance and surveillance. The RIVM participates in the resistance proficiency study of the WHO for WHO supra-national laboratories to monitor the quality of the resistance testing.

Around 30 laboratories in the Netherlands involved in the diagnosis of TB send all *M. tuberculosis* isolates to the RIVM for epidemiological typing to support the investigations on TB transmission by Municipal Health Services.

Methods

The current drug susceptibility testing (DST) most often used is the WHO recommended mycobacteria growth indicator tube (MGIT) system. In this approach bacteria are incubated in the presence of critical concentrations of drugs. The MGIT incubator automatically monitors the growth of the bacteria.

Since 2011, not all drug susceptibility testing for first line drugs is performed at the RIVM; a part (36%) of these tests is performed at regional- or peripheral laboratories. When resistance is observed however, this is reported to the national reference laboratory at the RIVM for verification and/or additional resistance testing.

Results

In the year 2018, 553 *M. tuberculosis* complex isolates were received at the RIVM for epidemiological typing, of which 356 (64%) were subjected to DST for first line drugs at the RIVM.

Figure 4.6.3.1 Trends in antibiotic resistance for *M. tuberculosis* 2003-2018.

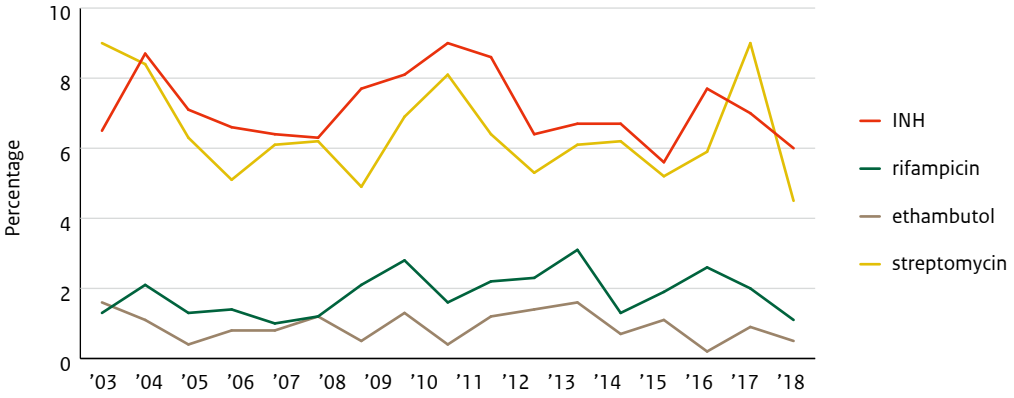
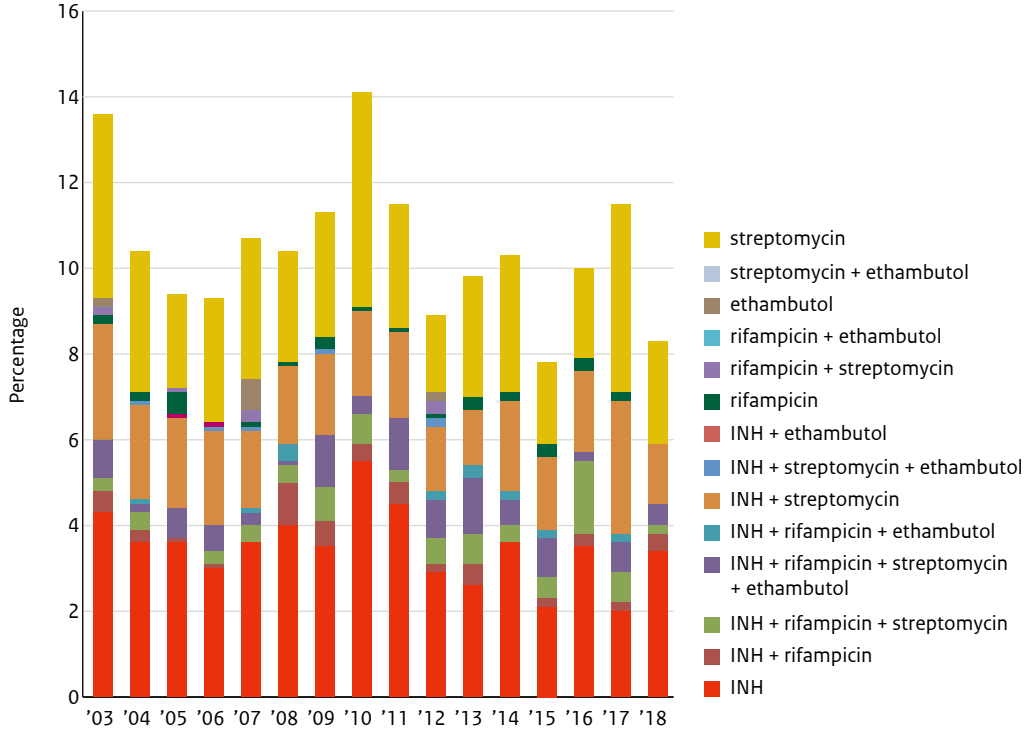


Figure 4.6.3.2 Trends in combined antibiotic resistance for *M. tuberculosis* 2003-2018.



In 2018, the number of TB notification cases was 806, of which 553 were confirmed by *M. tuberculosis* complex cultures that were received at the RIVM for epidemiological typing and in the majority of cases resistance testing.

In 2016 there was a clear increase in INH resistance to 7.7% (figure 4.6.3.1), but this decreased to 7.0% in 2017 and to 6.0% in 2018. In 2015 and 2016 the rifampicin resistance increased marginally from to 1.9% to 2.6%. In 2017 and 2018 rifampicin resistance again decreased from 2.0% to 1.1% of the cases. In 2018, in 0.5% of the cases ethambutol resistance was detected, which is a slight decrease.

In 2017, 10 (1.8%) isolates were reported as multidrug resistant tuberculosis (MDR-TB), defined as resistance to at least INH and rifampicin. In 2018, 6 MDR-TB cases were diagnosed (1.1%), while XDR-TB, defined as resistance to INH, rifampicine, an injectable and a fluoroquinolone was not diagnosed in the last year (figure 4.6.3.2).

In recent years mono-resistance to rifampicin was incidentally found; in 2017 in one case and in 2018 no mono-resistance to rifampicin was found.

Discussion

Worldwide, resistance is an important aspect of TB control. Because there was a slight increase in the notification of TB in the Netherlands in the period 2015-2016, due to a higher influx of asylum seekers and immigrants from high prevalence areas, it remains important to continue the surveillance on resistance. In 2017 the notification of TB declined with 11% mainly due to a reduced number of newly arrived residents. In 2018, presumably due to variation in the composition of the group of asylum seekers there was a slight increase in the notification of TB.

In 2018, 8.3% percent of the 553 isolates tested in the Netherlands revealed some form of resistance. This seems a bit lower than the percentage observed in previous years. Although the number of multidrug resistant isolates remained low and amounted to 6 cases, due to the extended hospitalization of patients and the cumbersome treatment this problem deserves special attention.

In 2016, a new project was initiated at the RIVM on structural Whole Genome Sequencing (WGS) of *M. tuberculosis* isolates. The detection of mutations in the 9 major resistance genes appears a reliable predictor of resistance to first line drugs. WGS will therefore be introduced to screen for resistance in *M. tuberculosis* isolates.

Conclusions

- Resistance to the antibiotics to treat tuberculosis remained almost stable over the last 5 years, and showed a slight decrease in 2018.
- MDR-TB remained stable in the recent years, (average 10 each year) and decreased to 1.1% in 2018.
- Tuberculosis notification increased with 6% in 2015 and 3% in 2016, but decreased with 11% in 2017 and again increased by 2% in 2018.

References

Evaluation of Mycobacteria Growth Indicator Tube (MGIT) for drug susceptibility testing for *Mycobacterium tuberculosis*. J.C. Palomino, H. Traore, K. Fissette, F. Portaels; Int J. Tuberc Lung Dis 1999 3(4) ;344-348.

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study. Walker[1], Kohl TA[2], Omar SV[3], Hedge J[4], Del Ojo Elias C[4], Bradley P[5], Iqbal Z[5], Feuerriegel S[6], Niehaus KE[7], Wilson DJ[4], Clifton DA[7], Kapatai G[8], Ip CL[5], Bowden R[5], Drobniewski FA[9], Allix-Béguec C[10], Gaudin C[10], Parkhill J[11], Diel R[12], Supply P[13], Crook DW[14], Smith EG[15], Walker AS[14], Ismail N[16], Niemann S[6], Peto TE[14]; Lancet Infect Dis. 2015 Oct;15(10):1193-202.

4.6.4 Influenza antiviral drugs

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 for treatment of influenza cases with severe course of disease. In the Netherlands the M2 ion channel blockers (M2B) amantadine and rimantadine acting against type A viruses only, and the neuraminidase enzyme inhibitors (NAI) oseltamivir and zanamivir acting against both type A and B viruses, are registered. The M2B prevent uncoating of the virus in the cell and thereby virus replication whereas the NAI prevent release of progeny virus from the cell limiting spread to and infection of other cells. To be able to decide which antivirals can be used and for early warning when antiviral resistant viruses emerge, monitoring of M2B and NAI susceptibility of seasonal human influenza viruses is performed since the 2005/2006 winter season.[1]

Methods

Monitoring of influenza antiviral susceptibility is embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinels, that 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). Since the 2009 A(H1N1)pdm09 pandemic, this system is extended to include viruses detected in hospital and peripheral laboratories with special attention for viruses detected in patients treated with antivirals who show prolonged shedding of influenza virus. These viruses are submitted to, and analysed at, the Erasmus Medical Centre location of the NIC. From the 2009/2010 season onwards, hospital laboratories voluntarily report antiviral resistant cases to the RIVM. Techniques used in the Netherlands to monitor antiviral resistance in influenza viruses include Sanger sequencing, whole genome Next Generation Sequencing, pyrosequencing or site-specific polymerase chain reaction (PCR) assays for known resistance markers for both the M2Bs and NAIs. 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 (IC50).

Results

Findings for the influenza seasons 2005/2006 through 2009/2010 are presented in NethMap 2016.[1] Table 4.6.4.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season. Figure 4.6.4.1 shows the prescriptions for oseltamivir, zanamivir and amantadine since 2010.In the 2018/2019 season, for results obtained so far, three patients harbouring viruses with the A(H1N1)pdm09 NA-H275Y amino acid substitution, either pure (n=1) or as a mixture with wildtype virus (n=2), indicative of highly reduced inhibition by oseltamivir were found. Two of them were admitted to an intensive care unit, of whom one was known to have been treated with oseltamivir. The third patient presented at the general practitioner and was not treated with antiviral medication suggesting spontaneous in-patient natural emergence of the H275Y variant.

Oseltamivir prescriptions increased slightly at the beginning of the 2018/2019 influenza epidemic in late December 2018. An increase in prescriptions is anticipated for the early months of 2019. Amantadine prescriptions stabilised during 2018 compared to previous years, but the vast majority of these prescriptions are for treatment of Parkinson's disease.

Discussion

As in the Netherlands, and globally, virtually all influenza type A viruses carry amino acid substitution M2-S31N causing resistance against M2B, the M2B are useless for influenza antiviral therapy and prophylaxis. In the Netherlands, and globally, the proportion of NAI reduced susceptible influenza viruses is very low.[2] Most of the NAI reduced susceptible viruses come from antiviral treated patients and do not spread. However, occasionally clusters of NAI reduced susceptible viruses are detected suggesting spread. Except for the emergence and sustained worldwide circulation of oseltamivir reduced susceptible former seasonal A(H1N1) in 2007/2008, these clusters did not result in sustained transmission of reduced susceptible virus. Nevertheless, these findings show that NAIs are still appropriate for prophylaxis and treatment and that it is important to monitor susceptibility of influenza viruses for the antivirals being used. A new single dose RNA polymerase inhibitor (Baloxavir marboxil; Xofluza®) has been approved in 2018 for use to treat uncomplicated influenza in Japan and the USA.[3] Approval by EMA for use in the Netherlands is anticipated.

Conclusions

- Over the last 9 seasons type A and type B influenza viruses remained susceptible to the neuraminidase inhibitors oseltamivir and zanamivir; whilst type A influenza viruses remained highly reduced susceptible for the M2 ion channel blockers.
- Sporadically, a neuraminidase inhibitor reduced susceptible virus has been detected, mostly associated with the use of antivirals prior to specimen collection or an amino acid substitution induced by virus isolation in cell culture.
- The prescriptions of oseltamivir and zanamivir remained low, with only slight increases during the influenza seasons. Prescriptions of amantadine showed a slightly decreasing trend over the past 8 years but seem to stabilize in 2018. However, due to a natural mutation that exists in virtually all influenza type A viruses amantadine cannot be used anymore to treat influenza.

Table 4.6.4.1 (Higly) reduced inhibition of influenza viruses by NAIs and M2Bs in the Netherlands, 2010/2011 - 2018/2019[1].

Season	A(H3N2)		A(H1N1)pdm09		B
	NAI	M2B	NAI	M2B	NAI
2010/2011	0/2	2/2 (100%)	0/58	40/40 (100%)	0/64
2011/2012	0/257	34/34 (100%)	2/7 (29%)[2]	7/7 (100%)	0/10
2012/2013	0/156	15/15 (100%)	3/125 (2.4%)[3]	10/10 (100%)	0/8
2013/2014	2/220 (<1%)[4]	31/31 (100%)	1/150 (<1%)[5]	20/20 (100%)	0/4
2014/2015	0/727	50/50 (100%)	1/130 (<1%)[6]	9/9 (100%)	0/42
2015/2016	0/44	4/4 (100%)	1/1191(<1%)[7]	73/73 (100%)	1/69 (1%)[8]
2016/2017	0/911	56/56 (100%)	2/11 (18%)[9]	2/2(100%)	0/14
2017/2018	0/355	13/13 (100%)	1/233(<1%)[10]	12/12 (100%)	0/156
2018/2019[11]	0/421	3/3 (100%)	3/331(<1%)[12]	ND	0/4

1 *Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; M2B = M2 ion channel blocker; ND = viruses available, but analysis was not done.*

2 *Two viruses with highly reduced inhibition by oseltamivir due to the H25Y amino acid substitution, isolated from two epidemiological unlinked not treated patients returning from holiday at the Spanish coast.*

3 *Three viruses with highly reduced inhibition by oseltamivir due to the H25Y amino acid substitution. Two isolated from epidemiological unlinked immunocompromised hospitalised patients treated with oseltamivir. No details available for the third patient.*

4 *Two clinical specimens from two patients with mixture of 292R and 292K amino acid composition; R292K is associated with highly reduced inhibition for oseltamivir and zanamivir. No patient characteristics or viral exposure data available.*

5 *One virus with highly reduced inhibition by oseltamivir due to the H275Y amino acid substitution. No patient characteristics or viral exposure data available.*

6 *One virus with highly reduced inhibition by oseltamivir due to mixture 275H/Y amino acid substitution. The patient was treated with oseltamivir prior to specimen collection.*

7 *One virus with highly reduced inhibition by oseltamivir due to mixture 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

8 *One virus with highly reduced inhibition by zanamivir and reduced inhibition by oseltamivir due to an E105K amino acid substitution. However, highly likely induced by virus isolation as in the clinical specimen this amino acid substitution was not detectable. The patient was not treated with antivirals prior to specimen collection.*

9 *Two viruses from one patient taken 10 days apart with both highly reduced inhibition by oseltamivir due to a H275Y amino acid substitution. The patient was treated with oseltamivir prior to specimen collection.*

10 *One virus with highly reduced inhibition by oseltamivir due to mixture 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

11 *Preliminary data, status by 22 May 2019.*

12 *Three viruses with highly reduced inhibition by oseltamivir due to H275Y (n=1) or mixture 275H/Y (n=2) amino acid substitution. Two patients were admitted to ICU of which one was treated with oseltamivir prior to specimen collection and the other had an unknown treatment status. One community patient had no prior treatment with oseltamivir.*

Figure 4.6.4.1 Prescriptions of amantadine and oseltamivir (A) and zanamivir (B). Shown are the Defined Daily Doses (ddd) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands.

References

[1] NethMap 2016. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2015. National Institute for Public Health and the Environment, June 2016.

[2] Lackenby A, Besselaar TG, Daniels RS, Fry A, Gregory V, Gubareva LV, Huang W, Hurt AC, Leang SK, Lee RTC, Lo J, Lollis L, Maurer-Stroh S, Odagiri T, Pereyaslov D, Takashita E, Wang D, Zhang W, Meijer A. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors and status of novel antivirals, 2016-2017. Antiviral Res. 2018 Sep;157:38-46.

[3] Hayden FG, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, Ishida T, Sekino H, Yamada K, Portsmouth S, Kawaguchi K, Shishido T, Arai M, Tsuchiya K, Uehara T, Watanabe A; Baloxavir Marboxil Investigators Group. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. N Engl J Med. 2018 Sep 6;379(10):913-923.

4.6.5 The antibiotic susceptibility profile of anaerobic bacteria

Introduction

Following the reports in earlier years, we report the profile of the anaerobic bacteria isolated from human clinical specimens in 2018, at the University Medical Center Groningen (UMCG).

Methods

All infection related anaerobic isolates, isolated from clinical specimens in the UMCG were identified using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The MIC for amoxicillin, amoxicillin-clavulanic acid (only gram-negative anaerobic bacteria), clindamycin, metronidazole (except for *Actinomyces*, *Bifidobacterium* and *Propionibacterium* isolates) and meropenem (only for *Bacteroides* and *Prevotella* isolates) was determined using Etest (bioMerieux, France). Isolates were inoculated on prereduced Brucella Blood Agar (Mediaproducts, the Netherlands) and incubated for 48 hours at 35˚ in an anaerobic cabinet (Don Whitley, UK) having an anaerobic atmosphere (80% N2, 10% CO2, 10 H2). After determination of the MIC, resistance was assessed using the breakpoints described in the EUCAST guidelines.

Results

The antibiotic susceptibility profiles of the different anaerobic genera for the tested antibiotics is summarized in Table 4.6.5.1.

Gram-negative anaerobic bacteria

In gram-negative anaerobic bacteria amoxicillin resistance was observed among all tested genera, except within the genus *Porphyromonas*. The resistance rates were similar to the detected rates in the previous years, table 4.6.5.2. Resistance to amoxicillin-clavulanic acid was only observed among isolates belonging to the genera *Bacteroides* and *Parabacteroides*.

In most gram-negative anaerobic genera resistance to clindamycin remained stable throughout the years. This year however clindamycin resistance among isolates belonging to the genus *Porphyromonas* was low compared to previous years, and we noticed for the first time clindamycin resistance in *Veillonella* isolates, table 4.6.5.2. Metronidazole resistance was observed among isolates of *Prevotella* and *Veillonella*. Meropenem resistance was observed in 2.7% of the tested *Bacteroides* strains, which is higher than in 2017 and 2016 (0% and 1.4%,respectively), table 4.6.5.2.

Gram-positive anaerobic bacteria

None of the tested gram-positive anaerobic isolates showed resistance to amoxicillin, while in previous years resistance was only encountered among *Clostridium* isolates, Table 4.6.5.3. Clindamycin resistance remained stable throughout the years. Metronidazole resistance was observed in just one *Clostridium* strain, but in none of the other tested strains. Among different genera of gram-positive anaerobic cocci (GPAC) only resistance to clindamycin was observed. Strains belonging to the genus *Peptostreptococcus* were sensitive to all tested antibiotics, in contrast to the other tested genera.

Table 4.6.5.1 The MIC50, MIC90 and percentage resistance of different anaerobic genera, isolated from human clinical specimens in 2018, for different kind of antibiotics.

	amoxicillin			amoxicillin-clavulanic acid			clindamycin			metronidazole			meropenem		
	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. (148-150)[a]	48	>256	94	0.5	3	1.3	2	>256	31.3	0.25	0.5	0	0.125	0.5	2.7
Bilophila spp. (11)	64	>256	100	1	2	0	0.5	0.75	0	0.023	0.19	0	n.a.[b]	n.a.	n.a.
Fusobacterium spp. (31)	0.047	6	16.1	0.047	0.19	0	0.064	0.19	0	0.016	0.064	0	n.a.	n.a.	n.a.
Parabacteroides spp. (18)	>256	>256	61.1	3	12	22.2	4	>256	50	0.25	0.75	0	n.a.	n.a.	n.a.
Porphyromonas spp. (14-16)[a]	<0.016	0.023	0	<0.016	0.016	0	<0.016	0.016	6.3	0.023	0.19	0	n.a.	n.a.	n.a.
Prevotella spp. (122-123)[a]	2	256	49.2	0.094	1	0	0.016	>256	13.4	0.19	0.75	0.8	0.032	0.094	0
Veillonella spp. (20)	0.75	2	5	0.75	2	0	0.125	0.25	10	1	4	5	n.a.	n.a.	n.a.
Gram-positive anaerobes															
Actinomyces spp. (171)	0.125	0.38	0	n.a.	n.a.	n.a.	0.125	24	11.7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bifidobacterium spp. (9)	0.094	0.75	0	n.a.	n.a.	n.a.	0.032	0.19	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Clostridium spp. (46)	0.094	1	0	n.a.	n.a.	n.a.	1.5	32	26.1	0.38	2	2.2	n.a.	n.a.	n.a.
Cutibacterium spp. (165-167)[a]	0.047	0.19	0	n.a.	n.a.	n.a.	0.047	0.125	6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
GPAC (197-200)[a]	0.064	0.25	0	n.a.	n.a.	n.a.	0.25	>256	14	0.19	0.5	0	n.a.	n.a.	n.a.
GPAC															
Anaerococcus spp. (36)	0.023	0.064	0	n.a.	n.a.	n.a.	0.047	2	11.1	0.19	0.5	0	n.a.	n.a.	n.a.
Finegoldia magna (65)	0.19	0.38	0	n.a.	n.a.	n.a.	0.75	>256	20	0.19	0.38	0	n.a.	n.a.	n.a.
Parvimonas micra (34-36)[a]	0.023	0.064	0	n.a.	n.a.	n.a.	0.25	2	8.3	0.064	0.25	0	n.a.	n.a.	n.a.
Peptoniphilus spp. (49)	0.016	0.094	0	n.a.	n.a.	n.a.	0.38	16	16.3	0.25	0.75	0	n.a.	n.a.	n.a.
Peptostreptococcus spp. (13-14)[a]	0.25	0.38	0	n.a.	n.a.	n.a.	0.064	0.38	0	0.064	0.19	0	n.a.	n.a.	n.a.

[a] *Not all strains were tested for all antibiotics.*
[b] *Not available.*

Table 4.6.5.2 An overview of the percentage resistance for different antibiotics within gram-negative anaerobic genera, per year.

		% resistance							
	Antibiotic	2018	2017	2016	2015	2014	2013	2012	2011
Bacteroides spp.	amoxicillin	94	97	94	92	93	91	98	98
	amoxi-clav	1	1	0	1	2	0	0	1
	clindamycin	31	24	18	21	20	20	27	27
	metronidazole	0	0,6	0,7	0	2	0	0	0
	meropenem	3	0	1	n.a.[b]	n.a.	n.a.	n.a.	n.a.
Parabacteroides spp.	amoxicillin	61	67	82	55	55	60	n.a.	n.a.
	amoxi-clav	22	0	6	17	9	0	n.a.	n.a.
	clindamycin	50	28	59	0	27	60	n.a.	n.a.
	metronidazole	0	0	0	0	0	0	n.a.	n.a.
Prevotella spp.	amoxicillin	49	41	52	41	51	60	33	42
	amoxi-clav	0	0	0	0	0	0	0	0
	clindamycin	6	9	13	17	11	4	10	8
	metronidazole	1	1	1	2	0	4	0	0
	meropenem	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
Fusobacterium spp.	amoxicillin	16	24	3	6	0	16	9	22
	amoxi-clav	0	8	3	6	0	5	0	0
	clindamycin	0	0	0	0	0	0	0	0
	metronidazole	0	0	0	0	0	0	0	0
Porphyromonas spp.	amoxicillin	0	15	6	22	n.a.	n.a.	n.a.	n.a.
	amoxi-clav	0	0	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	6	38	17	11	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	0	n.a.	n.a.	n.a.	n.a.
Bilophila spp.	amoxicillin	100	86	100	78	n.a.	n.a.	n.a.	n.a.
	amoxi-clav	0	7	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	0	0	0	0	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	0	n.a.	n.a.	n.a.	n.a.
Veillonella spp.	amoxicillin	5	5	0	0	22	0	0	n.a.
	amoxi-clav	0	0	0	0	20	0	0	n.a.
	clindamycin	10	0	0	0	0	0	0	n.a.
	metronidazole	5	0	0	0	0	0	0	n.a.

[a] *Not all strains were tested for all antibiotics.*
[b] *Not available.*

Table 4.6.5.3 An overview of the percentage resistance for different antibiotics within gram-positive anaerobic genera, per year.

		% resistance							
		2018	2017	2016	2015	2014	2013	2012	2011
Actinomyces spp.	amoxicillin	0	0	0	0	0	0	0	0
	clindamycin	12	5	7	7	11	0	0	8
GPAC	amoxicillin	0	0	0	0	0	0	0	0
	clindamycin	14	13	17	13	18	10	6	14
	metronidazole	0	0	0	0	0	1	0	0
Anaerococcus spp.	amoxicillin	0	0	n.a.[b]	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	11	13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Finegoldia magna	amoxicillin	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	20	21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Parvimonas micra	amoxicillin	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	8	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Peptonphilus spp.	amoxicillin	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	16	17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Peptostreptococcus spp.	amoxicillin	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Clostridium spp.	amoxicillin	0	3	14	7	14	0	10	0
	clindamycin	26	30	28	22	0	27	33	19
	metronidazole	2	0	0	0	0	0	0	0
Eggerthella lenta	amoxicillin	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
	metronidazole	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
Propionibacterium spp.	amoxicillin	0	0	0	0	0	0	0	0
	clindamycin	6	[4]	4	1	3	3	4	3

[a] *Not all strains were tested for all antibiotics.*

[b] *Not available.*

Discussion

Per year the resistance rates for the tested antibiotics differ for the encountered anaerobic genera. The observed amoxicillin resistance among *Fusobacterium* isolates, which were mainly identified as *Fusobacterium nucleatum/naviforme*, is due to the production of beta-lactamases (data not shown). Resistance to amoxicillin-clavulanic acid is high among *Parabacteroides* isolates, 22.2%, indicating possible resistance due to efflux pumps. The mechanism for metronidazole resistance among isolates of *Prevotella* and *Veillonella* was not determined. However, these strains will be tested for the presence of a nim gene or other resistance mechanisms.

Resistance to carbapenem antibiotics among *Bacteroides fragilis* strains is due to the production of a metallo-beta-lactamase encoded by the *cfiA* gene. The presence of this gene is only observed among *B. fragilis* strains, not among other *Bacteroides* strains resistant to meropenem. In a previous study, we showed that 15.8% of the *B. fragilis* strains, isolated at the UMCG, harbor the *cfiA* gene.[1] Of all tested *B. fragilis* strains 5.3% were shown to be phenotypically resistant to meropenem. For this year 3 *B. fragilis* isolates showed resistance to meropenem, 5.4% of all isolated *B. fragilis* strains. This rate of resistance is similar as the one observed in the previous study. However, also a *Bacteroides* ovatus strain was resistant to meropenem and one strain showed intermediate resistance. The resistance mechanisms among non-fragilis isolates remains unknown.

Conclusions

- Amoxicillin resistance was only observed among gram-negative anaerobic bacteria. It is recommended to perform beta-lactamase testing on these strains.
- Metronidazole resistance was observed for some, *Prevotella*, *Veillonella* and *Clostridium* strains. Microbiologists should be aware of this possibility.
- The resistance rate to meropenem among *Bacteroides* species was higher compared to previous years.
- The antibiotic susceptibility profile differs between GPAC genera, which makes it necessary to differentiate between these genera.

References

[1] Veloo ACM, Baas WH, Haan FJ, Coco J, Rossen JW. Prevalence of antimicrobial resistance genes in *Bacteroides* spp. and *Prevotella* spp. Dutch clinical isolates. Clin Microbiol Infect 2019; DOI: 10.1016/j.cmi.2019.02.017

4.6.6 *Clostridioides difficile*

Introduction

The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a National Reference Laboratory for *Clostridioides (C.) difficile* at the Leiden University Medical Centre (LUMC) soon after recognition of fluoroquinolone resistant *C. difficile* PCR ribotype 027 outbreaks in 2005. Since then, this laboratory has offered ad hoc typing services for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the Dutch sentinel *C. difficile* infections (CDI) surveillance programme has been initiated in 2009 in order to monitor CDI incidence rates and circulating ribotypes in an endemic situation. Antimicrobial susceptibility tests are regularly performed at the Reference laboratory and resistance to vancomycin, metronidazole and fidaxomycin was not detected until 2017. In December 2017, a first clinical *C. difficile* isolate PCR ribotype 014 was found in a patient who failed to metronidazole treatment (MIC=8 mg/L) (I.M. Boekhoud et al. submitted manuscript). The stable metronidazole resistance correlated with the presence of a transferable plasmid which was not found in susceptible isolates.

Methods

In the period 2017-2018, 22 acute care hospitals participated in the sentinel surveillance programme. In these hospitals, all hospitalized patients >2 years old with clinical signs and symptoms of CDI in combination with a positive test for *C. difficile* toxins or toxigenic *C. difficile* were included. Clinical data and outcomes after 30 days were registered. Isolates of all included CDI cases were sent to the LUMC for PCR ribotyping. Antibiotic resistance was determined by agardilution for a selection of *C. difficile* sentinel surveillance isolates.

Results

From May 2017 to May 2018, a mean CDI incidence rate of 2.90 cases per 10,000 patient-days was found through sentinel surveillance. The most frequently encountered PCR ribotypes were 014/020 (20.9%) and 002 (11.9%). No outbreaks of *C. difficile* in hospitals participating in the sentinel surveillance were reported.

Among samples submitted for ad hoc typing, PCR ribotype 014/020 was the predominant ribotype (26%), followed by PCR ribotype 027 (15%). In the previous year the predominant type was PCR ribotype 027 (17%). An outbreak due to PCR ribotype 027 was reported in the southwestern part of the Netherlands. Another outbreak due to PCR ribotype 017 took place in a hospital in the northwestern part of the Netherlands[1].

Antibiotic resistance was determined for 45 randomly selected *C. difficile* sentinel surveillance isolates, collected between January 2018 and December 2018 (Table 4.6.6.1). No resistance was detected to metronidazole, vancomycin or fidaxomicin using CLSI and EUCAST cut-off levels[2,3].

Discussion

The epidemiology of CDI is comparable with previous years, with only one outbreak due to Type 027. Resistance to antibiotics that are used for treatment of CDI is still very rare, though a plasmid mediated resistance to metronidazole (pCD-METRO) has been discovered in 2017. Using a newly developed PCR for detection of pCD-METRO, a large collection of human and animal strains were investigated. PCD-METRO was detected in toxigenic and non-toxigenic isolates from humans and animals in various countries. The presence of the plasmid always correlated with increased MIC levels to metronidazole. The clinical relevance of pCD-metro is currently studied.

Table 4.6.6.1 MIC50, MIC90 and range (mg/L) of 45 *C. difficile* sentinel surveillance isolates.

	MIC_{50}	MIC_{90}	Range
Ribotype 001 (n = 9)			
Fidaxomicin	<0.06	0.06	<0.06 – 0.06
Metronidazole	0.25	0.25	0.25 – 0.5
Vancomycin	0.125	0.25	0.06 – 0.25
Ribotype 014/020 (n =10)			
Fidaxomicin	<0.06	<0.06	<0.06 – 0.125
Metronidazole	0.5	0.25	0.25 – 0.25
Vancomycin	0.125	0.25	0.06 – 0.25
Ribotype 078/126 (n = 9)			
Fidaxomicin	<0.06	0.125	<0.06 – 0.125
Metronidazole	0.25	0.25	0.125 – 0.25
Vancomycin	0.25	0.25	0.125 – 0.25
Other ribotypes (n = 17)			
Fidaxomicin	<0.06	0.125	<0.06 – 0.125
Metronidazole	0.25	0.25	<0.06 – 0.5
Vancomycin	0.125	0.25	<0.06 – 0.25

Conclusion

- No resistance of *C. difficile* to metronidazole, vancomycin or fidaxomicin was found.
- Plasmid-mediated resistance to metronidazole (pCD-METRO) has been found in clinical isolates from various countries and will be included in the national surveillance from January 2019 onwards.

References

[1] Vendrik KEW, Crobach MJT, Terveer EM, Harmanus C, Sanders IMJG, Kuijper EJ, Notermans DW, Greeff de SC, Alblas J, Dissel v JT. 2018. Twelfth Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance. May 2017-May 2018.

[2] Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard-Eight Edition. [Document M11-A-8].

[3] EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. Available at http://www.eucast.org/clinical_breakpoints/.

4.6.7 *Aspergillus fumigatus*

Introduction

Acquired triazole resistance has emerged in the mold *Aspergillus fumigatus*, a saprophytic fungus that causes invasive and non-invasive diseases in humans depending on the immune status of the host. The triazoles voriconazole and isavuconazole represent first line treatment options for invasive aspergillosis, but the SWAB national guideline for invasive mycoses was revised in 2017, now recommending combination therapy in invasive aspergillosis, due to resistance rates exceeding 10% in five University Medical Centers (UMCs).[1] In 2018 five teaching hospitals were added to the surveillance network in order to determine the resistance frequency in non-UMCs and to increase the geographic spread of sampling sites.

Methods

In five UMCs and five teaching hospitals all clinical *A. fumigatus* isolates were screened for triazole resistance using a four-well agar plate (VIPcheck™, MediaProducts, Groningen, the Netherlands). Three agars contain medical triazoles, itraconazole, voriconazole and posaconazole, and one well acts as growth control. This method has been shown to be highly sensitive and specific to detect azole resistance.[2] Growth on the triazole containing well 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 was performed using the EUCAST microbroth dilution method. 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 2018 *A. fumigatus* isolates from 1,548 culture-positive patients were screened for triazole resistance, including 764 (range 81 to 238 per center) patients from UMCs and 784 (range 81 to 265 per center) patients from teaching hospitals. Overall 162 patients (10.5%) harbored a triazole-resistant isolate, with a resistance frequency of 14.7% (112 of 764 patients) in UMCs and 7.8% (50 of 784 patients) in teaching hospitals (Table 4.6.7.1). In the UMCs the resistance frequency remained stable compared with 2017, and in all UMCs the frequency exceeded 10%, ranging from 11.7% in Radboudumc, Nijmegen to 20.8% in LUMC, Leiden (Table 4.6.7.1). The resistance frequency was lower in teaching hospitals (range 4.9% to 10.6%), with only one center exceeding the 10% threshold. The underlying diseases of patients with an azole-resistant *A. fumigatus* isolate are shown in figure 4.6.7.1. Environmental resistance mutations, i.e. TR_{34}/L98H and TR_{46}/Y121F/T289A, were most frequently present in all centers accounting for 66% and 16% of resistance mutations, respectively. The proportion of TR_{34} and TR_{46} resistance mutations was higher in teaching hospitals compared with UMCs (88% versus 79%, p=0.04). Similar to previous years, 19% of triazole-resistant *A. fumigatus* isolates harbored no resistance mutations in the *Cyp51A*-gene in the UMCs, compared to 7% in teaching hospitals.

Discussion

In 2018 the detected triazole resistance frequency in UMCs was similar to 2017 with 14.7% of screened isolates showing a triazole-resistant phenotype. After several years of an increasing trend, the resistance frequency remained stable compared to the previous year. However, all five centers showed resistance frequencies exceeding the 10% threshold. For the first time five teaching hospitals participated in the resistance surveillance, showing a triazole resistance frequency of 7.8% in cultured *A. fumigatus* isolates. In only one teaching hospital the 10% threshold was exceeded. The difference in resistance frequency between UMCs and teaching hospitals remains unexplained. Although some variables were similar including the number of screened patients, more detailed research will be required to explain the observed differences in resistance frequency including continued surveillance. Patients with chronic lung diseases including cystic fibrosis remain an important group regarding triazole-resistant cultures. In both UMCs and teaching hospitals underlying resistance mutations were dominated by those associated with the environment accounting for 82% of resistance mutations found.
The recent ESCMID-ECMM-ERS Aspergillus guideline recommends to reconsider triazole monotherapy in regions with (environmental) resistance frequencies exceeding 10%.[3] The Dutch national SWAB-invasive mycoses guideline indeed recommends triazole/echinocandin or triazole/liposomal amphotericin B first line therapy in patients suspected for invasive aspergillosis. The 10% threshold was exceeded in the UMCs, but not in most teaching hospitals.

Conclusions

- The triazole resistance frequency in *A. fumigatus* in UMCs remained stable compared with 2017 at 14.7% of unselected culture positive patients harboring a resistant isolate.
- The triazole resistance frequency in *A. fumigatus* was 7.8% in teaching hospitals.
- In both UMCs and teaching hospitals triazole resistance mutations were dominated by those associated with environmental resistance selection, as they were found in 82% of triazole-resistant isolates.

References

[1] Dutch Working Party on Antibiotic policy. SWAB Guidelines for the management of invasive fungal infections. (http://www.swab.nl/swab/cms3.nsf/uploads/3AA7A56 CE879 587BC12581F80061297F/$FILE/SWAB%20Richtlijn%20 Mycosen%202017%20(final).pdf)

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[3] Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 2018; Suppl 1:e1-e38.

Table 4.6.7.1 Triazole resistance frequency in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers, 2013 to 2018, and 5 teaching hospitals, 2018.

	2013		2014		2015		2016		2017		2018	
	screened	azoleR (%)	screened	azoleR (%)	screened	azoleR (%)	screened	azoleR (%)	screened	azoleR (%)	screened	azoleR (%)
UMCs												
ErasmusMC, Rotterdam	231	10 (4.3)	265	10 (3.8)	22	7 (31.8)*	186	24 (12.9)	147	19 (12.9)	129	17 (13.2)
LUMC, Leiden	99	19 (19.2)	113	15 (13.3)	141	23 (16.3)	88	18 (20.5)	114	27 (23.7)	120	25 (20.8)
Radboudumc, Nijmegen	123	6 (4.9)	143	7 (4.9)	145	12 (8.3)	210	20 (9.5)	198	21 (10.6)	196	23 (11.7)
UMCG, Groningen	194	16 (8.2)	191	18 (9.4)	225	15 (6.7)	215	26 (12.1)	240	35 (14.6)	238	34 (14.3)
VUmc, Amsterdam	113	8 (7.1)	104	9 (8.7)	89	14 (15.7)	85	13 (15.3)	75	12 (16)	81	13 (16)
Total UMCs	**760**	**58 (7.6)**	**814**	**59 (7.2)**	**600**	**64 (10.7)****	**784**	**101 (12.9)**	**774**	**114 (14.7)**	**764**	**112 (14.7)**
Teaching hospitals												
Medisch Spectrum Twente, Enschede											88	5 (5.7)
St Antonius hospital, Nieuwegein											265	28 (10.6)
PAMM, Veldhoven §											81	4 (4.9)
CWZ, Nijmegen											155	11 (7.1)
Isala, Zwolle											195	13 (6.7)
Total teaching hospitals											**784**	**50 (7.8)**

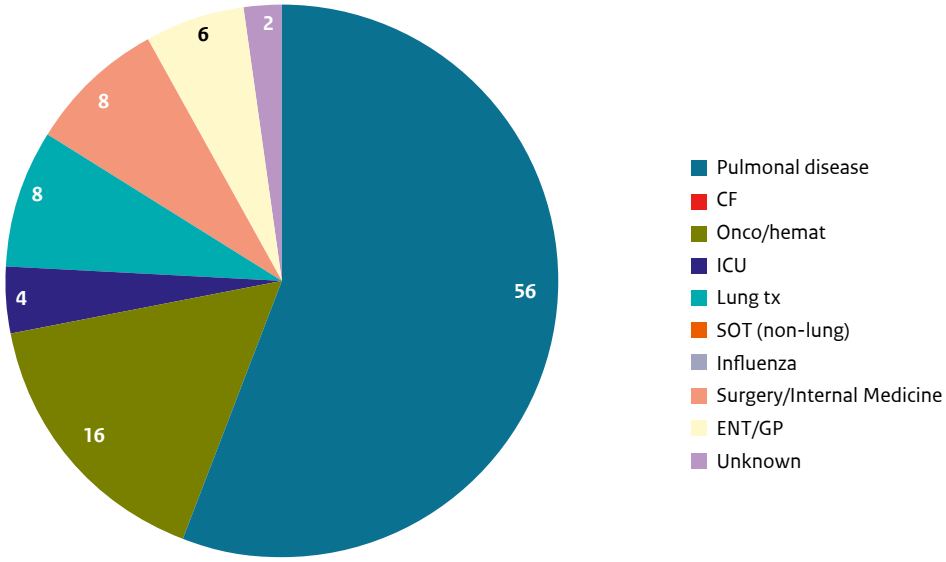
* *Resistance was screened for only in high-risk patients*

** *Resistance frequency was calculated based on the data of four centers.*

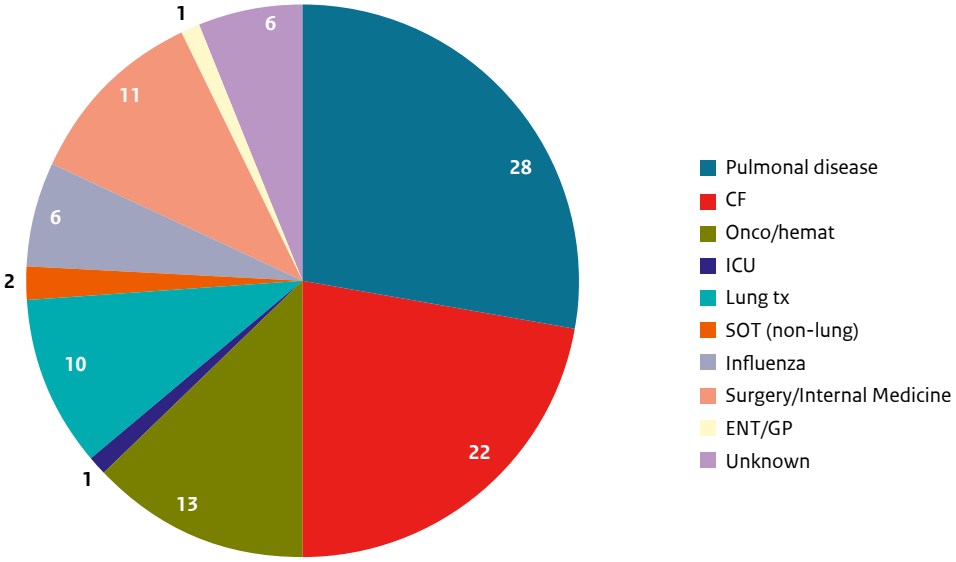
§ *PAMM screened A. fumigatus isolates from 4 hospitals: St. Anna hospital, Elkerliek hospital, Maxima Medical Center and Catharina hospital.*

Figure 4.6.7.1 Distribution of underlying diseases of patients with triazole-resistant *A. fumigatus* culture in 5 University Medical Centers (112 patients) and 5 teaching hospitals (50 patients).

Teaching hospitals



UMC's



5 Antimicrobial stewardship monitor in hospitals

Introduction

The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by antimicrobial stewardship teams in hospitals and 2) the quality of antimicrobial use in hospitals. Together with antibiotic consumption and resistance data, the antimicrobial stewardship monitor provides data on the impact of antimicrobial stewardship programs in hospitals in the Netherlands.

5.1 Stewardship activities employed by antimicrobial stewardship teams in hospitals

Methods

In 2018, an electronic survey was sent to all 77 acute care hospitals in the Netherlands to assess stewardship activities employed by antimicrobial stewardship teams in hospitals. The survey was aimed at measuring and improving the quality of antimicrobial use and was based on a systematic literature search including articles containing surveys on antimicrobial stewardship. It consisted of 39 questions categorized into four sections: 1) hospital characteristics; 2) organization of an antimicrobial stewardship program (ASP); 3) hospital resources for ASP; 4) stewardship activities. Results are presented as percentages of the responding hospitals. Trends were described comparing the data with 2016 and 2017.

Results

Hospital characteristics, organization of and hospital resources for an antimicrobial stewardship program

Thirty-five of 77 hospitals returned the survey, resulting in a response rate of 45%. The mean number of hospital beds was 613 (range 100-1339). Eight (23%) of the hospitals were university hospitals, 22 (63%) were non-university teaching hospitals and 5 (14%) non-teaching hospitals. All of the responding hospital had an A-team. These A-teams consisted of at least one hospital pharmacist, one medical microbiologist and 86% had at least one infectious disease specialist. Twenty-three percent of the A-teams employed a nurse, 14% an infection prevention specialist and residents/researchers were part of the A-team in 20% of the cases. Authorization by the hospital boards of directors had been

granted to 97% of the A-teams. Thirty-one and thirty-three hospitals provided data on total time spent on stewardship-related activities and salary support, respectively. The time spent by the A-team was a mean of 36.7 hours per week (range 4-134 hours). 79% of the hospital boards of directors provided a budget for the A-teams, with a median financial support of 0.7 FTE (range 0.1 – 3.1 FTE). IT support was available for 89% of the A-teams, although IT formation had been officially allocated to 11% of the A-teams and 46% of the A-teams explicitly indicated that they received only limited IT support. IT support was mainly used for the following antimicrobial stewardship-related activities: selection of specified patients (60%), data reporting (54% of the A-teams with IT support available), decision support (49%), and point prevalence survey (31%). Fourteen percent of the A-teams had antibiotic guardians (e.g. ambassadors) who propagate appropriate use of antimicrobials on all wards and an additional 26% had these on a limited number of wards. Some organizational characteristics and resources are compared with 2016 and 2017 in Table 5.1.1.

Table 5.1.1 Trends in A-team characteristics and monitoring between 2016 and 2018.

	2016	2017	2018
Survey response rate, N (%)*	42 (48%)	64 (80%)	35 (45%)
A-team characteristics			
Presence of an A-team	88%	94%	100%
≥1 clinical microbiologist	100%	100%	100%
≥1 hospital pharmacist	100%	100%	100%
≥1 infectious disease specialist	70%	68%	86%
≥1 nurse	5%	10%	23%
≥1 infection prevention specialist	10%	14%	14%
Time spent on stewardship per team, mean [hours per week], (range)	15.0 (1-47)	19.8 (3-58)	36.7 (4-134)
Budget provided by hospital board of directors	39%	41%	79%
Financial support, median [FTE], (range)	not available	0.5 (0.05-1.5)	0.7 (0.1 – 3.1)
*Occasional and continuous monitoring of***			
Restricted antimicrobials***	77%	91%	92%
Guideline adherence	71%	28%****	51%
IV-oral switch	76%	53%	80%
De-escalation	71%	34%	40%
Bedside consultation *S. aureus* bacteremia	53%	56%	72%
Therapeutic drug monitoring	63%	65%	69%
Correct diagnostics	58%	30%	34%

* *total number of hospitals in the Netherlands has decreased. Total number of hopsitals in 2016: 88, in 2017: 80, in 2018: 78*
** *meaning postprescription review for all objectives except bedside consultation*
*** *includes all types of interventions to improve the use of restricted antimicrobials*
**** *surveyed only for non-restricted antimicrobials in 2017*

Table 5.1.2 Number of hospitals that perform post-prescription review for stewardship activities (n=35).

	Total	Continuous (4-7 days a week)	Occasional (1-3 days a week)
No post prescription review, N (%)	3 (9)	n.a.	n.a.
Appropriateness of empirical antimicrobial use, N (%)	18 (51)	11 (31)	7 (20)
IV-oral switch, N (%)	28 (80)	21 (60)	7 (20)
De-escalation, N (%)	14 (40)	9 (26)	5 (14)
Discontinuation, N (%)	20 (57)	12 (34)	8 (23)
Therapeutic drug monitoring, N (%)	24 (69)	20 (57)	4 (11)
Surgical prophylaxis, N (%)	5 (14)	1 (3)	4 (11)
Correct diagnostics, N (%)	12 (34)	6 (17)	6 (17)

Table 5.1.3 Interventions in hospitals performed to monitor and improve the use of restricted antimicrobials (n=35).

Post-prescription review, N (%)	24 (69)
Education for residents, N (%)	16 (46)
Education for medical specialists, N (%)	7 (20)
Formulary restriction, N (%)	11 (31)
Computerized alert, N (%)	10 (29)
Check on diagnostics tests, N (%)	9 (26)
Post-authorization, N (%)	9 (26)
Pre-authorization, N (%)	6 (17)
Local opinion leaders, N (%)	3 (9)
Antibiotic checklist, N (%)	2 (6)
Antibiotic order forms, N (%)	5 (14)
Mandatory bedside consultation, N (%)	1 (3)
Stop orders, N (%)	0 (0)
Other activities, N (%)	1 (3)
No activities, N (%)	2 (6)

Stewardship activities

Thirty-five A-teams provided data on stewardship activities. Eighty percent of the A-teams received at least annually reports on cumulative antimicrobial susceptibility and reports on quantitative use of antimicrobials. 51% of the A-teams performed a point prevalence survey to assess the appropriateness of antimicrobials use at least annually. A-teams used post-prescription review for several stewardship objectives, as summarized in Table 5.1.2 and compared to previous years in Table 5.1.1. All but two of these 35 A-teams (94%) provided individual recommendations on stewardship objectives. This was done by telephone in 69% (range 6%-92%), face-to-face in 37% (range 11%-58%), and by computerized alerts or notes in the electronic medical chart in 56% (range 0%-100%) of the cases. Ninety-two percent of the A-teams had interventions in place to monitor and improve the use of restricted antimicrobials (Table 5.1.3). Table 5.1.4 summarizes the performance and monitoring of bedside consultation.

Table 5.1.4 Patient categories for which the hospital agreed to perform a compulsory bedside consultation by an infectious disease specialist and for which A-teams monitor the performance.

	Compulsory bedside consultation, N (% of 35 hospitals)	Monitoring of performance of bedside consultation, N (% of hospitals with indication for consultation)
No recommended bedside consultation	4 (11)	Not applicable
Staphylococcus aureus bacteremia	29 (83)	21 (72)
Infective endocarditis	18 (51)	3 (17)
Prothetic joint infection	14 (40)	5 (36)
Vascular prosthesis infection	13 (37)	3 (23)
Invasive fungal infection	15 (43)	Not asked

5.2 Quality of antimicrobial use in hospitals

Methods

All 77 acute care hospitals were invited by e-mail to share data on quality of antimicrobial use acquired by a point prevalence survey (PPS) in 2017 or 2018. Those hospitals that performed a PPS and were willing to share data for publication in NethMap received a short survey with questions on patient selection, quality of antimicrobial use, communication of the results and improvement interventions following the PPS. Different protocols for PPS were allowed as long as the appropriateness of antimicrobial use, defined as adherence of the prescription to the local guideline, was assessed.

Results

Sixteen hospitals responded to the questionnaire (21%) and received the second questionnaire. Eight hospitals provided useful data. The mean number of beds was 636. The PPS was performed in 2017 in three hospitals and in 2018 in five hospitals. All wards were included in all PSS, except one. That PPS included the wards cardiology, internal medicine, pulmonology, and surgery.

Lower respiratory tract infection was associated with the highest number of inappropriate therapeutic prescriptions in five hospitals, one hospital identified wound infections as point of improvement and two hospitals did not specify a syndrome associated with inappropriate treatment. Seven hospitals identified an antibiotic that was associated with the highest number of inappropriate therapeutic prescriptions: ciprofloxacin (3), amoxicillin-clavulanic acid (2), clindamycine (1), and cefuroxime (1). The wards associated with the highest number of inappropriate prescriptions were pulmonology (4), surgery (2), internal medicine (1), and pediatrics (1).

All hospitals have summarized the results of the PPS in a report. This report was sent to the wards by three hospitals, the medical staff by four, and to the hospital board of directors by four. Two hospitals did not distribute the report. Five hospitals started an improvement intervention based on the results of the PPS. Among these five hospitals were the four hospitals that sent a report to the hospital board of directors.

Conclusions

- The percentage of surveyed hospitals that have an A-team has increased to 100%
- Increasingly, nurses and infectious disease specialists are part of A-teams
- There is a steadily increase in budget provided to A-teams
- A point prevalence survey among eight hospitals has identified lowest guideline adherence for lower respiratory tract infections

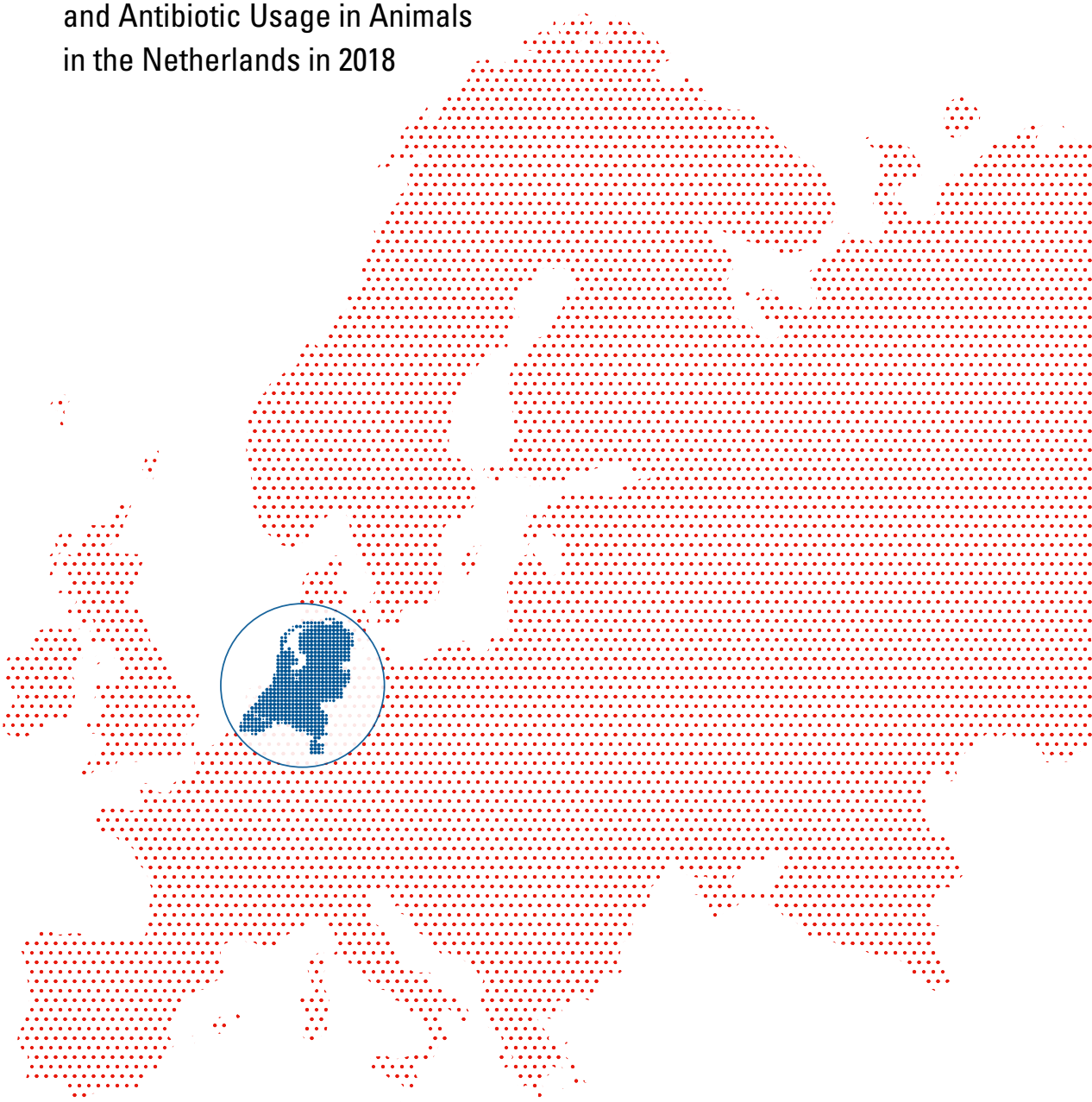
Discussion

Now all the 35 surveyed hospitals have an A-team, which are spending more time on stewardship activities compared to previous years. The main focus remains restricted antimicrobials, iv-oral switch, and therapeutic drug monitoring. More hospitals were provided with a budget by the hospital board of directors, although still 21% do not receive any budget, and the total financial support is lower than the national staffing standard for A-teams. The composition of A-teams has changed: more infectious diseases physicians and nurses are part of the A-teams.

The SWAB has continued the antimicrobial stewardship monitor with the aim to provide benchmarked feedback reports based on automated data extraction. Results will follow later this year. In addition, we asked hospitals to provide data from their PPS aimed at measuring the quality of antimicrobial use. Despite the fact that only large signals of inappropriate use can be picked up, most hospitals found targets for improvements and started improvement interventions based on the results of the PPS. Despite a recent SWAB guideline on this topic, lower respiratory tract infection was identified by most of them and inappropriate use was associated with a small group of antibiotics.

MARAN 2019

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2018



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

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MARAN 2019

Monitoring of Antimicrobial Resistance
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in 2018

June 2019

Colophon

This report is published under the acronym MARAN-2019 by Wageningen Bioveterinary Research (WBVR) in collaboration with the Food and Consumer Product Safety Authority (NVWA), the National Institute for Public Health and the Environment (RIVM) and the Netherlands Veterinary Medicines Institute (SDa). The information presented in MARAN-2019 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the occurrence of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
MARAN-2019 is published in a combined back-to-back report with NETHMAP-2019. The combined report is available on the website of WBVR at www.wur.nl 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).
MARAN-2019 can be ordered from the secretariat of WBVR, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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Contents

1 Summary

Antimicrobial usage

Sales of antimicrobial veterinary medicinal products (AMVPs) in 2018 (179 tonnes) showed a decrease of 1.1 % compared to 2017 (181 tonnes). In all sectors (dairy cattle, other cattle, veal valves, pigs and turkeys) but broilers a slight reduction in consumption has been realized. The method of estimation of number of animals was changed in 2018 for broilers, the increase of consumption might be (in part) attributable to this phenomenon.

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The small decrease in sales of AVMPs in the Netherlands in 2018 is consistent with an overall decrease as observed in the monitoring data on usage.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these AVMPs from other EU member states is not monitored in sales data, but if used in the monitored animal sectors, veterinarians are obliged to report these AVMPs.

Antimicrobial resistance

In 2018, *S.* Enteritidis (21%), followed by *S.* Typhimurium (19%) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,[5],12:i:- (12%), were most frequently isolated from humans suffering from salmonellosis. In pigs, *S.* Typhimurium and the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated. In poultry (including poultry products), the most frequently isolated serovars were *S.* Infantis (39%), *S.* Paratyphi B var. Java (*S.* Java, 16%) and *S.* Enteritidis (14%). The highest proportions of resistance were observed in the monophasic *S.* Typhimurium, *S.* Infantis, *S.* Paratyphi B var. Java, and to a lesser extent in *S.* Typhimurium. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Infantis (50%), *S.* Enteritidis (26%) and *S.* Typhimurium (15%). In 2018, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 0.9%, among seven different serovars, mainly isolated from human samples. Cefotaxime resistance was detected in one *Salmonella* Infantis isolate obtained from poultry products. No cefotaxime resistant isolates were found in fresh retail meat. In 2018 no carbapenemase producing *Salmonella* were found.

Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and increased slightly in 2018, compared to 2017. Resistance to macrolides was rarely detected amongst *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates. Ciprofloxacin resistance in *Campylobacter* isolates from human patients remained at a high level (with a further increase in 2018), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low. For *C. jejuni* and *C. coli* from human patients, resistance proportions were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

In human STEC O157 isolates proportions of resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim were substantially lower in 2018, compared to 2017 (from 16.1% to 4.7% for ampicilline, from 16.1% to 4.7% for sulfamethoxazole and tetracycline; no resistance for trimethoprim in 2018). The increasing tendency for resistance against these antimicrobials since 2009 did not continue in 2018. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 1.6% of human STEC O157 isolates. Similar to 2017, one ESBL-producing isolate was detected in 2018.

Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat. In In 2018, the resistance levels showed a tendency to stabilise (or increase for ampicillin) in broilers and veal calves and slightly decreased in pigs. In dairy cattle the resistance proportions remained at a constant low level. Resistance levels of *E. coli* in turkey meat were substantially higher than in chicken meat. The proportion of *E. coli* isolates resistant to extended spectrum cephalosporins was very low in faecal samples from broilers, pigs, dairy cattle and veal calves. Resistance to fluoroquinolones was at the same level as in 2017, and was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof. 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.

Selective culturing of ESBL/AmpC producing *E. coli* from broilers showed an ongoing decrease in the proportion of samples positive (prevalence) from 66% in 2014 to 23% in 2018. After a peak in the prevalence of ESBL/AmpC producing *E. coli* from rosé veal calves in 2016, little fluctuation was seen since then. However, since the peak in white veal calves in 2016, a steady increase is still ongoing.
The prevalence of ESBL/AmpC producing *E. coli* in Dutch retail meat has further decreased to 3.9% in 2018. No ESBL/AmpC producing *Salmonella* could be detected from Dutch retail meat. The proportion of ESBL/AmpC producing *Salmonella* from humans has further decreased to 0.8%, which is related to a decrease in ESBL/AmpC producing *S.* Kentucky.

No carbapenemase-producing *Enterobacteriaceae* were detected in livestock (n = 1206). Only $bla_{\text{OXA-48-like}}$ genes were detected in fifteen caecal samples (five veal calves, four slaughter pigs, three broilers and three dairy cow) and all associated with *Shewanella* spp..

In an ongoing prospective study of faecal samples of companion animals, two individual dog samples were positive for carbapenemase producing *E. coli*, harboring bla_{OXA-48} and $bla_{OXA-181}$ respectively. Both samples originated from different parts of the Netherlands. This was the second year such carbapenemase producing isolates were detected in medicated dogs in the Netherlands. Molecular analysis of the isolates is still ongoing but the analysis suggests that the bla_{OXA} genes are transferable because they are located on mobile genetic elements.

In 2018, the colistin resistance gene, *mcr*-1 was identified incidentally in *E. coli* from different livestock species by PCR screening. In veal calves, *mcr*-4 was detected in caecal samples of four animals. The finding of *mcr*-1 positive *E. coli* on poultry meat indicate a higher level in retail meat from chicken and turkey, related to imports from neighbouring countries. A significant higher prevalence of *mcr*-1 was detected in isolates from German broilers. No *mcr* genes were detected in *Salmonella*.

It can be concluded that the sales of antibiotics for animals of the last two years show small fluctuations, suggesting stabilisation compared to the steady decrease in the period 2011-2016.
The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) remains to be very minimal.
The data on usage are to a large extend reflected in the resistance data of 2018 where proportions of resistant *E. coli* stabilized in most livestock species. In broilers the proportion of samples (caeca and meat) positive for ESBL/AmpC-producing *E. coli* was again lower than in previous years. In contrast to broilers, in 2018 the prevalence of ESBL-carriers again increased in white veal calves. This shows that the measures implemented in Dutch livestock production to reduce the overall antibiotic use and to stop the use of 3rd-generation cephalosporins have been effective in reducing ESBL/AmpC-contamination of food-products. But, they have not been sufficiently effective in the veal calf sector, where ESBL occurrence increased. As in previous years, carbapenemase producing *Enterobacteriaceae* or the colistine resistance gene *mcr*-1, were not detected or found at low levels, respectively.

2 Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2018

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 on package level sold in 2018 in the Netherlands, as extracted from the Vetindex and supplemented with antimicrobial veterinary medicinal products (AVMP) data of non-FIDIN members. These data are estimated to cover approximately 98% of all sales in the Netherlands. Actual use can be different from the quantities sold due to stock piling and cross border use. Monitored use in the major livestock farming sectors (pigs, broilers, turkey, other poultry, veal calves, dairy- and other cattle, meat rabbits) covered 87.8% of sales in 2018.
The European Medicines Agency (EMA) collects harmonised systemic antibiotic usage data based on overall sales of veterinary antimicrobial agents through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project since September 2009. Sales figures from 1999 to 2008 were recalculated and adjusted according to the ESVAC protocol. Data as from 2011 are calculated according to the SDa method for all antimicrobial veterinary medicinal products, which means only active base substance mass (excluding mass of salts and esters) is calculated, including (unlike the ESVAC reports) 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 on in a following paragraph.

The average number of food-producing animals present in the Dutch livestock farming sector (pigs, poultry, veal calves, other cattle, sheep, goats, rabbits) shows annual variations (Table ABuse01). The goat sector consists of almost 75% dairy goats, and has grown since 2010, in contrary to the sheep sector which was reduced by 25% in 2018. The number of broilers derived from the national board of statistics (CBS) shows a steep reduction which is not reflected in the number of animals in the monitoring and benchmarking system of broilers.

Table ABuse01 Trends in livestock in the Netherlands in numbers (thousands); (Source: poultry, veal calves, goats and rabbits CBS, other Eurostat).

Number of animals * 1000	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Piglets (less than 20 kg)	4,809	4,649	4,797	4,993	4,920	5,115	5,408	4,986	5,522	5,307
Sows	1,100	1,098	1,106	1,081	1,095	1,106	1,053	1,022	1,066	970
Fattening pigs	6,199	6,459	6,200	4,189	4,209	4,087	4,223	4,140	3,967	4,033
Other pigs	2,100	2,040	2,021	1,841	1,789	1,765	1,769	1,733	1,741	1,624
Turkeys	1,060	1,036	990	827	841	794	863	762	671	657
Broilers	52,323	54,367	57,811	43,912	44,242	47,020	49,107	48,378	48,237	41,789
Other poultry	46,383	48,218	40,442	52,356	54,345	56,924	58,636	57,172	56,947	55,197
Veal calves	886	921	906	908	925	921	909	956	953	995
Other cattle	3,112	3,039	2,993	3,045	3,064	3,230	3,360	3,353	3,082	2,634
Dairy cattle	1,562	1,518	1,504	1,541	1,597	1,610	1,717	1,794	1,665	1,552
Sheep	1,091	1,211	1,113	1,093	1,074	1,070	1,032	1,032	1,015	743
Dairy goats	274	248	251	272	277	296	328	347	376	431
Other goats	100	105	130	125	136	136	142	153	156	157
Fattening rabbits	271	260	262	284	270	278	333	318	300	291
Dows	41	39	39	43	41	43	48	45	43	41

2.1.2 Trends in total sales

Figure ABuse01 and Table ABuse02 show the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. Total sales decreased by 63.8 % over the years 2009-2018, the Governmental 70% reduction goal has not been reached yet.
Sales of antimicrobial veterinary medicinal products in 2018 (179 tonnes) showed a decrease of 1.1 % compared to 2017 (181 tonnes). The gap between sales data and usage in monitored sectors has never been bigger since 2013, although more sectors are monitored in 2018 than in 2013. Further investigations are initiated, addressing the accuracy of the sales data, usage in unmonitored sectors and completeness of usage data in monitored sectors.
As demonstrated in Figure ABuse02 some groups of antimicrobials show a fluctuating pattern over the years, with an overall decreasing tendency, and some variation from year to year (penicillins, tetracyclines and cephalosporins of 1st and 2nd generation). A steady decrease over the years is noted for fixed combinations (mainly mastitis injectors), the critically important antimicrobials fluoroquinolones and cephalosporins of 3rd and 4th generation, and for trimethoprim/sulfonamides. Sales of amphenicols dropped in 2017 and in 2018 after increases in earlier years. Also, sales of 1st and 2nd generation cephalosporins (-15%) decreased. The sales of polymyxins (mainly colistin) and aminoglycosides surprisingly increased with, respectively, 29.8% and 19.4%. Quinolones also increased (16.0%). The biggest increase was noted for pleuromutilins sales; this class of antimicrobials is still exclusively used in veterinary medicine and has increased every year since 2016, in 2018 with 45.9%.

Figure ABuse01 Antimicrobial veterinary medicinal product sales 1999-2018 in kg (thousands).

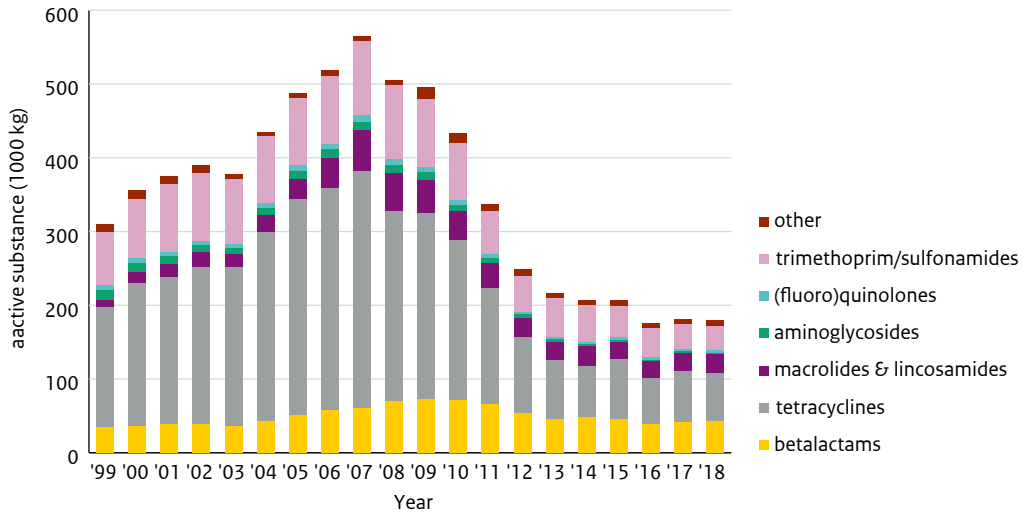
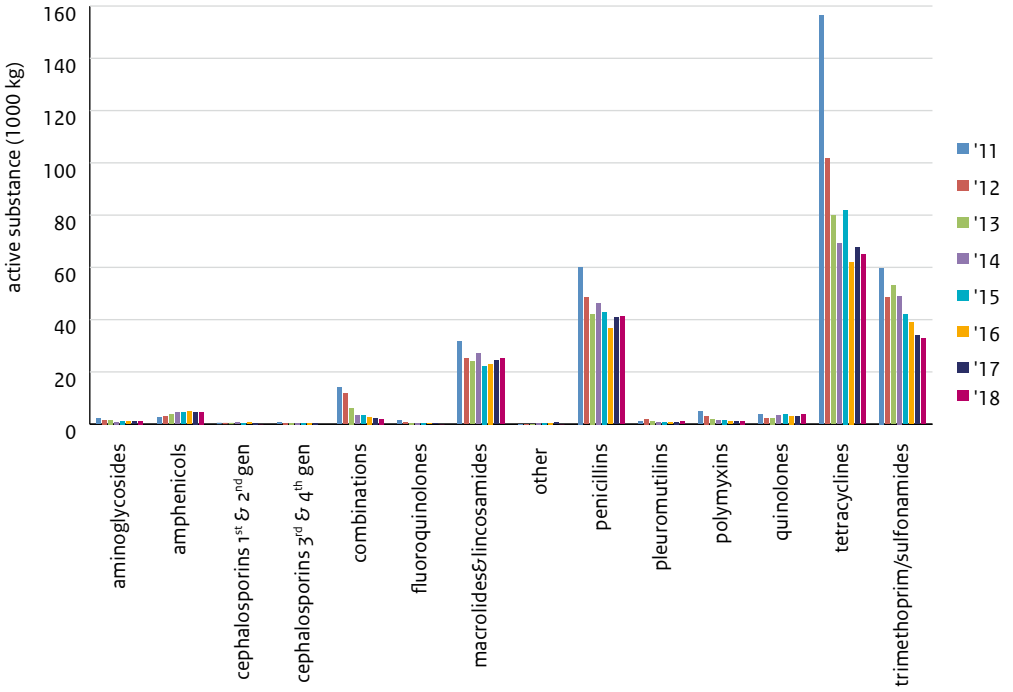


Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2018 in kg (thousands) (FIDIN, 2018).

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18
betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65
macrolids & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25
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
(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
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33
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
total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179

Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmaco-therapeutic class 2011-2018 in kg (thousands).



Tetracyclines

The fraction of doxycycline decreased to 42.5% of the total sales of tetracyclines (49% in 2017, 47% in 2016, 42% in 2015, 41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

Penicillins

Second place in mass, penicillin sales was stable in 2018 in comparison to 2017. The distribution of broad and narrow spectrum penicillins (in mass sold) is the same as last year, 75-25.

Trimethoprim/sulfonamides

The use of trimethoprim/sulfonamides decreased with 3% in 2018, it ranks third in mass sold.

(Fluoro)quinolones

The sales of fluoroquinolones decreased with 20kg (7.9%) in 2018. An overall reduction of 84.5% was realized in comparison with 2011. 56% of the sales are applied in the monitored sectors. The sales of quinolones increased in 2018, the absolute mass is comparable to the mass sold in 2011, these substances are exclusively applied in the food producing sectors.

Cephalosporins

The sales of 1st and 2nd generation cephalosporins increased steeply in 2014 due to underreporting in previous years; two veterinary medicinal products for companion animals were reported for the first time. Sales of these AVMPs were relatively stable over the period 2015 to 2017. The sales of 3rd and 4th generation cephalosporins halved in 2016 and are stable since then. A reduction of 99.8% has been achieved since 2011. The availability of these product on the market has diminished steeply as a result from this decrease. For food producing animals no products are available anymore, in case of urgency AVMPs have to be imported.

Polymyxins

Colistin sales and use increased in 2018 with 30%, the major application is in oral AVMPs. Compared to 2011 a reduction of 75% has been accomplished.

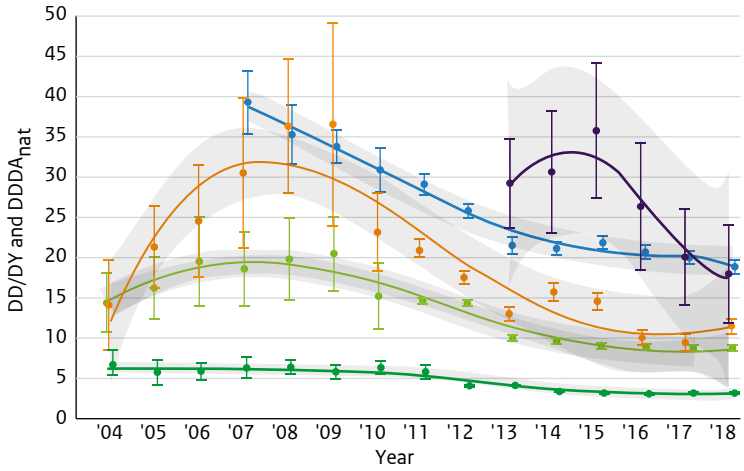
2.2 Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands

Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands for sentinel farms. These data were converted to the number of defined doses per animal year (DD/AY). The calculation method is similar to the method applied in human drug use. Applied antimicrobial veterinary medicinal products are converted to treated animal mass*days by national conversion factors (determined by the nationally authorized dosages and pharmacokinetics of the drug to compensate for duration of action) and related to animal mass present on a farm. Results are calculated for a period of a year and expressed as the number of days an average animal is treated in that year on that particular farm. The sentinel data (2004-2010) are weighted by farm related variables to obtain figures representative for the whole population of farms in a sector.
Since 2011, husbandry related antimicrobial consumption is monitored at *all* farms in the largest sectors of food production animals: pigs, veal calves, broilers, cattle (since 2012) and turkeys (since 2013). Since 2016 rabbits are also monitored but due to several continuance difficulties usage data are still not suitable for trend observations. Since 2017 also antimicrobials use in other poultry sectors than broilers and turkey is made available to the SDa.
While the calculation method for treated body mass (numerator) is the same, totalized for all farms per sector, the denominator represents the whole sector, and this measure is referred to as Defined Daily Doses Animal ($DDDA_{NAT}$). Table ABuse03 shows the animal populations veterinary medicinal products consumption data reported in 2013 – 2018 (pigs, veal calves, cattle, broilers, turkeys). Table ABuse04 gives animal weights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In all sectors (dairy cattle, other cattle, veal valves, pigs and turkeys and rabbits) but broilers a reduction in consumption has been realized. As mentioned earlier, the number of broilers from CBS showed a steep reduction in 2018 which might have caused the increase of the $DDDA_{NAT}$, whereas the $DDDA_F$ is fairly stable. The $DDDA_F$ calculations are based on a growth curve and therefore correct for age at treatment and breed (slow growing or regular). Animal numbers provided by the sector are used for the $DDDA_F$ and are substantially higher than CBS numbers.
The trends in the number of defined daily dosages animal for the veal farming, sows/piglets farming, fattening pigs farming and broiler farming sectors as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2018 as $DDDA_{NAT}$) are depicted in Figure Abuse03, and specification of applied antimicrobial groups in the different sectors for 2013-2018 is presented in Figure Abuse04. $DDDA_{NAT}$ in 2011 is estimated by the 2011/2012 $DDDA_F$ ratio (weighted by average animal kgs present per farm). For veal calves all observations of 2007-2010 were recalculated with the average dosages of AVMPs instead of maximum dosages as were applied for veal calves exclusively until 2013. For broilers the $DDDA_{NAT}$ in 2011 was estimated by the 2011/2012 treatment days ratio (treatment days are weighted by the number of animal days per farm) and the $DDDA_{NAT}$ in 2012 was estimated by treatment days adjusted by the 2013 treatment days/$DDDA_{NAT}$ ratio. From 2011 to 2018, CBS data for number of animals are used in the calculations for broilers, turkeys, veal calves and rabbits, and EUROSTAT data for pigs and dairy cattle. Confidence limits (CLs) are obtained from the corresponding CLs for $DDDA_F$ *in casu* weighted treatment days per year.

Table ABuse03 Weight per sector in kg (thousands) for DDD_{NAT} calculation.

Sector	2012	2017	2018
pigs	710,688	690,093	663,267
veal calves	156,602	163,935	171,692
dairy cows	924,600	999,000	931,200
other cattle	597,900	542,000	541,000
broilers	43,846	48,237	43,242
turkeys	4,961	4,023	3,815
rabbits	872	901	866

Figure ABuse03 Animal-defined daily dosages for 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 2011-2018 as $DDDA_{NAT}$) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area).



For benchmarking purposes, every farm in the Netherlands is periodically provided with the number of defined daily doses animal per year ($DDDA_F$) of the farm through internet portals of the sector quality systems. Consumption is calculated with a detailed denominator, to facilitate benchmarking and avoid misclassification. Table ABuseo6 depicts the animal bodyweights applied in the calculation of the denominator of $DDDA_F$ by the SDa.

For more details, annual reports of the SDa can be consulted (http://autoriteitdiergeneesmiddelen.nl/en/publications).

Table ABuse04 Applied bodyweights for $DDDA_{NAT}$ calculation.

species	category	Standard Weight (kg)
Veal Calves		172
Pigs	Piglets (< 20 kg)	10
	Sows	220
	Fattening pigs	70.2
	Other pigs	70
Broilers		1
Turkeys		6
Cattle	Dairy cows	600
	Other cows	500
Rabbits	Dow+kits	8.4
	Fattening rabbits	1.8
	Other rabbits	3.4

Figure ABuse04 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by pharmaco-therapeutic groups per animal sector over the years 2013-2018.

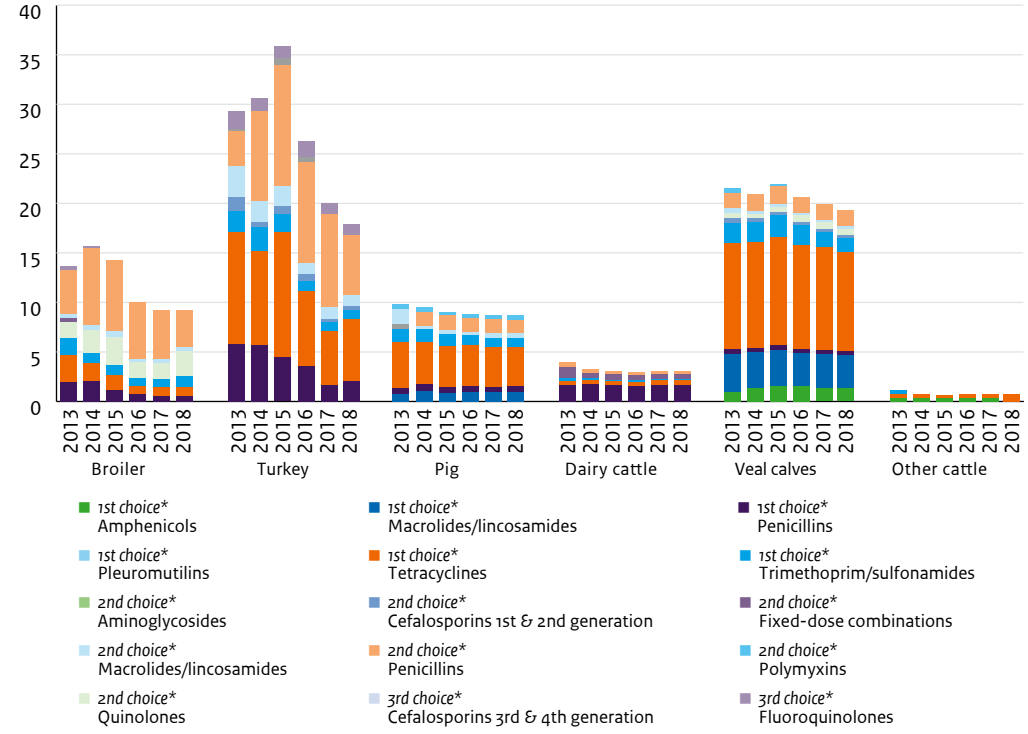


Table ABuse05 Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2014-2018.

	Animalsector														
	Veal calves					Dairy cattle					Other cattle				
Year	2014	2015	2016	2017	2018	2014	2015	2016	2017	2018	2014	2015	2016	2017	2018
Number of farms with prescriptions	2061	1978	1928	1868	1856	17747	17737	17529	17121	16499	13359	12971	12548	12790	12328
Pharmacotherapeutic group															
First choice*	**18.23**	**18.99**	**17.94**	**17.30**	**16.45**	**2.39**	**2.27**	**2.23**	**2.35**	**2.40**	**0.95**	**0.86**	**0.91**	**0.92**	**0.94**
% 1st choice of total	86.20%	86.09%	85.90%	85.90%	86.38%	72.56%	73.06%	74.03%	76.94%	78.99%	82.60%	86.00%	84.95%	84.19%	86.67%
Amphenicols	1.52	1.63	1.59	1.44	1.36	0.06	0.06	0.06	0.05	0.05	0.10	0.10	0.11	0.11	0.10
Macrolides/lincosamides	3.53	3.70	3.35	3.43	3.28	0.09	0.09	0.06	0.05	0.05	0.18	0.15	0.15	0.16	0.14
Other	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
Penicillins	0.43	0.42	0.48	0.46	0.44	1.62	1.50	1.52	1.69	1.76	0.09	0.09	0.10	0.11	0.10
Pleuromutilins	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
Tetracyclines	10.66	11.01	10.47	10.35	10.08	0.39	0.37	0.35	0.32	0.32	0.47	0.42	0.44	0.45	0.53
Trimethoprim/sulfonamides	2.08	2.22	2.05	1.61	1.28	0.24	0.25	0.24	0.24	0.23	0.11	0.10	0.10	0.09	0.06
Second choice*	**2.90**	**3.04**	**2.92**	**2.80**	**2.57**	**0.90**	**0.83**	**0.78**	**0.70**	**0.64**	**0.20**	**0.14**	**0.16**	**0.17**	**0.14**
% 2nd choice of total	13.71%	13.80%	13.97%	13.90%	13.50%	27.30%	26.79%	25.83%	22.94%	20.88%	17.36%	13.95%	15.01%	15.72%	13.28%
Aminoglycosides	0.34	0.19	0.23	0.23	0.21	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cefalosporins 1st & 2nd generation	*	*	*	*	*	0.02	0.02	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Combinations	0.01	0.00	0.00	0.01	0.00	0.48	0.42	0.38	0.34	0.29	0.04	0.03	0.03	0.04	0.03
Macrolides/lincosamides	0.19	0.18	0.19	0.23	0.29	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.03
Penicillins	1.71	1.91	1.77	1.75	1.68	0.38	0.37	0.34	0.31	0.29	0.09	0.07	0.06	0.08	0.06
Polymyxins	0.15	0.19	0.07	0.02	0.02	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Quinolones	0.49	0.58	0.66	0.57	0.37	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.03	0.02	0.01
Third choice*	**0.02**	**0.02**	**0.03**	**0.04**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
% 3rd choice of total	0.09%	0.11%	0.13%	0.19%	0.12%	0.14%	0.15%	0.14%	0.11%	0.13%	0.04%	0.05%	0.05%	0.09%	0.05%
Cefalosporins 3rd & 4th generation	0.00	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fluoroquinolones	0.02	0.02	0.03	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	**21.15**	**22.05**	**20.88**	**20.13**	**19.04**	**3.30**	**3.11**	**3.01**	**3.06**	**3.04**	**1.15**	**1.00**	**1.07**	**1.10**	**1.08**

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018.*

Table ABuse05 (continued) Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2014-2018.

	Animalsector														
	Pigs					Broilers					Turkeys				
Year	2014	2015	2016	2017	2018	2014	2015	2016	2017	2018	2014	2015	2016	2017	2018
Number of farms with prescriptions	6072	5824	5462	5297	4975	797	816	849	852	834	41	40	47	45	39
Pharmacotherapeutic group															
First choice*	**7.45**	**6.97**	**6.88**	**6.61**	**6.68**	**5.16**	**3.76**	**2.49**	**2.36**	**2.64**	**17.75**	**19.18**	**12.29**	**8.11**	**9.16**
% 1st choice of total	78.22%	77.10%	77.54%	75.99%	77.18%	32.72%	25.79%	24.42%	25.08%	22.26%	57.73%	53.37%	46.49%	40.22%	52.46%
Amphenicols	0.17	0.18	0.24	0.25	0.25	*	*	*	*	*	*	*	*	*	*
Macrolides/lincosamides	0.92	0.78	0.82	0.76	0.77	*	*	*	*	*	*	*	*	*	*
Other	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Penicillins	0.61	0.57	0.58	0.55	0.68	2.12	1.20	0.70	0.59	0.51	5.80	4.49	3.70	1.64	2.22
Pleuromutilins	0.09	0.08	0.07	0.09	0.12	*	*	*	*	*	*	0.12	*	0.10	0.10
Tetracyclines	4.34	4.14	4.07	4.05	3.86	1.70	1.49	1.01	0.95	1.21	9.58	12.57	7.63	5.51	6.05
Trimethoprim/sulfonamides	1.33	1.20	1.10	0.90	1.01	1.34	1.07	0.78	0.82	0.92	2.37	2.01	0.95	0.86	0.79
Second choice*	**2.07**	**2.07**	**1.99**	**2.09**	**1.98**	**10.43**	**10.75**	**7.63**	**6.99**	**9.15**	**11.71**	**15.56**	**12.54**	**10.99**	**7.66**
% 2nd choice of total	21.76%	22.89%	22.45%	24.01%	22.82%	66.15%	73.73%	74.86%	74.34%	77.11%	38.08%	43.29%	47.45%	54.50%	43.92%
Aminoglycosides	0.01	0.01	0.00	0.01	0.03	0.03	0.02	0.01	0.03	0.02	0.40	0.71	0.69	0.05	0.00
Cefalosporins 1st & 2nd generation	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Combinations	0.05	0.04	0.03	0.02	0.02	0.06	0.11	0.05	0.01	0.02	*	*	*	*	*
Macrolides/lincosamides	0.17	0.25	0.26	0.37	0.37	0.35	0.48	0.25	0.20	0.29	2.12	1.98	1.18	1.30	1.14
Penicillins	1.45	1.36	1.39	1.41	1.23	7.80	7.23	5.78	5.00	6.09	9.09	12.13	10.05	9.37	6.37
Polymyxins	0.34	0.38	0.28	0.26	0.31	0.05	0.06	0.04	0.03	0.05	0.08	0.63	0.61	*	*
Quinolones	0.05	0.03	0.02	0.03	0.02	2.13	2.86	1.51	1.72	2.68	0.02	0.10	0.01	0.26	0.15
Third choice*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.18**	**0.07**	**0.07**	**0.05**	**0.07**	**1.29**	**1.20**	**1.60**	**1.06**	**0.63**
% 3rd choice of total	0.02%	0.00%	0.00%	0.00%	0.00%	1.13%	0.48%	0.72%	0.58%	0.63%	4.19%	3.34%	6.06%	5.28%	3.62%
Cefalosporins 3rd & 4th generation	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Fluoroquinolones	0.00	0.00	0.00	0.00	0.00	0.18	0.07	0.07	0.05	0.07	1.29	1.20	1.60	1.06	0.63
Total	**9.52**	**9.03**	**8.87**	**8.70**	**8.66**	**15.76**	**14.59**	**10.19**	**9.40**	**11.87**	**30.74**	**35.94**	**26.42**	**20.16**	**17.45**

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018.*

Table ABuse06 Applied bodyweights for DDDAF calculation.

Species	Category	Specifications	Age	Standard weight (kg)
Calves	White veal		0 - 222 days	160
	Red veal startup		0 - 98 days	77.5
	Red veal fattening		98 - 256 days	232.5
	Red veal combination		0 - 256 days	205
Pigs	Sows/piglets	Sows (all female animals after 1st insemination) and boars		220
		Suckling piglets	0 - 25 days	4.5
		Gilts	7 months - 1st insemination	135
	Weaned piglets		25 - 74 days	17.5
	Fattening pigs / gilts	Fattening pigs	74 days - 5 months	70
		gilts	74 days - 7 months	70
Broilers			0 - 42 days	1
Turkeys		male	0 - 20 weeks	10.5
		female	0 - 17 weeks	5.6
Cattle	Dairy cows /	female	>2 years	600
	Suckler cows /	female	1 - 2 years	440
	Bulls for meat /	female	56 days - 1 year	235
	Rearing animals	female	<56 days	56.5
		male	>2 years	800
		male	1 - 2 years	628
		male	56 days - 1 year	283
		male	<56 days	79
Rabbits	Dow+kits	combined weight		8.4
		Dow	> 3 - 5 months	
		Kits	0 - 4.5 weeks	
	Fattening rabbits		4.5 - 13 weeks	1.8
	Other rabbits	female	11 weeks - 5 months	3.4

2.3 Usage 2016-2018 expressed in the number of international units DDD_{VET} of the European Surveillance of Veterinary Antimicrobial Consumption in pigs, veal calves, cattle, broilers and turkeys in the Netherlands per animal-year

The usage is also expressed as internationally established number of ESVAC with the denominator of the $DDDA_{NAT}$ (live weight). This measure is included because it potentially facilitates international comparisons. The use is calculated excluding the locally administered veterinary medicinal products for mastitis and metritis, which are included in the Dutch system, but in the ESVAC system are only accounted for in the defined course dose (DCD_{VET}) calculation.

In general, both methods result in comparable consumption. In the Dutch system, veterinary medicinal products consisting of a combination of active substances result in only one treatment day, while in the ESVAC approach application of such product results in one treatment day for every active substance. This difference in the group trimethoprim/sulfonamides affects all sectors, except turkeys. In turkeys a product with one sulfonamide is predominantly applied, with a much lower authorized dose in the Netherlands than the average dose in Europe. Table Abuse07 depicts the results of antimicrobial consumption in European DDD_{VET} per (live weight) animal-year.

DDD_{VET} results decreased for all sectors, except for broilers, and is for 2018 consistent with de $DDDA_{NAT}$ calculation, although the increase for broilers is less extensive (10%).

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The small decrease in sales of AVMPs in the Netherlands in 2018 is consistent with an overall decrease as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized drugs.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these AVMPs from other EU member states is not monitored in sales data, but if used in the monitored animal sectors, veterinarians are obliged to report these AVMPs.

Table ABuse07 number of DDD_{VET}/animal year in monitored sectors 2016-2018.

	Broilers			Turkeys			Pigs		
	2016 #DDD_{VET}	2017 #DDD_{VET}	2018 #DDD_{VET}	2016 #DDD_{VET}	2017 #DDD_{VET}	2018 #DDD_{VET}	2016 #DDD_{VET}	2017 #DDD_{VET}	2018 #DDD_{VET}
First choice*	**4.02**	**3.71**	**4.30**	**16.12**	**11.37**	**12.82**	**6.91**	**6.62**	**6.38**
% 1st choice of total	**34.80%**	**34.36%**	**32.19%**	**57.72%**	**49.48%**	**60.76%**	**79.13%**	**77.72%**	**77.73%**
Amphenicols	0.00	*	*	0.00	*	*	0.18	0.19	0.18
Macrolides/lincosamides	0.24	*	*	1.28	*	*	0.81	0.85	0.82
Penicillins	0.68	0.58	0.50	3.64	1.61	2.18	0.57	0.54	0.54
Pleuromutilins	*	*	*	*	0.14	0.14	0.07	0.10	0.13
Tetracyclines	1.32	1.27	1.67	10.71	9.20	10.14	3.46	3.42	3.12
Trimethoprim/sulfonamides	1.78	1.86	2.13	0.49	0.42	0.37	1.81	1.51	1.59
Second choice*	**7.47**	**7.03**	**8.98**	**10.21**	**10.54**	**7.65**	**1.82**	**1.90**	**1.83**
% 2nd choice of total	**64.59%**	**65.15%**	**67.27%**	**36.55%**	**45.89%**	**36.24%**	**20.87%**	**22.28%**	**22.27%**
Aminoglycosides	0.00	0.03	0.02	0.20	0.01	0.01	0.00	0.00	0.01
Cefalosporins 1st & 2nd generation	0.00	*	*	0.00	*	*	0.00	*	*
Combinations	1.08	1.23	0.03	0.01	0.19	*	0.02	0.02	0.01
Macrolides/lincosamides	0.09	0.02	0.25	0.00	*	1.23	0.08	0.03	0.02
Penicillins	0.00	0.19	6.73	0.00	1.40	6.30	0.41	0.53	0.53
Polymyxins	6.28	5.53	0.03	9.56	8.95	0.00	0.97	1.01	0.90
Quinolones	0.03	0.02	1.92	0.44	0.00	0.11	0.34	0.31	0.36
Third choice*	**0.07**	**0.05**	**0.07**	**1.60**	**1.06**	**0.63**	**0.00**	**0.00**	**0.00**
% 3rd choice of total	**0.61%**	**0.49%**	**0.54%**	**5.73%**	**4.63%**	**2.99%**	**0.00%**	**0.00%**	**0.00%**
Cefalosporins 3rd & 4th generation	0.00	*	*	0.00	*	*	0.00	*	*
Fluoroquinolones	0.07	0.05	0.07	1.60	1.06	0.63	0.00	0.00	0.00
Total	**11.56**	**10.78**	**13.35**	**27.93**	**22.98**	**21.11**	**8.73**	**8.52**	**8.21**

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018.*

Table ABuse07 (continued) number of DDD_{VET}/animal year in monitored sectors 2016-2018.

	Dairy cattle (excluding intramammary and intrauterine administrations)			Veal calves			Other cattle		
	2016 #DDD_{VET}	2017 #DDD_{VET}	2018 #DDD_{VET}	2016 #DDD_{VET}	2017 #DDD_{VET}	2018 #DDD_{VET}	2016 #DDDVET	2017 #DDD_{VET}	2018 #DDD_{VET}
First choice*	**0.95**	**0.92**	**0.87**	**19.51**	**18.52**	**17.19**	**0.95**	**0.95**	**0.92**
% 1st choice of total	**90.33%**	**89.76%**	**88.69%**	**78.93%**	**87.61%**	**88.07%**	**81.28%**	**86.12%**	**88.58%**
Amphenicols	0.04	0.04	0.04	1.22	1.11	1.05	0.09	0.08	0.08
Macrolides/lincosamides	0.03	0.03	0.03	3.81	3.94	3.76	0.17	0.19	0.16
Penicillins	0.15	0.15	0.15	0.26	0.26	0.25	0.05	0.05	0.04
Pleuromutilins	*	*	*	*	*	*	*	*	*
Tetracyclines	0.24	0.22	0.21	10.88	10.61	10.06	0.47	0.48	0.54
Trimethoprim/sulfonamides	0.47	0.48	0.44	3.34	2.61	2.08	0.17	0.15	0.10
Second choice*	**0.10**	**0.10**	**0.11**	**5.18**	**2.59**	**2.31**	**0.22**	**0.15**	**0.12**
% 2nd choice of total	**9.34%**	**9.97%**	**11.00%**	**20.97%**	**12.23%**	**11.82%**	**18.68%**	**13.81%**	**11.38%**
Aminoglycosides	0.01	0.01	0.01	0.09	0.09	0.09	0.01	0.01	0.00
Cefalosporins 1st & 2nd generation	0.00	*	0.00	0.00	*	*	0.00	*	0.00
Combinations	0.00	0.00	0.00	0.85	0.74	0.00	0.04	0.03	0.02
Macrolides/lincosamides	0.04	0.04	0.02	0.00	0.01	0.19	0.03	0.03	0.02
Penicillins	0.01	0.01	0.01	0.12	0.14	1.53	0.01	0.01	0.02
Polymyxins	0.04	0.05	0.07	4.05	1.59	0.02	0.13	0.07	0.06
Quinolones	0.01	0.00	0.00	0.07	0.02	0.48	0.01	0.00	0.00
Third choice*	**0.00**	**0.00**	**0.00**	**0.02**	**0.03**	**0.02**	**0.00**	**0.00**	**0.00**
% 3rd choice of total	**0.33%**	**0.27%**	**0.30%**	**0.10%**	**0.16%**	**0.11%**	**0.03%**	**0.07%**	**0.04%**
Cefalosporins 3rd & 4th generation	0.00	0.00	0.00	0.00	*	*	0.00	*	*
Fluoroquinolones	0.00	0.00	0.00	0.02	0.03	0.02	0.00	0.00	0.00
Total	**1.05**	**1.03**	**0.98**	**24.72**	**21.15**	**19.52**	**1.17**	**1.10**	**1.04**

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018.*

3 Resistance data

This chapter describes susceptibility test results as determined in 2018 for the food-borne pathogens *Salmonella enterica* enterica, *Campylobacter* spp. and *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, and therefore, depending on the antibiotic, non-wild type susceptible isolates (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 resistance percentages of *Salmonella* isolates. These isolates were obtained from humans suffering from clinical enteral infections/acute gastroenteritis and food-producing animals and food products from animals, as potential sources for distribution to humans via the food chain, and animal feeds as potential source for food-producing animals.

Highlights

1. In 2018 *S.* Enteritidis (21%) followed by *S.* Typhimurium (19%) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,[5],12:i:- (12%), were most frequently isolated from humans suffering from salmonellosis.
2. In pigs, *S.* Typhimurium and the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated.
3. In poultry (including poultry products), the most frequently isolated serovars were *S.* Infantis (39%), *S.* Paratyphi B var. Java (*S.* Java, 16%) and *S.* Enteritidis (14%).
4. The highest proportions of resistance were observed in the monophasic *S.* Typhimurium, *S.* Infantis, *S.* Paratyphi B var. Java, and to a lesser extent in *S.* Typhimurium.
5. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Infantis (50%), *S.* Enteritidis (26%) and *S.* Typhimurium (15%).
6. In 2018, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 0.9%, among seven different serovars, mainly isolated from human samples. Cefotaxime resistance was detected in 2.8% of the *Salmonella* isolates obtained from poultry products. No cefotaxime resistant isolates were found in fresh retail meat.
7. In 2018 no carbapenemase producing *Salmonella* were found.

Salmonella serovar prevalence

In the Netherlands, an extensive laboratory surveillance of *Salmonella* is carried out by the Dutch National Institute of Public Health and the Environment (RIVM), the EU reference laboratory (EU-RL) for *Salmonella* (EC 882/2004). Table S01 shows a summary of the serotyping results of *Salmonella* isolated from humans and farm animals (pigs, cattle and poultry).

A selection of all human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories (N = 1141) was sent to WBVR for susceptibility testing. Also, 577 isolates from other sources were tested. These were isolates from pigs (N = 49) and cattle (N = 50) mainly sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections in animals. Also, isolates from broilers (N = 29) and layers (N = 29) were tested, which were mainly nonclinical *Salmonella* isolates derived from a diversity of monitoring programs on farms, slaughterhouses and at retail. Furthermore, there were isolates from a diversity of other sources (N = 290) from animal feed and food products, and other animals from animal husbandry (e.g. sheep, goats).

In addition, NVWA tested 62 *Salmonella* isolates obtained from raw meats (mainly poultry), spices, herbs and seafood. Furthermore, 37 isolates were included, from (EC/2073.2005) verification projects, from broiler neck skins and 29 carcass swabs from slaughter pigs.

In 2018 several relatively small clusters of human salmonellosis were detected, predominantly *S.* Typhimurium (including monophasic), followed by *S.* Enteritidis and *S.* Goldcoast and very small clusters of *S.* Chester, *S.* Infantis, *S.* Saintpaul and *S.* Stanley. The Goldcoast outbreak was linked to a specific pig slaughterhouse. As a result a large scale forward tracing was conducted by NVWA and eventually a recall action was executed. The other clusters did not reach special public health concern and were not examined epidemiologically or traced to a source.

The most frequently isolated serovars from humans suffering from salmonellosis in 2018 were the same as in previous years: *S.* Enteritidis (21%), followed by *S.* Typhimurium (19%), and the monophasic variant of Typhimurium (*S. enterica subspecies enterica* 1,4,[5],12:i:-) (12%).

S. Typhimurium and its monophasic variant were mainly associated with pigs and cattle, but were also found in poultry. *S.* Enteritidis was isolated from broilers, chicken meat and layers, and was not found in cattle or pigs in 2018 (Table S01).
The most isolated serovar from pigs was *S.* Typhimurium and its monophasic variant. For cattle, these were *S.* Typhimurium and *S.* Dublin. Many different serovars were found in poultry and poultry meat in 2018. The most isolated serovar was *S.* Infantis (43%), followed by *S.* Paratyphi B var. Java (*S.* Java, 19%) and *S.* Kedougou (16%). *S.* Heidelberg, which was the most frequently isolated serovar from poultry in 2017, was not found in 2018.
Reported travel, on average 10%, contributed up to 40% of the cases of human salmonellosis over the years 2015-2018, but differed per serovar. Relatively high contributions of travel (≥30%) were noted for the serovars Kentucky, Typhi/Paratyphi A,B,C, Stanley, Virchow, Corvallis, Orion and Weltevreden. It should be noted that the contribution of travel as presented in Table S01 is only indicative of the true contribution, because travel is underreported by an estimated factor of about two.

Resistance proportions

The in November 2013 implemented EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), includes susceptibility testing of mandatory panels of antimicrobials. For the monitoring of *Salmonella* and *E. coli* three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice, were added to the panel since the implementation of this legislation, and three antimicrobials of less importance for treatment of human infections (florfenicol, kanamycin and streptomycin) were removed from the panel (Table S02). Tigecycline is structurally related to tetracyclines, but has a broader spectrum of activity. Azithromycin is a potent macrolide and in human medicine often used instead of erythromycin for treatment of infections by Gram-positive bacteria, due to the effectiveness of a once-daily administration during a few days. Given its activity against *Enterobacteriaceae* and its favourable pharmacokinetics, it is also used for typhoidal *Salmonella* cases for which *in vivo* efficacy has been demonstrated. Meropenem belongs to the carbapenems, which are last resort antimicrobials that are used to treat infections with multi-drug resistant bacteria. Colistin has been used widespread in veterinary medicine for prevention and treatment of diarrhoeal diseases in livestock. In human medicine, colistin can be used for treatment of human infections with multidrug-resistant carbapenemase producing bacteria. For this reason, the use of colistin in veterinary medicine has been reduced in Dutch livestock. Moreover, the recent finding of a plasmid mediated colistin resistance gene (*mcr*-1) resulted in even more attention for this compound.
Like in previous years, colistin resistance was not reported in *Salmonella* in 2018 (Table S02). That is because an epidemiological cut-off value that can be applied for all *Salmonella* serovars is lacking for colistin, which makes the results difficult to interpret. Using the former ECOFF of 2 mg/L (which is also the clinical breakpoint) resistance rates would have been highly influenced by differences in natural susceptibility (e.g. wildtype strains of *S.* Enteritidis and *S.* Dublin are less susceptible to colistin). As a result, colistin resistance would have been over-reported in *Salmonella*. Therefore, all *Salmonella* with elevated colistin MIC-values (colistin MIC > 2 mg/L for most *Salmonella* and MIC > 4 mg/L for Dublin and Enteritidis) were screened with PCR for the presence of *mcr*-genes (see section 4.3).

Table S01 Most prevalent *Salmonella* serotypes isolated in 2017 and 2018 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

		Travel	Humans		Pigs		Cattle	
		2015-2018	2017	2018	2017	2018	2017	2018
N Total			1255	1209	163	91	80	61
N tested	Tested		1222	1141	50	83	40	55
Enteritidis	601	13%	322	300			1	
Typhimurium	575	4%	200	231	56	29	28	24
Typhimurium (monofasisch)	423	4%	196	158	86	25	10	10
Infantis	173	11%	44	35	1	3		1
Paratyphi B. var. Java	76	26%	20	23			1	
Derby	68	6%	11	19	5	12		
Dublin	67	2%	7	22	1	1	27	15
Kentucky	62	30%	40	9				
Kedougou	62	n.a.		1				
Typhi/Paratyphi A,B,C	55	32%	25	29				
Newport	55	20%	24	26			1	1
Brandenburg	49	3%	7	18	2	5	1	
Livingstone	49	4%	4	2	2	1		
Montevideo	49	22%	9	3			3	
Yoruba	48	n.a.						
Bovismorbificans	47	6%	29	9	2	1	1	
Goldcoast	42	3%	6	27	1	3	1	
Agona	40	25%	11	9			1	
Chester	35	18%	16	20				
Give	32	17%	6	5				
Senftenberg	32	20%	1	5				
Stanley	31	31%	13	17				
Saintpaul	29	25%	9	22				
Virchow	28	32%	11	13				
Mbandaka	26	27%	2	2				
Napoli	21	8%	10	13				
Schwarzengrund	21	29%	6	3				
London	20	6%	3	2	1	1		2
Rissen	20	16%	3	4		3		
Thompson	20	3%	7	2				
Braenderup	19	24%	8	8				
Corvallis	19	34%	9	8				
Ohio	19	10%	3	4				
Agbeni	19	0%	16	5				
Orion	18	40%						
Anatum	17	28%	2	3				
Weltevreden	16	30%	6	4				
Bredeney	15	22%	3	7		1		
Oranienburg	15	19%	8	5				
Tennessee	14	3%		1				

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2017 and 2018 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2017	2018	2017	2018	2017	2018	2017	2018
N Total	272	172	160	107	24	50	926	802
N tested	66	132	8	95	8	33	318	305
Enteritidis	35	24	6	3	5	21	47	32
Typhimurium	19	6	7	4	4	2	107	49
Typhimurium (monofasisch)	21	4	5		7	1	78	29
Infantis	18	67	10	56		1	33	34
Paratyphi B. var. Java	26	27	22	27			7	18
Derby	1	1		1			15	32
Dublin	1						1	1
Kentucky							8	18
Kedougou	6	20	2	1		19	65	16
Typhi/Paratyphi A,B,C								
Newport	1	1	1	1			2	1
Brandenburg	5	2	5	1		1	22	17
Livingstone		3		2		1	109	82
Montevideo	2				1		24	21
Yoruba	1		1				42	85
Bovismorbificans	2							7
Goldcoast		2		2			4	5
Agona	2	4	1	4			14	14
Chester							2	2
Give							21	13
Senftenberg	2	1				1	22	19
Stanley							4	1
Saintpaul							5	
Virchow	2		1				3	3
Mbandaka	1	1	1	1			16	16
Napoli							3	
Schwarzengrund	8		8				17	5
London							2	19
Rissen							3	12
Thompson	11	1			4	1	8	3
Braenderup		1				1	2	1
Corvallis		1		1			2	2
Ohio							13	5
Agbeni								
Orion	1		1				17	6
Anatum	3		3				15	23
Weltevreden							1	6
Bredeney	1				1		2	9
Oranienburg							3	3
Tennessee							7	27

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2017 and 2018 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

		Travel	Humans		Pigs		Cattle	
		2015-2018	2017	2018	2017	2018	2017	2018
N Total			1255	1209	163	91	80	61
N tested	Tested		1222	1145	50	83	40	55
Poona	13	27%	7	4				
Hadar	12	27%	5	7				
Javiana	12	20%	12	4				
Kottbus	12	22%	4	7			1	
Bareilly	10	19%	4	5				
Mikawasima	10	2%	2	7				
Muenchen	10	16%	6	5	1			
Panama	10	9%	6	1	2	1		
Goettingen	7	8%	2	2			1	2
Heidelberg	5	10%	1	4				
Indiana	5	10%		2				
Minnesota	5	8%		2				
Jerusalem	4	n.a.						
Other	274	18%	109	86	3	5	3	6

MIC-distributions and resistance percentages of 1718 *Salmonella's* from different sources tested for susceptibility in 2018 are presented in Table S02. The resistance rates were approximately at the same level as in 2016 and 2017, with a slight increase for ciprofloxacin and nalidixic acid. The highest resistance proportions were again observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, trimethoprim and chloramphenicol. The proportions of resistance to ciprofloxacin and cefotaxime/ceftazidime seem to fluctuate a little since 2013. Again, no resistance was detected to the carbapenem antibiotic meropenem, indicating that carbapenemase producers were not present in the tested isolates (see also chapter 4.2). As in previous years, low proportions of resistance were found for tigecycline (1.0%) and azithromycin (0.8%), almost exclusively in human isolates.

Table S03 presents resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2018. There was a considerable variation between the resistance profiles of the different serovars. Because of the substantial differences between resistance proportions of S. Java from humans and broilers data are presented in separate columns. High resistance proportions were observed in the monophasic S. Typhimurium (almost 90% resistance to tetracycline and >75% to ampicillin and sulfamethoxazole), S. Infantis (>50% resistance to tetracycline, sulfamethoxazole, ciprofloxacin and nalidixic acid) and S. Paratyphi B var. Java from broilers (100% resistance to trimethoprim), and to a lesser extent in S. Typhimurium (48% resistance to ampicillin). Most serovars have acquired resistance against more than one antimicrobial. The most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2017 and 2018 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs) poultry, broilers (including poultry products) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2017	2018	2017	2018	2017	2018	2017	2018
N Total	272	172	160	107	24	50	926	802
N tested	66	132	8	95	8	33	318	305
Poona							3	1
Hadar							5	2
Javiana							1	
Kottbus								2
Bareilly								1
Mikawasima								1
Muenchen							2	1
Panama							2	13
Goettingen								
Heidelberg	74	2	73	2			10	7
Indiana		1		1			1	5
Minnesota	3		3					8
Jerusalem	8		5				1	
Other	18	3	5		2	1	155	125

Fluoroquinolone resistance

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, 17.7% of *Salmonella* isolates demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02), which was somewhat higher than in 2017 (13.8%). The dominant serovars of ciprofloxacin resistant isolates were S. Infantis (50%) from both humans and broilers, S. Enteritidis (26%) from humans and S. Typhimurium (15%) from humans.

Table S06 shows that the proportion of isolates resistant to ciprofloxacin in chicken meat was very high (69%), but not as high as in 2017 (89%). These isolates were obtained from broiler meat and broiler meat preparations from retail and meat industry. In chicken meat S. Infantis (N=18) was the most predominant isolate followed by S. Paratyphi B var. Java (N = 5). The high proportion of resistance to fluoroquinolones in poultry meat reflects the frequent usage of fluoroquinolones in the poultry production chain within EU.

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N=1718) tested for antibiotic susceptibility during 2018.

"Salmonella N = 1718"	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	95% CI
Ampicillin							37.9	36.0	2.7	0.1			0.1	23.2					23.3	21.4 - 25.4
Cefotaxime					97.3	1.8				0.9									0.9	0.5 - 1.5
Ceftazidime						95.1	4.1	0.1	0.4	0.1	0.2								0.8	0.4 - 1.3
Gentamicin						78.2	17.6	0.9	0.1	0.6	0.5	0.5	1.6						3.3	2.5 - 4.3
Tetracycline								71.7	2.6	0.3	0.1	0.5	2.5	22.3					25.4	23.4 - 27.6
Sulfamethoxazole										35.4	33.2	5.5	1.2	0.9	0.1	0.1	0.1	23.6	23.9	21.9 - 26.0
Trimethoprim					70.5	16.9	1.3	0.1	0.1				11.2						11.2	9.8 - 12.8
Ciprofloxacin	21.5	58.9	1.9	1.5	8.1	5.1	2.2	0.2	0.1	0.1	0.5								17.7	15.9 - 19.6
Nalidixic acid									75.3	7.0	2.4	2.0	0.4	0.8	12.1				15.3	13.6 - 17.0
Chloramphenicol										83.1	9.4	0.2	0.4	0.9	5.9				7.5	6.3 - 8.9
Azitromycin*								0.3	40.6	54.4	4.0	0.1	0.3	0.3					0.8	0.4 - 1.3
Colistin**							55.4	36.0	7.4	1.2									-	-
Meropenem		86.7	13.3																0.0	0 - 0.2
Tigecycline***					46.6	45.5	6.9	1.0											1.0	0.6 - 1.6

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 S03 Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2018 (N tested).

	Enteritidis (275)	Typhimurium (302)	1,4,[5],12:i:- (189)	Infantis (108)	Paratyphi B var Java, human (24)	Paratyphi B var Java, broiler (24)	Derby (49)	Dublin (41)	Goldcoast (33)	Brandenburg (31)	Kedougou (30)	Newport (30)	Yoruba (25)
Ampicillin	11.3	48.3	78.8	13.9	0.0	50.0	6.1	2.4	9.1	3.2	0.0	10.0	0.0
Cefotaxime	0.0	1.3	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.7	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.4	3.3	8.5	6.5	0.0	12.5	0.0	0.0	3.0	0.0	0.0	3.3	0.0
Tetracycline	7.3	36.4	89.9	50.0	0.0	8.3	22.4	2.4	9.1	9.7	0.0	13.3	0.0
Sulfamethoxazole	4.4	35.1	75.7	51.9	8.3	70.8	16.3	0.0	12.1	12.9	0.0	6.7	0.0
Trimethoprim	0.4	15.2	15.3	31.5	8.3	100.0	18.4	0.0	12.1	0.0	0.0	3.3	0.0
Ciprofloxacin	25.8	12.9	9.0	50.0	0.0	54.2	6.1	4.9	3.0	12.9	0.0	16.7	0.0
Nalidixic acid	25.5	9.6	5.8	52.8	0.0	50.0	6.1	0.0	3.0	12.9	0.0	6.7	0.0
Chloramphenicol	1.1	18.2	16.4	8.3	0.0	8.3	10.2	2.4	6.1	0.0	0.0	6.7	0.0
Azithromycin	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	1.0	1.6	7.4	0.0	4.2	0.0	2.4	0.0	0.0	0.0	0.0	0.0

ESBL's 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 (WHO, factsheet 139, 2005). In 2018, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 15/1718 (0.9%), among seven different serovars, all but two isolated from human samples. The predominant serovars were *S.* Typhimurium (N=4) and *S.* Infantis (N=5). The other serovars were *S.* Kentucky, (N=2), *S.* Muenchen (N=1), *S.* Corvallis (N=1), *S.* Enteritidis (N=1) and *S.* Saintpaul (N=1).
In chicken meat samples only one isolate ESBL-suspected *S.* Infantis was found (Table S06).
No cefotaxime resistance was detected in samples from other fresh meat products.

Table S04 Resistance (%) of *S.* Typhimurium (N tested) isolated from humans, cattle, pigs and other sources in 2018.

	S. Typhimurium (302)[a]			
	Humans (230)	Cattle (24)	Pigs (28)	Other sources (20)[b]
Ampicillin	47.0	54.2	64.3	35.0
Cefotaxime	1.7	0.0	0.0	0.0
Ceftazidime	0.9	0.0	0.0	0.0
Gentamicin	2.2	20.8	0.0	0.0
Tetracycline	32.6	58.3	50.0	35.0
Sulfamethoxazole	31.7	58.3	50.0	25.0
Trimethoprim	13.5	8.3	35.7	15.0
Ciprofloxacin	14.8	8.3	3.6	10.0
Nalidixic acid	10.4	8.3	3.6	10.0
Chloramphenicol	17.8	25.0	21.4	10.0
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.3	0.0	0.0	0.0

a Monophasic variants (1,4,[5],12:i:-) are excluded.

b Other sources include broilers, layers, goats, horses, food and feed products.

Table S05 Resistance (%) of *S.* Enteritidis (N tested) isolated from humans and broilers in 2018.

	S. Enteritidis (275)	
	Humans (259)	Other sources (16)[a]
Ampicillin	11.6	6.3
Cefotaxime	0.0	0.0
Ceftazidime	0.0	0.0
Gentamicin	0.4	0.0
Tetracycline	7.3	6.3
Sulfamethoxazole	4.2	6.3
Trimethoprim	0.0	6.3
Ciprofloxacin	26.3	18.8
Nalidixic acid	25.9	18.8
Chloramphenicol	1.2	0.0
Azithromycin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

a Other sources include broilers, layers, goats, food and feed products.

S. Typhimurium

Table S01 shows that *S.* Typhimurium represented 19.1% (231/1209) of all human *Salmonella* isolates as characterized by RIVM in 2018. This is more than in 2017 (15.9%) and 2016 (17.0%), and approximately the same as in 2015 (19.4%). *S.* Typhimurium is a common serotype in animals. If the monophasic Typhimurium variant is included, *S.* Typhimurium may be regarded as the most dominant serotype in humans and food-producing animals like pigs and cattle.
Table S04 shows that resistance in *S.* Typhimurium was very high for ampicillin, tetracycline and sulfamethoxazole, for chloramphenicol in cattle and pig isolates and for trimethoprim in pig isolates and to a lesser extent in isolates from other sources (including broilers, sheep, goats, food and feed) and human isolates. Resistance to chloramphenicol was also found in isolates from humans and other sources, at a somewhat lower level.
About 16% of the *S.* Typhimurium isolates exhibited the resistance profile Ampicillin-Chloramphenicol-Sulfamethoxazole-Tetracycline (ACSuT). Although streptomycin is not tested anymore, these figures indicate that the proportion of the penta-resistant phenotype (ACSuST) based on the chromosomal *Salmonella* Genomic Island 1, is lower than in previous years (20% in 2017 and 26% in 2016). Resistance to the clinically important drug cefotaxime was not detected in animal isolates and only at a very low level in human isolates (1.7%). The resistance percentage to fluoroquinolones in human isolates was higher than in 2017 (14.8% in 2018; 7.8% in 2017), but was lower than in 2016 (19.2%). In 2017, resistance to fluoroquinolones was not found in cattle and pig isolates, but in 2018 two cattle isolates (8.3%) and one pig isolate (3.8%) were resistant to ciprofloxacin. Borderline resistance to tigecycline was only observed in human isolates (N = 3), and not in isolates from cattle, pigs and other sources. These isolates all exhibit slightly elevated MIC-values caused by an unknown resistance mechanism (if any).

Resistance proportions in *S.* Typhimurium isolates from human samples showed an increasing tendency until 2010, after which they showed a tendency to decrease until 2013. Since 2013, resistance proportions seem to fluctuate from year to year. In 2018, the resistance proportions for almost all antimicrobials were lower than in 2017, except for ciprofloxacin. Resistance proportions for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated since 2014 (Figure S01).
Resistance proportions in *S.* Typhimurium isolates from pig and cattle samples (Figure S01) varied considerably over the years. These proportions seemed to decrease from 2013, but an increase was seen in 2016, and in 2017 for the cattle isolates. In 2018, resistance for almost all antimicrobials decreased in both pig and cattle isolates, except for ciprofloxacin (cattle and pigs) and chloramphenicol (pigs). However, these figures should be interpreted with care, because of the relatively small number of isolates per year.

Table S06 Resistance (%) of *Salmonella* enterica isolated from different types of raw meat, spices and seafood in the Netherlands in 2018.

	Chicken Retail N = 16	Chicken Imported N = 102	Other meat[a] N = 12	Other products[b] N = 16
Ampicillin	25.0	71.6	5.9	12.5
Cefotaxime	0.0	67.6	0.0	0.0
Ceftazidime	0.0	67.6	0.0	0.0
Gentamicin	6.3	5.9	0.0	0.0
Tetracycline	56.3	74.5	0.0	12.5
Sulfamethoxazole	75.0	77.5	0.0	0.0
Trimethoprim	56.3	5.9	5.9	0.0
Ciprofloxacin	68.8	89.2	17.6	12.5
Nalidixic acid	68.8	86.3	17.6	12.5
Chloramphenicol	0.0	3.9	0.0	0.0
Azithromycin	6.3	5.9	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	6.3	18.6	0.0	0.0

a Other meat includes pork (n = 5) , beef (n = 3), lamb (n = 1), crocodile (n = 2) and frog (n = 1).

b Other products includes seafood (n = 7), fish (n = 2) and spices (n = 7).

S. Enteritidis

In the Netherlands, human infections caused by S. Enteritidis are mainly related to the consumption of contaminated eggs and, to a lesser extent, of poultry meat products and travel abroad. MLVA-typing is used to differentiate between types isolated from Dutch broilers and humans. In 2018, the four dominant MLVA-types (02-10-07-03-02, 03-09-05-04-01, 02-11-07-03-02 and 03-10-05-04-01) were found in isolates from humans, layers and broilers and were similar to the most predominant MLVA types in 2013 to 2016 and in 2018 involved in small clusters of infection in humans.

Table S03 shows that resistance in S. Enteritidis is relatively low, compared to many other *Salmonella* serovars. Table S05 presents resistance proportions in S. Enteritidis isolates from human samples and other sources (including broilers, layers, goats, food and feed products). The resistance percentage for fluoroquinolones in human isolates was 26.3%, and for ampicillin a resistance rate of 11.6% was found. The resistance percentages for tetracycline and sulfamethoxazole in human isolates increased, compared to 2017 (2.3% and 2.0% respectively for tetracycline and sulfamethoxazole in 2017; 7.3% and 4.2% in 2018). For all other antimicrobials resistance proportions of human S. Enteritidis isolates were very low or not detected. The resistance percentages in the isolates of other sources were high for the fluoroquinolones (18.8%). Lower resistance percentages were measured for ampicillin, tetracycline, sulfamethoxazole and trimethoprim (all 6.3%).

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999-2018.

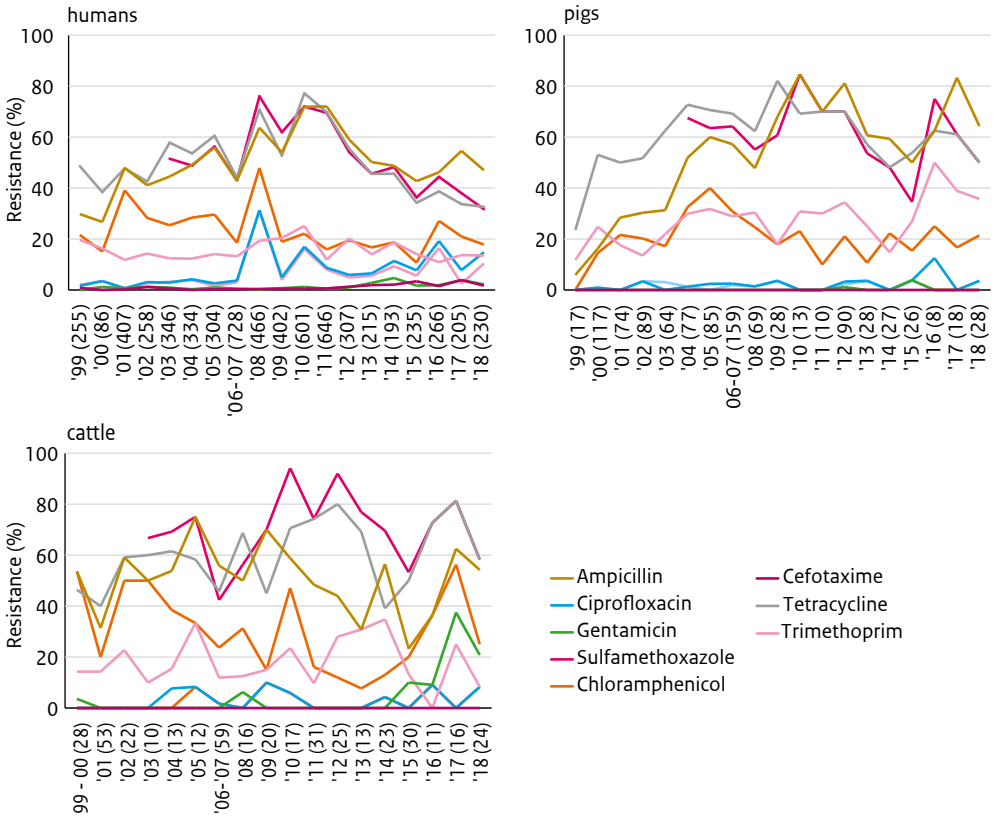
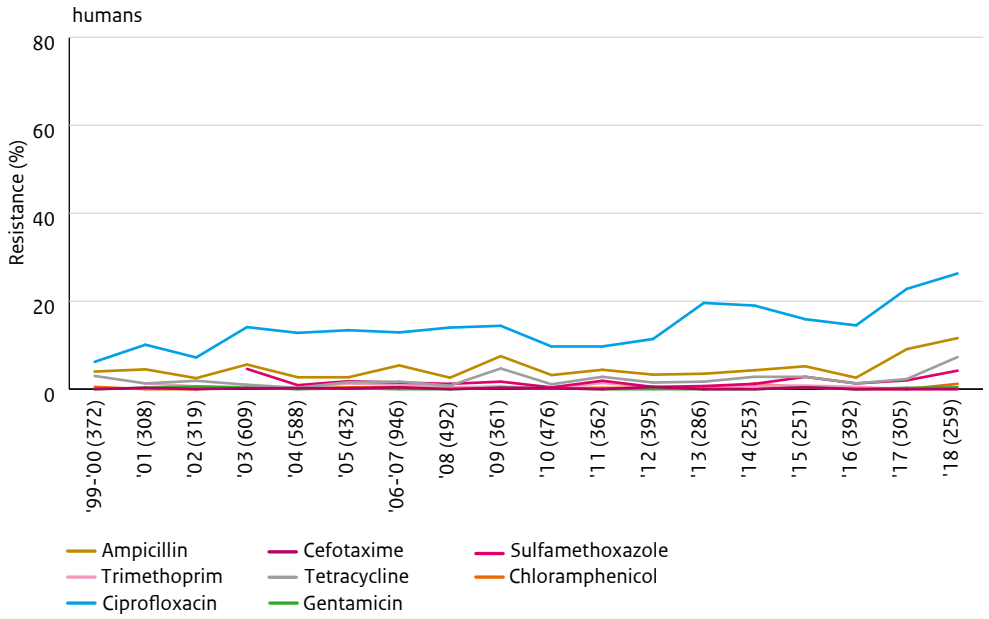


Figure S02 summarizes the trends in resistance of *S.* Enteritidis over the years in human isolates. Resistance for most antimicrobials is still low, but increased in 2018, compared to 2017. In general, resistance proportions in human isolates seem to be stable over years, with an increasing trend for ciprofloxacin resistance since 2010.

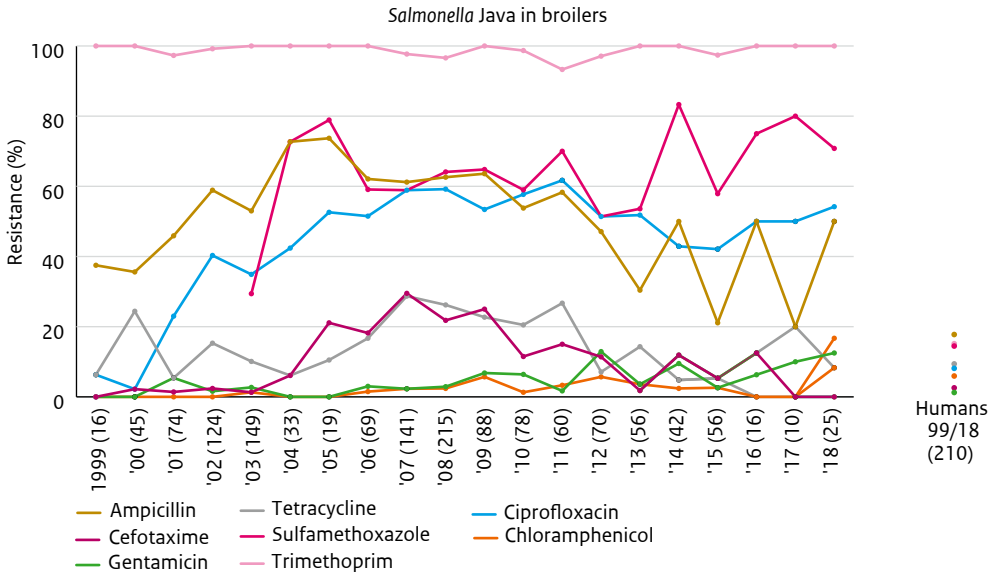
Figure S02 Trends in resistance (%) of *S.* Enteritidis isolated from humans from 1999-2018.



S. Paratyphi B var. Java (*S.* Java)

Since 2016, *S.* Java was not the most predominant serovar isolated in broiler production anymore, as it was in the period before 2015. Resistance percentages of human and broiler isolates of *S.* Java are presented in Figure S03. Since 2012, the resistance proportions seem to fluctuate, and a real increasing or decreasing trend cannot be seen. As in previous years, resistance to trimethoprim was 100%. In 2016 and 2017, resistance to chloramphenicol was not detected, but in 2018 the resistance percentage to chloramphenicol was 8.3%. The resistance level for ciprofloxacin further increased to 54.2% in 2018. The majority of the *S.* Java strains, isolated from human infections, were trimethoprim susceptible and therefore not considered to be related to the clone that spread in Dutch poultry and most probably predominantly travel related.

Figure S03 Trends in resistance (%) of S. Paratyphi variant Java isolated in humans and broilers from 1999-2018.

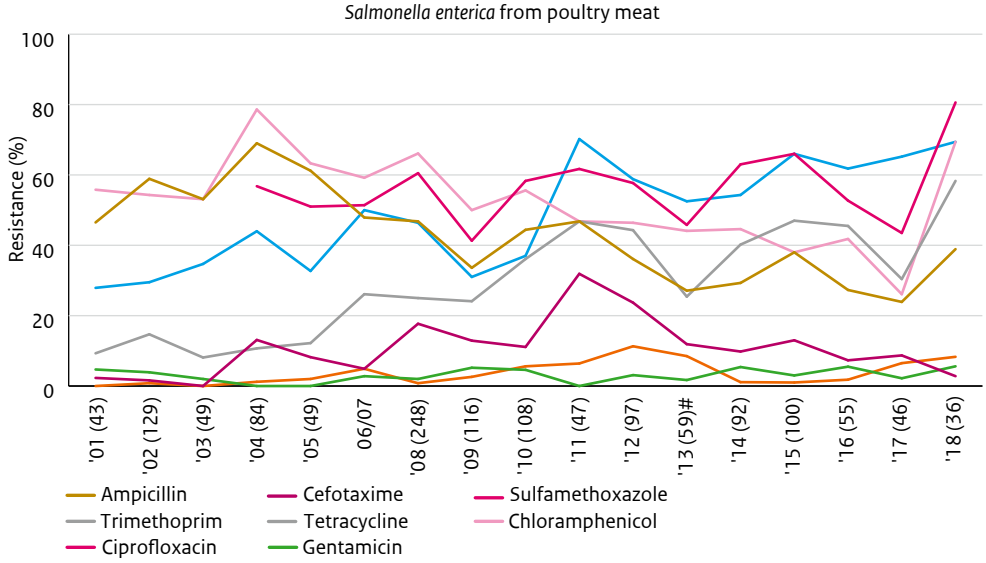


Salmonella from chicken meat, other meat sources and spices

Table S06 and Figure S04 show resistance data of *Salmonella* isolates from raw meat (chicken and other), herbs, spices and seafood. S. Infantis (70%) was the most prevalent serovar found in chicken meat in 2018, followed by S. Paratyphi B variation Java (27%). Isolates from other meat samples were resistant at lower levels than isolates from chicken meat (Table S06). Resistance proportions for the quinolones (ciprofloxacin and nalidixic acid) were very high in isolates from chicken meat (69.4%); resistance proportions in isolates from other meat samples were a bit higher than in 2017, but the number of isolates was low. Borderline resistance to tigecycline was observed in only one S. Infantis isolate from chicken meat and in one S. Infantis isolate from other meat (crocodile meat sample). Resistance to cephalosporins (cefotaxime and ceftazidime) was detected in one chicken meat sample (2.8%, S. Infantis), but not in the other meat samples.

Only 16 isolates were retrieved from "other products" (herbs, spices, sea food), so the resistance proportions in Table S06 are therefore not representative for those products in general. Resistance in isolates from these products was only found for sulfamethoxazole (18.8%), ampicillin (6.3%) and tetracycline (6.3%). For the other antimicrobials no resistance was detected in the isolates from other products.

Figure S04 Trends in resistance (%) of *Salmonella* enterica isolated from poultry meats in the Netherlands from 2001-2018.



The overall resistance proportions of *Salmonella* from poultry meat over the years are presented in Figure S04. Resistances fluctuate since 2001, with an increasing trend for ciprofloxacin; the resistance proportion for tetracycline also increased since 2001, was decreasing since 2015, but increased substantially in 2018. In 2013, a substantial reduction in resistance proportions was observed for most antimicrobials. However, after 2013 resistance proportions tended to increase again for sulfamethoxazole, ciprofloxacin, tetracycline, ampicillin and cefotaxime, with a slight decrease for most of them in 2016 and 2017. In 2018, the resistance percentages for almost all antimicrobials increased again, compared to 2017. The increase in 2014/2015 could reflect the relatively high proportion of strains from imported poultry meat preparations included. It should be noticed that the fluctuating resistance proportions during the years, could be influenced by the varying proportions of retail broiler meat sampled per year originating from Dutch poultry farms.

3.1.2 Campylobacter

This chapter describes the occurrence and trends in antimicrobial resistance in *Campylobacter jejuni* and *C. coli*. Isolates were obtained from food animals, meat and from humans suffering from acute gastroenteritis. Data on human isolates were derived from sixteen regional public health laboratories. As a result of prioritization and changes in legislation, from 2014 onwards the surveillance of antimicrobial resistance in *Campylobacter* focusses mainly on poultry (and poultry meat). In 2018, no additional samples from other animals species were collected.

The MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2018 from caecal samples of broilers are presented in table C01. Table C02 shows resistance percentages of *C. jejuni* and *C. coli* isolated from broilers and poultry meat. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products over the last 14 to 18 years are presented in Figures C01 and C02. National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and from 2008 onwards also in Table C03.

Highlights

1. Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and increased slightly in 2018, compared to 2017.
2. Resistance to macrolides was rarely detected amongst *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat.
3. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
4. Ciprofloxacin resistance in *Campylobacter* isolates from human patients remained at a high level (with a further increase in 2018), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.
5. For *C. jejuni* and *C. coli* from human patients, resistance proportions were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Resistance proportions

EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), implemented in November 2013, includes susceptibility testing of mandatory panels of antimicrobials. Since the start of the monitoring programme of *Campylobacter* spp., six out of twelve antimicrobials (ampicillin, chloramphenicol, clarithromycin, tulathromycin, sulfamethoxazole and neomycin) are no longer included. Most of the remaining antimicrobials in the panel: ciprofloxacin, gentamicin, erythromycin and tetracycline, represent antimicrobial classes, which are used in human medicine for treatment of campylobacteriosis.

Overall, in 2018 resistance proportions were higher in *C. coli* than in *C. jejuni* isolates (Table C01 and C02). Table C02 shows that resistance against gentamicin was not detected in any of the *C. jejuni* and *C. coli* isolates.

In 2018, the highest proportions of resistant *C. jejuni* and *C. coli* from broilers were found for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table C01). Table C02 shows that resistance percentages were high in isolates from both broilers and poultry meat, with the highest resistance percentages for the *C. coli* isolates.

Over the last 10 years, resistance levels of *C. jejuni* from broilers and poultry meat for erythromycin, streptomycin and gentamicin were very low to zero. Surprisingly, in 2018 the resistance percentage for streptomycin was 10.3% in broilers and 8.0% in poultry meat. Resistance to erythromycin was 0.9% in isolates from poultry meat, but was not detected in isolates from broilers. Resistance to tetracycline is increasing since 2012 in both broilers and poultry meat (64.1% in broilers and 59.8% in poultry meat in 2018). Resistance to ciprofloxacin showed fluctuation over the years, with high resistance percentages, and was for the first time over 70% in broilers (Figure C01).

The resistance levels in *C. coli* isolates from broilers and poultry meat showed more fluctuation over years than in *C. jejuni*, which is most probably affected by the lower number of isolates in the survey (Figure C02). Like the years before, resistance in *C. coli* from broilers and poultry meat could not be detected for gentamicin. Resistance in *C. coli* was low for erythromycin and streptomycin in 2016, but showed a sudden increase in 2017 for both broilers and poultry meat. In 2018, the resistance percentages for erythromycin decreased, which was also the fact for the resistance percentage for streptomycin in broilers, but the resistance percentage for streptomycin in poultry meat increased to 24.1%. Resistance percentages for ciprofloxacin in broilers and poultry meat have been fluctuating since 2001, and were still high in 2018. These percentages might not be very representative, because of the low number of *C. coli* isolates tested (N = 62 for broilers and N = 29 for poultry meat). Figure 2 shows that resistance to tetracycline in broilers seems to follow the same trend as ciprofloxacin resistance, at approximately equal percentages; the resistance percentage for tetracycline in poultry meat was almost at the same level in 2018 as in 2017 (increased from 69.4% in 2017 to 72.4% in 2018), whereas the resistance percentage in broilers decreased from 80.0% in 2017 to 69.4% in 2018.

Table C01 MIC distribution (in %) for *Campylobacter jejuni* (N = 156) and *C. coli* (N = 62) isolated from caecal samples of broilers in 2018.

C. jejuni, *broilers*	MIC (%) distribution mg/L												R%	95% CI
(N = 156)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	25.6	3.2	0.6	0.0	0.0	1.3	18.6	37.2	13.5				70.5	62.7 - 77.5
Nalidixic acid				0.0	16.0	14.1	3.2	0.0	0.0	0.6	66.0		66.7	58.7 - 74.0
Erythromycin				66.7	31.4	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0 - 2.3
Gentamicin	56.4	42.9	0.6	0.0	0.0	0.0	0.0	0.0					0.0	0 - 2.3
Streptomycin		3.8	41.7	43.6	0.6	0.0	0.6	0.0	9.6				10.3	6.0 - 16.1
Tetracycline			34.6	1.3	0.0	0.0	0.0	1.9	1.9	6.4	53.8		64.1	56.0 - 71.6

C. coli, *broilers*	MIC (%) distribution mg/L												R%	95% CI
(N = 62)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	14.5	8.1	0.0	0.0	0.0	24.2	25.8	21.0	6.5				77.4	65.0 - 87.1
Nalidixic acid				0.0	0.0	9.7	9.7	3.2	0.0	0.0	77.4		77.4	65.0 - 87.1
Erythromycin				71.0	16.1	8.1	0.0	0.0	0.0	0.0	0.0	4.8	4.8	1.0 - 13.5
Gentamicin	4.8	77.4	17.7	0.0	0.0	0.0	0.0	0.0					0.0	0 - 5.8
Streptomycin		0.0	3.2	71.0	21.0	0.0	0.0	0.0	4.8				4.8	1.0 - 13.5
Tetracycline			21.0	6.5	3.2	0.0	0.0	0.0	0.0	1.6	67.7		69.4	56.4 - 80.4

Table C02 Resistance (%) of C. *jejuni* and *C. coli* isolated from faecal samples of broilers and from poultry meat in 2018.

	C. jejuni		*C. coli*	
	Broilers	Poultry meat	Broilers	Poultry meat
N =	156	112	62	29
Ciprofloxacin	70.5	65.2	77.4	75.9
Nalidixic acid	66.7	61.6	77.4	75.9
Erythromycin	0.0	0.9	4.8	10.3
Gentamicin	0.0	0.0	0.0	0.0
Streptomycin	10.3	8.0	4.8	21.1
Tetracycline	64.1	59.8	69.4	72.4

Fluoroquinolones

The high proportion of *Campylobacter* spp. isolates from animal origin resistant to the fluoroquinolones (Figures Co1 and Co2) and especially from human patients (Figure Co3) is a serious concern for public health. The proportion of *C. jejuni* isolates from broilers resistant to fluoroquinolones was in 2018 for the first time higher than 70% (70.5%), after remaining at a continuous high level during the last decade. The proportion of fluoroquinolone resistance in *C. jejuni* from poultry meat was also high (65.2% in 2018).

Also in the *C. coli* isolates from broilers a continuation of even higher levels of ciprofloxacin resistance were observed (77.4% in 2018), although this was lower than in 2017, when 94.4% of isolates were resistant for ciprofloxacin. The proportion of resistance of *C. coli* isolates from poultry meat strongly fluctuates in time due to the low number of isolates included in the survey. After measuring extremely high resistance proportions to fluoroquinolones in 2017 (94.4% for both ciprofloxacin and nalidixic acid), in 2018 the resistance proportions decreased to 75.9% for both antimicrobials. The resistance levels for fluoroquinolone in human campylobacter isolates were high again (63.6%). As a result figure Co3 shows a continuously increasing trend of ciprofloxacin resistance in *Campylobacter* spp. isolated from human patients.

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and poultry meat in the Netherlands.

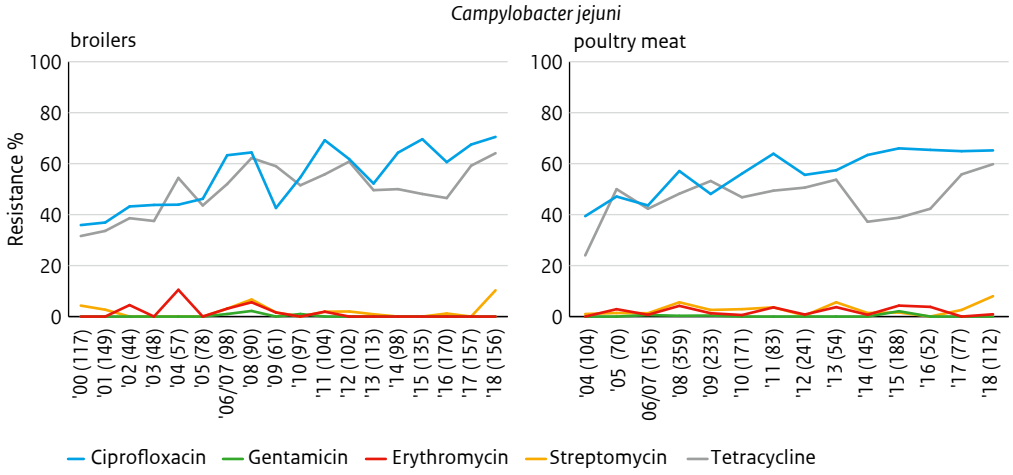


Figure C02 Trends in resistance (%) of *Campylobacter coli* isolated from broilers and poultry meat in the Netherlands.

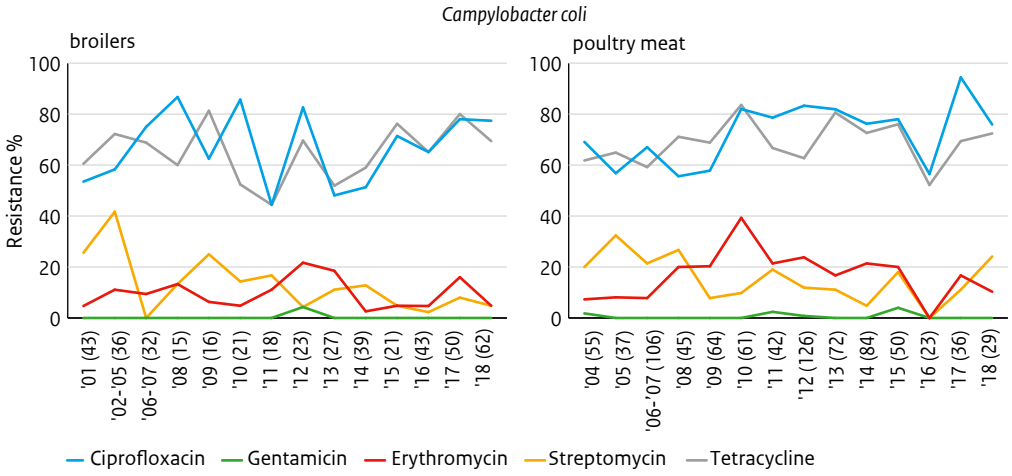


Table C03 Domestically acquired and travel related resistance in C. *jejuni* and *C. coli* isolated from humans from 2008-2018 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2008-2013							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	16603	54.5	1261	55.3	834	66.7	96	61.5
Tetracycline	9132	24.3	780	37.3	178	37.6	27	51.9
Erythromycin	14022	2.3	1044	9.5	633	4.1	74	20.3

	2014-2018							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	12833	59.3	910	65.8	1063	77.4	123	81.3
Tetracycline	9653	43.1	643	64.9	767	58.9	89	67.4
Erythromycin	11320	2.0	767	15.1	967	3.2	114	33.3

	Campylobacter spp. (R%)					
	2018	2017	2016	2015	2014	2008/13
Fluoroquinolone	63.6	62.6	58.3	61.4	60.6	55.3
Tetracycline	50.2	47.6	42.0	42.3	43.9	26.3
Erythromycin	4.0	3.5	2.6	2.9	3.2	3.0

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. The proportion of resistance to macrolides reported in animals and humans in 2018 was low, as in 2017. It could not be detected in *C. jejuni* from caecal samples of broilers in 2018, and only 0.9% of *C. jejuni* isolates from poultry meat were classified as resistant (Table C02). Table C03 shows that 2.0% of human isolates from 2014-2018 was resistant for erythromycin. It should be noted that for human isolates a lower breakpoint for resistance has been applied for erythromycin (≥ 1.5-2.0 mg/L); for animal and meat isolates the EUCAST epidemiological cut-off values were used (> 4 mg/L for *C. jejuni*, and > 8 mg/L for *C. coli*).

Erythromycin resistance percentages were somewhat higher in *C. coli* isolates. Resistance was detected in 4.8% of isolates from broilers and 10.3% of isolates from poultry meat (table C02). 15.1% of human *C. coli* isolates from domestically acquired infections was detected as resistant for erythromycin.

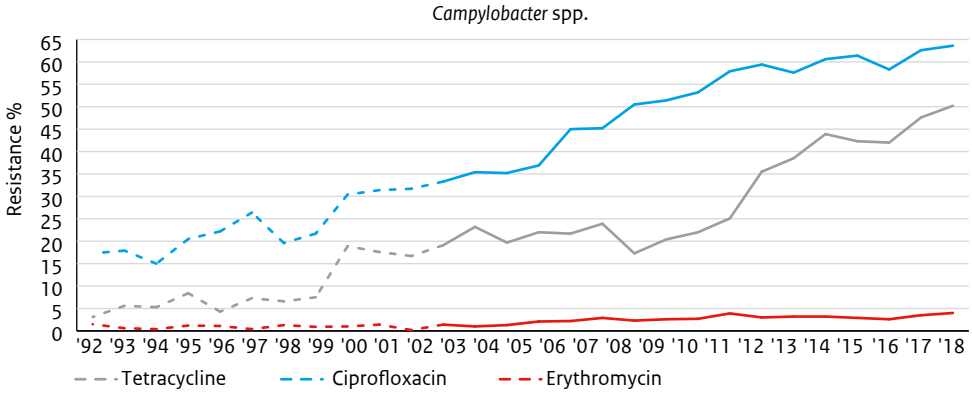
Broiler chickens and poultry meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from broilers as well as from chicken meat samples. In 2018 no samples were collected from laying hens, ducks and turkey meat.

As shown in TableCo2, the proportions of resistance for tetracycline and the quinolones in *C. jejuni* isolates from poultry meat were at the same high level as for the isolates from caecal samples of broilers. The proportion of resistance for the *C. coli* isolates from broilers and poultry meat for tetracycline and quinolones were even a bit higher. Resistance to gentamicin was not observed in both *C. jejuni* and *C. coli* isolates. Resistance to erythromycin was rarely observed in *C. jejuni*, but more frequently found in *C. coli*. Resistance to streptomycin was higher than to erythromycin in *C. jejuni* isolates from broilers, but still at low levels (10.3%). Resistance to streptomycin was higher in *C. coli* isolates from poultry meat (21.1%) than in isolates from broilers (4.8%).

In general, higher resistance rates were observed for almost all antimicrobials in *C. coli* isolates from broilers and poultry meat, compared to *C. jejuni* isolates from the same sources. Overall, Figure Co1 and Figure Co2 show similar trends in resistance proportions of both *C. jejuni* and *C. coli* in broilers and poultry meat.

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2002 at the regional Public Health. Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900-2900.



Campylobacter in humans

Table Co3 and Figure Co3 show data on resistance levels for ciprofloxacin, tetracycline and erythromycin. Figure Co3 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance in *Campylobacter* spp. isolated from human patients, with a slight decrease for tetracycline in 2015 and 2016, and for ciprofloxacin in 2016, but an increase again for both antimicrobials in 2017 and 2018. Resistance to erythromycin seemed to stabilize around 3% since 2011, but increased to 4.0% in 2018.
Table Co3 shows resistance levels for *Campylobacter* spp. isolates, specified whether the infection was acquired domestically or abroad. Resistance levels were higher for all three antimicrobials in travel related infections compared to those domestically acquired for *C. jejuni* isolates. For *C. coli* this was also the fact, but with a smaller difference between travel related and domestically acquired infections. However, these percentages were based on a relatively low number of isolates. The resistance levels in human *Campylobacter* spp. isolates for all three antimicrobials show an increasing trend since 2013.

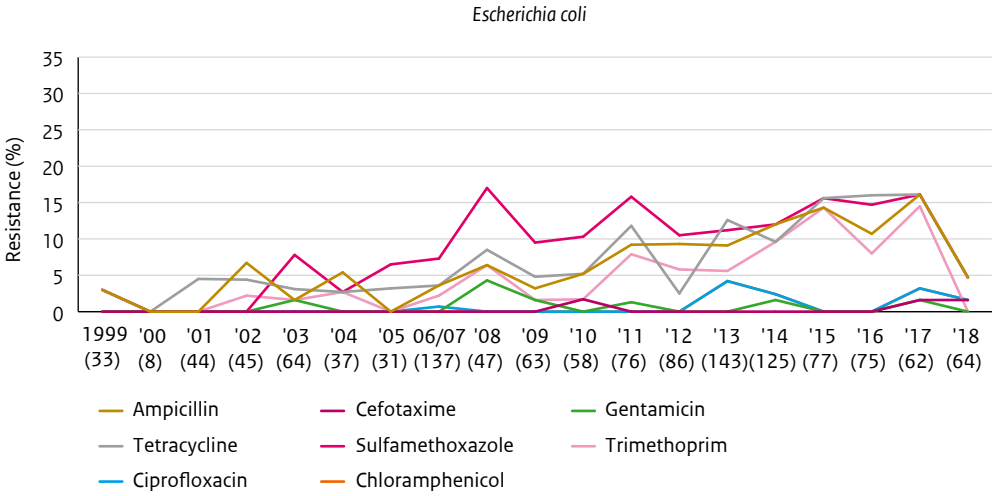
3.1.3 Shiga-toxin producing *E. coli* (STEC)

Highlights

1. Proportions of resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim in human STEC O157 isolates were substantially lower in 2018, compared to 2017 (from 16.1% to 4.7% for ampicilline, from 16.1% to 4.7% for sulfamethoxazole and tetracycline; no resistance for trimethoprim in 2018). The increasing tendency for resistance against these antimicrobials since 2009 did not continue in 2018.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 1.6% of human STEC O157 isolates.
3. Similar to 2017, one ESBL-producing isolate was detected in 2018.

Human isolates (64) of Shiga-toxin producing *E. coli* O157 (STEC O157) isolates were tested for susceptibility. Isolates were obtained from regional public health laboratories within the national laboratory surveillance of STEC. Table STECo1 shows the MIC results for all *E. coli* O157 isolates from humans; Figure STECo1 presents the trends over time.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2018.



Human STEC O157 isolates

Resistance proportions of human isolates showed a substantial decrease for most antibiotics in 2018, compared to 2017. Since approximately 2009, resistance proportions for ampicillin, tetracycline and trimethoprim showed a tendency to increase, whereas resistance against sulfamethoxazole was high since 2008, but fluctuating (Figure STEC01). After a decrease in 2016 for ampicillin, sulfamethoxazole and trimethoprim, levels of resistance increased in 2017 (from 10.7% to 16.1% for ampicillin, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim), but decreased in 2018 to levels lower than in 2016 (to 4.7% for ampicillin, to 4.7% for sulfamethoxazole, and no resistance to trimethoprim was detected). The resistance level for tetracycline decreased to 4.7% in 2018. Resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016, was 3.2% for both antimicrobials in 2017, and 1.6% (one positive isolate) in 2018. As in 2017, one cefotaxime resistant, ESBL-producing isolate was detected harbouring a $bla_{\text{CTX-M-15}}$ gene.

Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=64) isolated from humans the Netherlands in 2018.

"E. coli N = 64"	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	95% CI
Ampicillin							0.0	12.5	81.3	1.6	0.0	0.0	0.0	4.7					4.7	1.0 - 13.1
Cefotaxime					98.4	0.0	0.0	0.0	0.0	1.6									1.6	0 - 8.4
Ceftazidime						98.4	0.0	0.0	0.0	1.6									1.6	0 - 8.4
Gentamicin						71.9	23.4	4.7	0.0	0.0	0.0	0.0							0.0	0 - 5.6
Tetracycline								89.1	6.3	0.0	0.0	0.0	0.0	4.7					4.7	1.0 - 13.1
Sulfamethoxazole										95.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	4.7	1.0 - 13.1
Trimethoprim					96.8	3.2	0.0	0.0	0.0	0.0	0.0	0.0							0.0	0 - 5.6
Ciprofloxacin	67.2	31.3	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0									1.6	0 - 8.4
Nalidixic acid									96.9	1.6	0.0	0.0	0.0	0.0	1.6				1.6	0 - 8.4
Chloramphenicol										78.1	20.3	0.0	0.0	0.0	1.6				1.6	0 - 8.4
Azithromycin								23.4	73.4	3.1	0.0	0.0	0.0						0.0	0 - 5.6
Colistine							98.4	1.6	0.0	0.0	0.0								0.0	0 - 5.6
Meropenem		100.0																	0.0	0 - 5.6
Tigecycline					96.9	3.1	0.0	0.0	0.0	0.0									0.0	0 - 5.6

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.

3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals and meat products. 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 flora in the surveillance, no enterococci are reported since 2017.

EFSA[1] prescribes the sampling strategy and isolation methodology of bacteria from caeca of randomly picked 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 poultry by collecting samples from caeca of individual veal calves at slaughterhouses, and resistance levels were reported separately for white and rosé veal calves.

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 per cent resistance in e.g. *E. coli* indicates that in all animals of that animal species 1% of the *E. coli* bacteria are resistant. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in individual animals.

[1] Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. http://www.efsa.europa.eu/en/efsajournal/pub/141r.htm.

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 in the Netherlands.
EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in 2014. This includes susceptibility testing by broth microdilution according to ISO 20776-1:2006 with mandatory panels of antimicrobials. Results are interpreted with epidemiological cut-off values (ECOFF's) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In this report non-wild type susceptible isolates are classified as resistant. These isolates all harbour an acquired resistance mechanism, but may for some antibiotics not be clinically resistant.

Highlights 2018

1. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat.
2. Resistance levels in indicator *E. coli* from caecal samples showed a tendency to stabilise (or increase for ampicillin) in broilers and veal calves and to slightly decrease in pigs. In dairy cattle the resistance proportions remained at a constant low level.
3. Resistance proportions in *E. coli* from turkey meat were substantially higher than in chicken meat.
4. The proportion of *E. coli* isolates resistant to extended spectrum cephalosporins was very low in faecal samples from broilers, pigs, dairy cattle and veal calves.
5. Resistance to fluoroquinolones was at the same level as in 2017, and was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
6. For almost all antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.

Resistance levels

Table Eco01 shows resistance levels, presented as MIC-distributions, of 1198 *E. coli* isolates obtained from caecal samples from broilers, pigs, veal calves and faecal samples of dairy cows. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1998 to 2018 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02. Table Eco03 presents resistance percentages of 286 *E. coli* isolates collected from raw chicken, turkey meat and vegetables. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2018 isolated from raw meat products of chicken and turkey.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1198) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2018.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1198	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							1.8	23.3	47.0	4.3			0.3	23.5					23.7	21.3 - 26.2
Cefotaxime					99.4		0.1	0.1		0.4									0.6	0.2 - 1.2
Ceftazidime						99.4		0.1	0.2		0.3								0.6	0.2 - 1.2
Gentamicin						42.6	49.9	5.2	0.5	0.1	0.7	0.5	0.6						2.3	1.6 - 3.4
Tetracycline								59.9	12.8	0.2	0.1	0.3	8.0	18.7					27.1	24.6 - 29.7
Sulfamethoxazole										76.5	0.3	0.1			0.1	0.1		23.0	23.1	20.8 - 25.6
Trimethoprim					33.9	43.7	3.8		0.2				18.4						18.6	16.5 - 20.9
Ciprofloxacin	76.0	14.5	0.2	0.8	5.3	1.8	0.3	0.2	0.1	0.4	0.4								9.3	7.7 - 11.1
Nalidixic acid									90.0	1.3	0.3	0.0	0.7	4.0	3.8				8.4	6.9 - 10.2
Chloramphenicol										82.2	8.9	0.8	0.9	3.1	4.0				8.8	7.3 - 10.6
Azithromycin*								3.6	47.8	44.7	3.4	0.1	0.3	0.2					0.5	0.2 - 1.1
Colistin							99.0	1.0	0.0										0.0	0.0 - 0.3
Meropenem		99.7	0.3																0.0	0.0 - 0.3
Tigecycline					83.8	16.2													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*

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 2018.

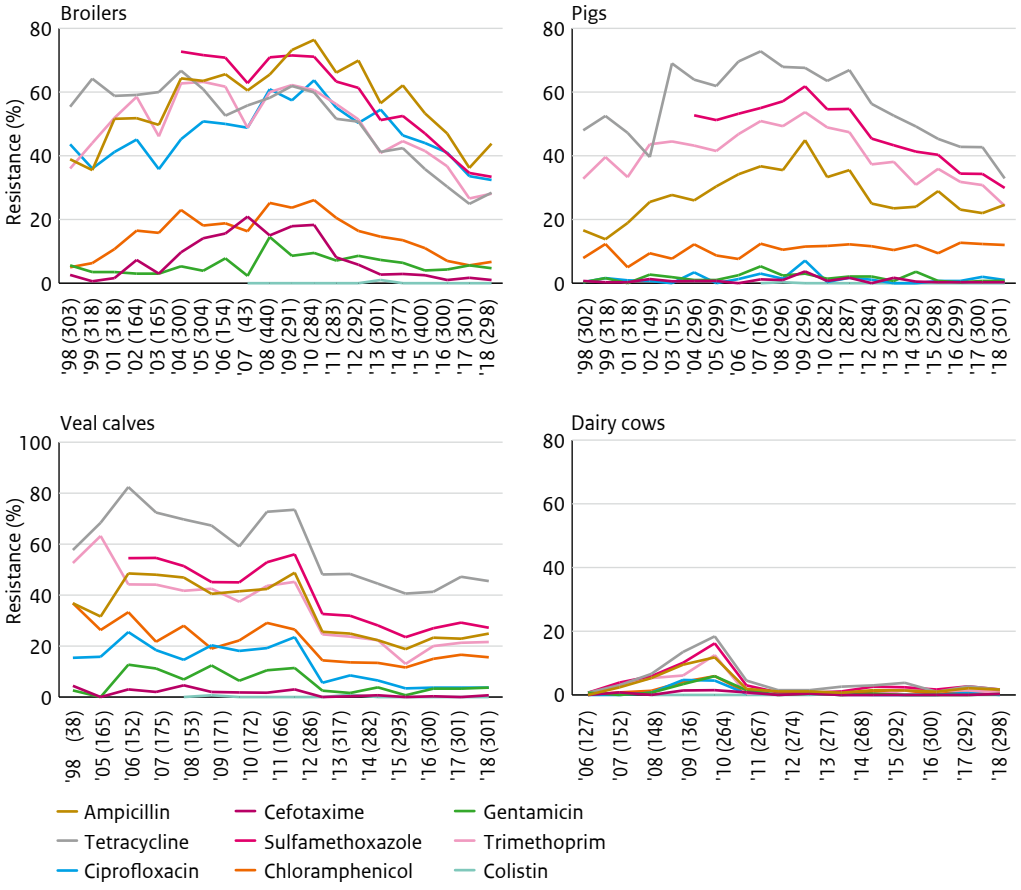
Faecal samples	Broilers	Pigs	Dairy	Veal calves	
	N = 299	N = 301	N = 298	White, N = 209	Rosé, N = 91
Ampicillin	43.8	24.6	1.7	31.9	8.8
Cefotaxime	1.0	0.3	0.3	0.5	1.1
Ceftazidime	1.0	0.3	0.3	0.5	1.1
Gentamicin	4.7	0.7	0.3	4.8	1.1
Tetracycline	28.4	32.9	1.7	58.1	16.5
Sulfamethoxazole	33.4	29.9	1.7	34.8	9.9
Trimethoprim	28.1	24.3	0.7	28.6	5.5
Ciprofloxacin	32.4	1.0	0.0	5.2	0.0
Nalidixic acid	30.4	0.7	0.0	3.8	0.0
Chloramphenicol	6.7	12.0	1.0	20.0	5.5
Azithromycin	0.3	0.7	0.0	1.4	0.0
Colistin	0.0	0.0	0.0	0.5	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0

For most drugs or drug classes, resistance levels varied substantially between the different animal species (Table Eco02). Highest resistance levels were found in broilers, slaughter pigs and white veal calves, lower levels in rosé veal calves, and hardly any resistance was observed in isolates from dairy cattle. This pattern was also observed in previous years. Overall, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used classes in veterinary medicine in The Netherlands.

Fluoroquinolones

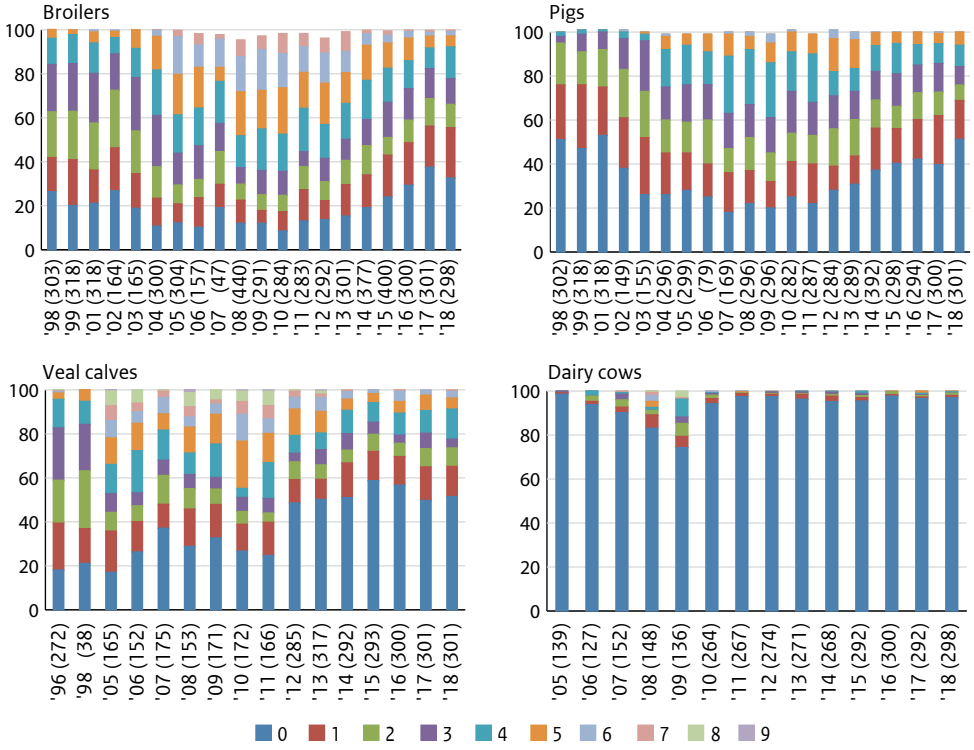
Highest resistance levels for fluoroquinolones were found in *E. coli* from broilers: 32.4% resistance to ciprofloxacin and 30.4% resistance to nalidixic acid in isolates from Dutch broilers. This was approximately at the same level as in 2017 (with a decreasing trend for both drugs since 2013). In 2018, high level resistance (MIC >1 mg/L) to ciprofloxacin in broilers was detected in 3.7% (11/299) of the isolates. Resistance to ciprofloxacin in 2018 was 5.2% in *E. coli* isolates from white veal calves, 1.0% in pigs, and could not be detected in isolates from dairy cows and rosé veal calves.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from faecal samples of broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2018.



Resistance to fluoroquinolones in *E. coli* from meat was tested for chicken and turkey meat samples and vegetable samples from retail in The Netherlands (Table Eco03). No samples from meat imported from outside the EU were analysed for indicator *E. coli* in 2018. Figure Eco03 shows that resistance in chicken products at retail was approximately at the same level as in 2017: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 27.4% (26.9% in 2017) and 25.0% (23.1 % in 2017), respectively. Resistance percentages in isolates from turkey products were substantially higher than in 2017 (increased from 30.6% to 42.1% for ciprofloxacin and from 25.0% to 36.8% for nalidixic acid). However, these results should be interpreted carefully because of the low number of samples analysed over the years. Resistance percentages in isolates from vegetables were very low: only 1.1% of isolates (1 of 92 isolates) showed resistance to ciprofloxacin and nalidixic acid. The resistance percentages of *E. coli* from meat were somewhat higher for ciprofloxacin than for nalidixic acid. This is due to the increase of plasmid mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid.

Figure Eco02 Proportions of isolates resistant (%) to 0 - 9 antimicrobial classes among *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2018.



Cefotaxime

Resistance levels to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL/pAmpC producing *E. coli*, were very low in all tested animal species: broilers, pigs, dairy cows and veal calves. Proportions of resistant *E. coli* were 1.0% in broilers, 0.3% in pigs and dairy cows, 0.5 % in white veal calves and 1.1% in rosé calves for both cefotaxime and ceftazidime. These resistance percentages were comparable to the percentages in 2017. For broilers, this indicates a stabilised low level after a decreasing trend from 2013 to 2016 (Figure Eco01).

Resistance to cefotaxime in randomly isolated commensal *E. coli* obtained from turkey meat samples from retail could not be detected (Table Eco03). From chicken meat samples, cefotaxime resistance was detected in 1.1% of the *E. coli* isolates, which was a bit higher than in 2017, when no cefotaxime resistance was detected in chicken meat samples. Comparison with resistance levels of 2016 and earlier cannot be done, because then also retail samples from imported poultry meat (from outside EU) were included.

The small proportion of cefotaxime resistant *E. coli* from chicken meat samples, in randomly isolated strains cultured on non-selective media, suggests that the concentration of *E. coli* resistant to Extended Spectrum Cephalosporins (ESC) on meat is low. The proportion of fresh chicken meat samples in which ESC-resistant *E. coli* were found using selective media sharply decreased from 31.4% in 2017 to 13.7% in 2018 to (see chapter 4). One has to consider the fact that part of the retail meat included in the sampling originates from EU countries outside the Netherlands where resistance prevalences might be higher.

Importantly, the prevalence of broilers carrying ESC-resistant *E. coli* further decreased from 50.3% of the animals sampled in 2016 and 32.6% in 2017 to 23.0 % in 2018 (see chapter 4). The decrease in prevalence and concentrations of ESC-resistant *E. coli* in broilers and on poultry meat is an important finding because it suggests that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is also decreasing. In contrast, in veal calves a third year with relatively high prevalence of animals positive for ESC-resistant *E. coli* in their GI-tract was found. In white veal calves the prevalence increased from 40.5 % in 2017 to 47.6 % of the animals sampled (compared to 26.9% in rosé). The prevalence in 2018 of animals positive for ESC-resistance in pigs and dairy cattle were very similar to 2017 with 11.0 and 10.6% respectively.

Broiler chickens

In 2018, commensal *E. coli* isolated from caecal samples from broiler chickens showed resistance in relatively high proportions for most antimicrobials tested and proportion of resistance increased for some antimicrobial classes. (Table Eco02). Resistance proportions showed a tendency to increase for ampicillin (from 36.2 % in 2017 to 43.8% in 2018), for tetracycline (from 24.9% to 28.4%) and for trimethoprim (from 26.6% to 28.1%). Resistance proportions for sulfamethoxazole (33.4%) and ciprofloxacin (32.4%) were also high, but slightly lower than in 2017. Cefotaxime resistance was low in 2018 (1.0%), which was comparable to 2017 (1.7%).

Slaughter pigs

Resistance proportions for tetracycline, sulfamethoxazole and trimethoprim in *E. coli* isolates from pigs, sampled in 2018, were substantially lower than in 2017 (tetracycline from 42.7% in 2017 to 32.9% in 2018, sulfamethoxazole from 34.3% to 29.9% and trimethoprim from 30.8% to 24.3%). The resistance percentage for ampicillin increased slightly from 22.0% in 2017 to 24.6% in 2018. The proportion of isolates resistant to these four antibiotics shows a decreasing tendency since 2011, which stabilized from 2015 onwards (Figure Eco01). Resistance to the 3rd generation cephalosporins was very low since 2014 (0.3% in 2018).

Veal calves

Resistance data on white and rosé veal calves are reported separately, because of the difference in production systems. White veal calves are fattened on a milk diet with a required minimal uptake of roughage, while rosé veal calves are also fed corn silage, straw or pelleted feed. Most antibiotics are administered during the starting period in both production systems. On average, in white veal calves more antibiotics are used than in rosé calves and rosé calves are slaughtered at an older age, which results in a longer time period with relatively low antibiotic exposure. This results in a difference in resistance levels at slaughter between the two husbandry types. 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 Eco01 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.
In 2018, highest resistance levels in veal calves were against tetracycline (58.1% and 16.5% for white and rosé respectively), sulfamethoxazole (34.5% and 9.9%), trimethoprim (28.6% and 5.5%) and chloramphenicol (20.0% and 5.5%).
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 and 2018, which better reflected the proportions of slaughtered white and rosé calves in The Netherlands. This explains part, but not all of the apparent increase in resistant rates of *E. coli* in veal calves in 2016 and 2017 compared to 2015. *E. coli* isolates resistant to 3rd generation cephalosporins were detected at low rates in 2018 with 0.5% in white veal calves and 1.1 % in rosé veal calves (TableEco02).

Dairy cattle

Resistance in *E. coli* isolated from dairy cattle was, as always, very low compared to resistance proportions observed in pigs, broilers and veal calves (Table Eco02), reflecting the low use of antibiotics in this husbandry system. Resistance proportions were comparable to previous years. The overall resistance rates were not higher than 1.7%.

Multidrug resistance

Due to the implementation of new antimicrobial susceptibility testing panels for *E. coli*, the data to determine multidrug resistance have been adjusted backwards starting from 2014 and is based on resistance against the following antimicrobial classes: aminopenicillins (ampicillin), 3rd gen. cephalosporins (cefotaxime), carbapenems (meropenem), aminoglycosides (gentamicin), tetracyclines (tetracycline), sulfonamides (sulfamethoxazole), 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.
The proportion of multidrug resistant isolates (resistant to three or more classes of antibiotics) was in 2018 comparable to 2017. In broilers, the proportion of multidrug resistance isolates was 34.1%, which was higher than in 2017 (31.4%), but lower than in 2016 (41.0%). The proportion of multidrug resistance was at relatively high levels in pigs (24.1% in 2018, 27.3% in 2017) and veal calves (26.4% in 2018, 26.7% in 2017). In dairy cattle multidrug resistance in *E. coli* was again rarely detected with 1.0% of the isolates showing resistance to three or more classes of antimicrobials.
The percentage of completely susceptible *E. coli* isolates increased for pig, calf and dairy isolates, but decreased slightly for broilers, after an increasing trend from 2010 to 2017 (Figure Eco02).

Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw chicken meat, turkey meat and vegetables at retail in the Netherlands in 2018.

Meat products	Chicken N = 175	Turkey N = 19	Vegetables N = 92
Ampicillin	39.4	68.4	5.4
Cefotaxime	1.1	0.0	0.0
Ceftazidime	1.1	0.0	0.0
Gentamicin	2.3	5.3	0.0
Tetracycline	26.3	78.9	5.4
Sulfamethoxazole	32.6	42.1	4.3
Trimethoprim	27.4	42.1	4.3
Ciprofloxacin	27.4	42.1	1.1
Nalidixic acid	25.1	36.8	1.1
Chloramphenicol	2.9	21.1	1.1
Azithromycin	0.0	15.8	1.1
Colistin	1.1	10.5	1.1
Meropenem	0.0	0.0	0.0
Tigecycline	0.0	5.3	0.0

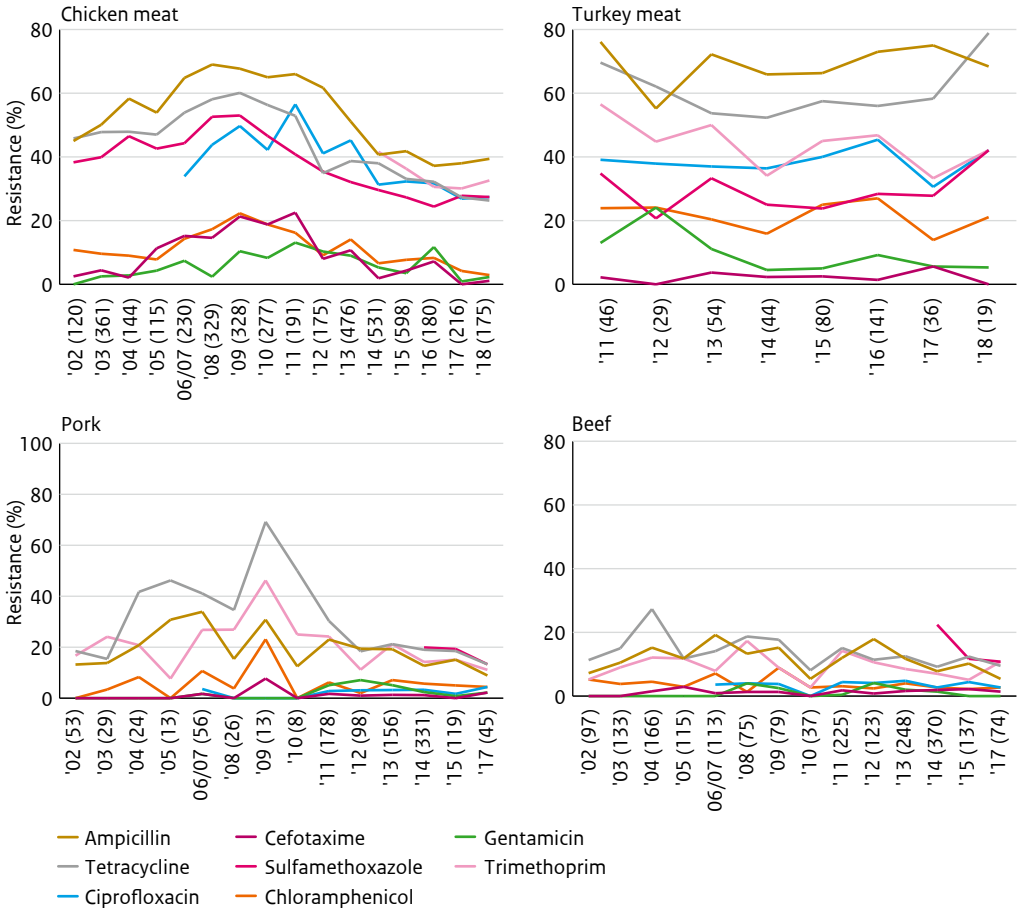
E. coli in raw-meat and vegetables

Table Eco03 presents resistance percentages of *E. coli* isolated from raw chicken and turkey meat and vegetables, sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail can include meat produced in The Netherlands, but also other EU countries. Meat products imported from outside the EU were not analysed for indicator *E. coli* in 2018. All vegetables were sampled as fresh products at retail and originated from within EU.

Fig Eco03 shows the trends in resistance in the meat samples. Resistance percentages in chicken meat show a tendency to decrease from 2010 onward, and seems to stabilise with some fluctuations since 2015. In turkey meat, resistance rates have been at a constant high level since 2011, with a decrease in 2017 for sulfamethoxazole, ciprofloxacin and chloramphenicol, and an increase in 2018 for almost all measured antibiotics. However, considering the low number of turkey meat samples analysed in 2018 and in previous years, results must be interpreted with care. Cefotaxime resistance could not be detected in *E. coli* isolates from turkey meat in 2018, and was at a very low level in chicken meat samples. Fluctuations in resistance rates of meat samples might be caused by a year-to-year variation in the proportion of retail poultry meat produced outside of the Netherlands included in the survey.

In vegetables, resistance levels of *E. coli* isolates were low to very low. No resistance was detected to cefotaxime and ceftazidime. The percentage of isolates resistant to ampicillin was 5.4%, which was equal to the resistance percentage to tetracycline. For sulfamethoxazole and trimethoprim the resistance percentage was 4.3% for both drugs. This was the first time that vegetable samples were tested, so trends in results could not be determined.

Figure Eco03 Trends in proportion of resistance (%) of *E. coli* isolated from raw chicken meat and turkey meat in the Netherlands from 1998-2018.



4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2018

Highlights

1. The proportion of randomly isolated *E. coli* from faecal samples of food-producing livestock in the Netherlands carrying ESBL/AmpC genes continued to decline since 2010 to a current proportion below 1%.
2. Selective culturing of ESBL/AmpC producing *E. coli* from broilers showed an ongoing decrease in the prevalence from 66% in 2014 to 23% in 2018.
3. After a peak in the prevalence of selectively cultured ESBL/AmpC producing *E. coli* from rosé veal calves in 2016, little fluctuation was seen since then.
4. Since the peak in prevalence of selectively cultured ESBL/AmpC producing *E. coli* from white veal calves in 2016, a steady increase is still ongoing to a current prevalence of 47.6%.
5. The proportion of Dutch retail meat positive for ESBL/AmpC producing *E. coli* (by selective culturing) has further decreased to 2.8% in 2018. In chicken meat at retail the prevalence decreased to 13.7%.
6. No ESBL/AmpC producing *Salmonella* could be detected from Dutch retail meat.
7. The proportion of ESBL/AmpC producing *Salmonella* from human *Salmonella* isolates has further decreased to 0.8%, which is related to a decrease in ESBL/AmpC producing S. Kentucky.
8. No carbapenemase-producing *Enterobacteriaceae* were detected in livestock.
9. Carbapenemase producing *E. coli* isolates were detected in two dogs carrying $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-181}}$ respectively.
10. In 2018, *mcr-1* was identified at low-level in *E. coli* from different livestock species. A significant higher prevalence of *mcr-1* was detected in German broilers (24.4%) compared to Dutch broilers (0.3%). In veal calves, *mcr-4* was found in four faecal samples.
11. No mcr genes were detected in *Salmonella*.

4.1 ESBL/AmpC-producing bacteria

4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2018

As prescribed by EFSA guidelines[1], surveillance of random, non-selectively isolated *E. coli* from faecal samples of food producing livestock animals is performed for resistance against extended-spectrum cephalosporins (ESC). Caecal samples were taken at slaughter for broilers, veal calves and slaughter pigs while faecal samples were taken at farms for dairy cows. The threshold of a minimum of 170 isolates per animal species was met, where veal calves and dairy cows are considered separate. Based on the epidemiological cut-off values described by EUCAST, see also Chapter 3, when a reduced susceptibility phenotype against cefotaxime or ceftazidime was determined, isolates were considered to be ESBL/AmpC suspected. The percentages of cefotaxime resistant isolates from randomly isolated *E. coli* are shown in Figure ESBL01. A trend over time can be observed in broilers, where the percentage of resistant isolates among randomly isolated *E. coli* increased for several years since 2003 but has been decreasing since 2011. This rapid decrease is associated with the strong decrease in the use of antibiotics since 2010, and specifically, the (off-label) use of ceftiofur at hatcheries. For slaughter pigs, dairy cows and veal calves, the percentage has never been above 5% since the monitoring programme has started (1998), and for all animal species the percentage has been at or below 1% over the past 5 years.

Table ESBL01 shows the quantitative results for all 1198 random isolates that were tested from faecal samples in 2018. Seven isolates displayed reduced susceptibility to cefotaxime (MIC > 0.25 mg/L, see also 3.2.1). Three isolates from broilers, two isolates from veal calves, 1 isolate from slaughter pigs and 1 isolate from dairy cows were suspected of producing an ESBL or AmpC gene product. All ESBL suspected isolates were analysed for the presence of ESBL/AmpC genes using an in-house developed RT-PCR (Geurts *et al.* 2017) or the Check-Points CT101 micro-array (Check-Points, Wageningen, the Netherlands). All detected genes were confirmed by PCR amplification and sequencing. Any negative confirmed but resistant isolates were examined for mutations in the chromosomal AmpC promoters that could lead to resistance through overexpression of this gene. Results of this molecular typing are included in Table ESBL01. The reduction in prevalence leads to a smaller diversity in the ESBL/AmpC genes that were detected, compared to previous years. All three isolates from broilers were shown to encode for bla_{SHV-12}. The two isolates from veal calves encode the CTX-M-1 group genes $bla_{CTX-M-1}$ (n=1) and $bla_{CTX-M-15}$ (n=1). Two chromosomal AmpC promoter mutations were confirmed in the two single isolates from slaughter pigs and dairy cows. The plasmid mediated AmpC gene bla_{CMY-2} was not detected for the second year in random *E. coli* isolates. The total proportion of ESBL/AmpC producing isolates was 0.6%, resulting in a proportion below 1% for four years in a row and re-confirming the decline of ESBL/AmpC in random *E. coli* isolates from livestock.

[1] European Food Safety Authority; Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA Journal 2012; 10(6):2742.

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows.

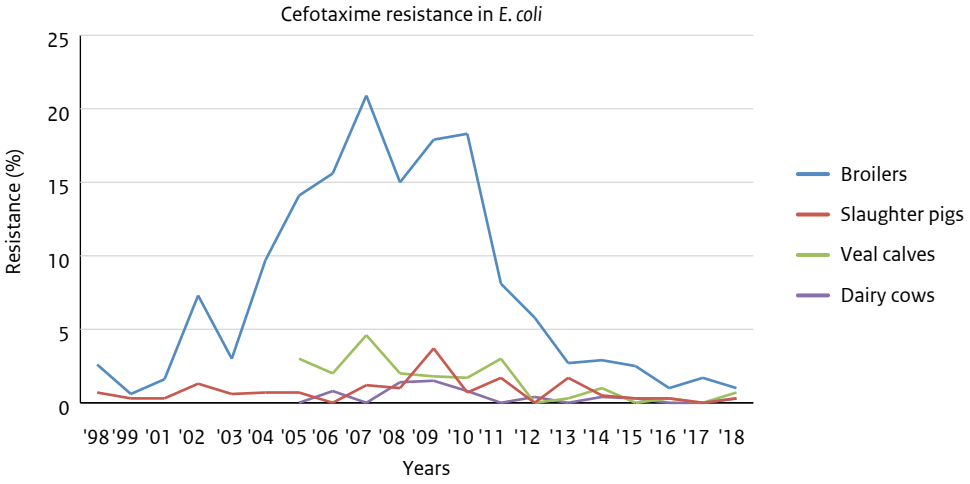


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2018.

	ESBLs isolated from						ESBL-genes detected											
Year	cBroilers	Veal calves	Slaughter pigs	dDairy cows	Turkeys	Total ESBL suspected (n)	aCTX-M-1-group	CTX-M-2	CTX-M-9-group	TEM-52c	TEM-20	bSHV-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	1026	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	1002	5.9
2011	23	5	5	0	6	39	9			8		9	2	3	3	5	1096	3.6
2012	26	2	0	1	n.t.	29	8			4		8		5		4	1328	2.2
2013	13	1	4	0	n.t.	18	7			4		3		3	1		1371	1.3
2014	11	3	2	0	n.t.	16	8			1		4			1	2	1519	1.1
2015	10	0	1	1	n.t.	12	3		2	1		1		2	3		1283	0.9
2016	3	1	1	0	n.t.	5	2			1				1	1		1492	0.3
2017	5	0	0	0	n.t.	5	2			1			2				1194	0.4
2018	3	2	0	0	n.t.	7	2					3			2		1198	0.6
Total	274	29	31	8	6	350	137	19	3	39	2	45	11	44	22	28		

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 Selective isolation of ESBLs in 2018

In parallel with the isolation of random, non-selective *E. coli*, selective isolation of ESBL/AmpC-producers is performed from caeca for broilers, veal calves and slaughter pigs or from faecal samples taken at farms for dairy cows. This screening is performed according to the EURL-AR protocols, briefly described here: 1 gram of faecal material is mixed in 9 ml of Buffered Peptone Water (BPW) and incubated overnight at 37 °C, followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime. Selective isolation of ESBL/AmpC producing *E. coli* from meat samples is performed by mixing 25 gram of meat with 225 ml of BPW and incubating overnight at 37 °C, followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime and on Brilliance ESBL Agar (Oxoid, part of ThermoFisher Scientific). From each plate, single colonies with typical *E. coli* morphology were selected for bacterial species identification using the MALDI-TOF (Bruker Biotyper). Confirmed *E. coli* isolates were screened for the identification of ESBL/AmpC as described for the random *E. coli*.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in faeces

A total of 1201 faecal samples were screened in 2018 for the presence of ESBL/AmpC producing *E. coli*, each representing a single slaughter batch (broilers, slaughter pigs and veal calves) or farm (dairy cattle). Colonies of confirmed *E. coli* that were isolated from MacConkey agar containing 1 mg/L cefotaxime are reported as ESBL suspected isolates, including those isolates that contain chromosomal *ampC* gene promoter mutations that lead to resistance through overexpression of this gene. Figure ESBL02 displays the prevalence of ESBL/AmpC producing *E. coli* over time (2014-2018). For broilers, a decreasing trend can be observed over time for the proportion of positive samples, with a prevalence that started in 2014 at 66.0%, which decreased to 23.0% in 2018. In slaughter pigs, the prevalence varied somewhat through time between 11.0% to 16.3% and was 11.0% again in 2018. In dairy cows, the prevalence had a slow rise from 6.0% to 13.2% between 2014-2016 but has since stabilised and was 10.6% in 2018. Since 2016, a rise in the prevalence in both white and rosé veal calves was noticed from 17.3% and 10.0% in 2015 to 33.9% and 28.7% in 2016, respectively. Since then, the prevalence in rosé veal calves has somewhat stabilised and was 26.9% in 2018. For the white veal calves, the prevalence has continued to rise further and was 47.6% in 2018. While the reduction in the prevalence of ESBL/AmpC producing *E. coli* in broilers follows the trend previously seen in the randomly isolated *E. coli* that is correlated with a reduction in the use of antimicrobials, currently no explanation is available yet for the rising prevalence in veal calves.

Figure ESBL02 Trends in prevalence of ESBL/AmpC-producing *E. coli* in faecal samples of broilers, pigs, white and rosé veal calves and dairy cows from 2014-2018 determined by using selective isolation.

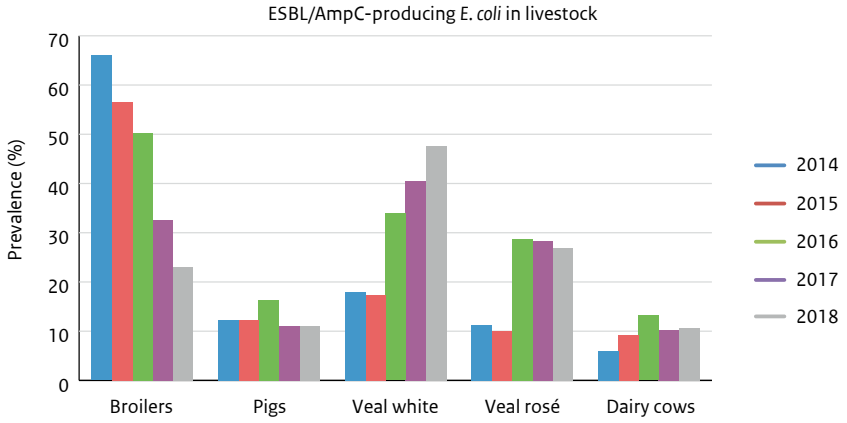


Table ESBL02 Prevalence of *E. coli* isolates showing reduced susceptibilty to cefotaxime derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves and dairy cows taken at slaughter in 2018.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence (%) ESBL confirmed
Broilers	296	68	68	23.0
Pigs	301	56	33	11.0
Veal calves				
white	210	104	100	47.6
rosé	93	27	25	26.9
Dairy cows	301	39	32	10.6
Total	1201	294	258	21.5

A complete overview of the molecular characterisation of ESBL and AmpC gene variants from faecal samples from livestock is presented in Table ESBL03. As shown in previous years (MARAN 2014-2018), a great variation of gene variants occur in Dutch livestock, some of which are spread evenly among animal species while others appear to have host-specific preferences, possibly linked to the plasmids on which the genes are encoded (Ceccarelli *et al.* 2019). As previously reported, $bla_{CTX\text{-}M\text{-}1}$ is the most dominant ESBL among all animal species that were monitored (100 out of 294 isolates), followed by $bla_{CTX\text{-}M\text{-}15}$ (n=62) $bla_{TEM\text{-}52c}$ (n=18), $bla_{SHV\text{-}12}$ (n=17) and $bla_{CMY\text{-}2}$ (n=17). While $bla_{CTX\text{-}M\text{-}15}$ was formerly mostly associated with human ESBL carriers (Dorade-Garcia *et al.* 2018) the relative prevalence compared to other ESBL gene variants appears to be rising, specifically in *E. coli* isolated from white veal calves. Chromosomal *ampC* promoter mutations that confer cefotaxime resistance were present in all animals species except broilers, with somewhat increasing relative prevalences of 41.1% in slaughter pigs and 17.9% in dairy cows.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in raw meat

The prevalence of ESBL/AmpC producing *E. coli* in raw meat were determined via selective culturing as described above and results are shown in Table ESBL04. When comparing the results from the years between 2013 until 2018 the prevalence of ESBL/AmpC producing *E. coli* has steadily and significantly declined, from 23% overall prevalence in 2013 to 2.8% in 2018. Partially, this is due to the inclusion of results from several new types of meat samples in which ESBL/AmpC prevalence is low, such as sheep and goat since 2016 and frog, crocodile and fish and shrimps since 2017, although sample numbers for several of these sources are low. While the prevalence for ESBL/AmpC producing *E. coli* always fluctuates over time, some remarkable reductions in the detection of ESBL/AmpC producing *E. coli* were seen in 2018. In veal, the prevalence went down from 7.5% in 2017 to 3.4% in 2018. In pork, the prevalence went down from 1.5% in 2017 to 0% in 2018. In fresh chicken meat from the Netherlands/EU the prevalence went down from 31.6% in 2017 to 13.7% in 2018 while meat imported from outside the EU went down from 56.1% in 2017 to 1.3% in 2018. Fresh turkey meat went down from 15.8% in 2017 to 9.5% in 2018. Finally, the prevalence in fish and shrimps went down from 12.5% in 2017 to 2.6% in 2018.

Using molecular typing methods the ESBL/AmpC encoded genes in the resistant isolates of a total of 65 isolates was determined, see Table ESBL05. For veal and beef, the most prevalent ESBL genes that were detected were $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}55}$, all of which are also regularly found in the faecal samples of veal calves and dairy cows, indicating potential contamination of the meat from faecal material during the slaughter and butchering processes. These genes were all also regularly found on beef or veal in previous years.

As seen in previous years, chicken meat showed the highest prevalence and variability of ESBL/AmpC genes that were encoded in resistant *E. coli*. $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}52cVar}$ and $bla_{CMY\text{-}2}$ were most prevalent on the chicken meat. As for beef and veal, these are also the most prevalent genes that were found in chicken faecal samples, indicating potential contamination of the meat from faecal material.

Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves, and dairy cows in 2018. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Slaughter pigs	Veal calves White	Veal calves Rose	Dairy cows	Total
CTX-M-1 group							
	CTX-M-1	28	15	40	7	10	100
	CTX-M-3	1				2	3
	CTX-M-15	1		35	12	14	62
	CTX-M-32	2		2	3		7
	CTX-M-55			4		1	5
	CTX-M-222	1					1
CTX-M-2 group							
	CTX-M-2		1				1
"CTX-M-9 group"							
	CTX-M-9		1	2		3	6
	CTX-M-14			5	3	1	9
	CTX-M-14b			1			1
	CTX-M-65			4			4
TEM							
	TEM-52c	6	7	5			18
	TEM-52cVar	3	2				5
SHV							
	SHV-2a	1					1
	SHV-12	15	2				17
CMY							
	CMY-2	9	5	2		1	17
Chromosomal ampC							
	ampC-type-3		23	2	2	6	33
	ampC-type-9					1	1
	ampC-type-18			2			2
	n.t	1					
Total		**68**	**56**	**104**	**27**	**39**	**294**

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat products in the Netherlands in 2018.

Animal source		N screened	N ESBL/AmpC positive (confirmed)	% ESBL/AmpC positive
Beef				
	fresh meat	626	8	1.3
Veal				
	fresh meat	266	9	3.4
Pork				
	fresh meat	314	0	0.0
Chicken				
	fresh meat [a]	291	40	13.7
	import [b]	75	1	1.3
Turkey				
	fresh meat [c]	21	2	9.5
	import [d]	-	-	
Lamb				
	fresh meat	280	1	0.4
Sheep				
	fresh meat	1	0	0.0
Goat				
	fresh meat	4	0	0.0
Fish and shrimps				
	fresh meat	304	8	2.6
Crocodile				
	fresh meat	6	1	16.7
Frog				
	fresh meat	4	0	0.0
Total		**2192**	**62**	**2.8**

a. Fresh broiler retail meat originates from animals produced within EU (mainly, but not exclusively from the Netherlands)

b. Imported frozen meat preparations originates from countries outside EU (mainly from South America or Asia)

c. Fresh turkey retail meat originates from animals produced within EU (but often not from the Netherlands)

d. Imported frozen turkey meat preparations originates from countries outside EU (mainly from South America or Asia)

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2018.

ESBL gene	Chicken	Turkey	Beef	Veal	Lamb	Crocodile	Fish and shrimps	Total
CTX-M-1 group								
CTX-M-1	11	2	4	2				19
CTX-M-15	1			2				3
CTX-M-55				3			2	5
CTX-M-2 group								
CTX-M-2		1	1					2
CTX-M-9 group								
CTX-M-14				1		1	1	3
CTX-M-14b					1			1
CTX-M-65				1				1
TEM								
TEM-52	1							1
TEM-52c	2							2
TEM-52cVar	8							8
SHV								
SHV-2a	1							1
SHV-12	11							11
CMY								
CMY-2	5		1					6
Chromosomal ampC								
ampC-type-3			2					2
ampC-type-11	1							
Total	**41**	**3**	**8**	**9**	**1**	**1**	**3**	**65**

ESBL/AmpC-producing *Salmonella*

Surveillance of extended-spectrum cephalosporin (ESC) resistant *Salmonella enterica* occurs in isolates from humans and meat sources in the Netherlands. The meat samples that were tested at NVWA in 2018 had a reduced prevalence of *Salmonella*. As such, no ESBL/AmpC producing *Salmonella* was detected from meat.

RIVM sent 1718 *Salmonella* isolates from human origin for susceptibility testing to cefotaxime and ceftazidime to WBVR. Table ESBL06 shows the results of the molecular typing per *Salmonella* serovar for the 14 isolates that were cefotaxime and ceftazidime resistant in 2018. Table ESBL07 displays the results of the molecular typing of all *Salmonella* collectively isolated from humans and meat from 2007 until 2018. The prevalence of ESBL/AmpC producing *Salmonella* fluctuates over time between 0.8% and 4.0% where 2018 had the lowest prevalence since 2007. The proportion of ESBL/AmpC producing *Salmonella* in the different serovars has somewhat changed compared to previous years. While *S.* Kentucky was the most prevalent ESBL/AmpC producing serovar in 2016 (n=9/35) and 2017 (n=18/31), in 2018 only two ESBL/AmpC producing isolates from this serovar were detected. In 2018, *S.* Infantis was the most ESBL/AmpC producing prevalent serovar (n=5) followed by *S.* Typhimurium (n=4), both of which were also prevalent in 2017 and 2016 (*S.* Infantis 2017 n=2/31, 2016 n=5/35, *S.* Typhimurium 2017 n=8/31, 2016 n=4/35). This change in dominant ESBL/AmpC producing serovars appears not to be caused by an increase in *S.* Infantis and *S.* Typhimurium but by a decrease in ESBL/AmpC producing *S.* Kentucky.

In 2018, ESBL/AmpC producing *Salmonella* originated from a total of 6 different serovars.

The decrease in the presence of ESBL/AmpC producing *Salmonella* has resulted in less diversity in the genes that were detected. Several genes of low prevalence such as bla_{TEM-52}, bla_{SHV-12} and bla_{DHA-1} were not detected in 2018.

In conclusion, when looking at the prevalence of randomly isolated ESBL/AmpC producing *E. coli* in Dutch livestock the decrease that started in 2011 still slowly continues. The decreasing trend in the proportion of chicken caecal samples positive for ESBL/AmpC producing *E. coli* continues in 2018 while for slaughter pigs and dairy cows the prevalence remains stable. This is in contrast with the situation in veal calves where for rosé veal calves, a spike in 2016 was seen, followed by a somewhat stable prevalence in 2017 and 2018 while for the white veal calves, a similar spike in 2016 is followed by a steady increase in the prevalence. Both in fresh and imported meat, the prevalence of ESBL/AmpC producing *E. coli* has shown a further decrease in 2018. The prevalence of ESBL/AmpC producing *Salmonella* from humans has also decreased while in meat these bacteria could not be detected in 2018.

Table ESBL06 Beta-lactamases in *Salmonella* isolated from humans in 2018.

	CTX-M-1 group		CTX-M-3 group	CTX-M-8 group	CTX-M-9 group				
Serovar	**CTX-M-15**	**CTX-M-55**	**CTX-M-3**	**CTX-M-8**	**CTX-M-9**	**CTX-M-14b**	**CTX-M-65**	**CMY-2**	**Total**
Corvallis		1							1
Infantis							5		5
Kentucky							1	1	2
Muenchen				1					1
Saintpaul	1								1
Typhimurium			1		2			1	4
Total	**1**	**1**	**1**	**1**	**2**	**0**	**6**	**2**	**14**

Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2018.

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	Total ESBL	Total *Salmonella* tested	% ESBL of total *Salmonella*
2007	9	13				17	2	4	2			47	1514	3.1
2008	25	12		1	1	13	1		6	2		61	2149	2.8
2009	12	4			2	3		1	9			31	2232	1.4
2010	8	3			1	2		3	4			21	1715	1.2
2011	5	3			1	1		2	13			25	1444	1.7
2012	14	5			2	2			10	1		34	1795	1.9
2013	1	3		5	4	5	1		36			55	1369	4.0
2014	6			2	3	1			21			33	1688	2.0
2015	13	2			6	1			12			34	1761	1.9
[f]2016	7				15	2			10		1	36	2117	1.7
[g]2017	3				23			1	3		1	31	1697	1.8
2018	2		1	1	8				2			14	1718	0.8
Total	**105**	**45**	**1**	**9**	**66**	**47**	**4**	**11**	**128**	**3**	**2**	**422**	**21199**	**2.0**

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 only human isolates were molecularly characterised.

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

In 2015 a sensitive molecular method was applied to screen for carbapenemase producers, extended spectrum beta-lactamases that can also hydrolyse carbapenems (MARAN 2016 for method details). This is important in an environment with a very low anticipated prevalence of carbapenem resistance. All faecal samples of livestock sent by NVWA to WBVR for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in BPW and after incubation five individual samples were pooled, centrifuged and DNA isolated from the pellet. A commercial RT-PCR (Check-Points, CarbaCheck MDR RT) that can detect the most important carbapenemase gene families (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{OXA-48)}$ was used according to manufacturer's instructions. If RT-PCR gave suspicious or positive results, a step-wise analysis was performed to confirm the results:

1. Five conventional PCRs were performed on purified DNA of the 5 individual samples of the pool;
2. If PCR was positive, genes were identified with Sanger sequencing;
3. 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 (for *Shewanella* spp).

Carbapenemase screening in 2018 (n=1206) resulted in fifteen $bla_{OXA-48-like}$ positive faecal samples in the RT-PCR (five white veal calves, four slaughter pigs, three broilers and three dairy cows). $bla_{OXA-48-like}$ genes are known to be chromosomally associated with *Shewanella* spp. In 11 samples the presence of $bla_{OXA-48-carrying}$ *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: $bla_{OXA-48b}$ (n=5), $bla_{OXA-48b-like}$ (n=3), $bla_{OXA-204}$ (n=1), and $bla_{OXA-252}$ (n=2). These results confirm the findings of previous years, as no carbapenemase-producing *Enterobacteriaceae* were isolated from livestock in the Netherlands. $bla_{OXA-48-like}$ genes have also been found in faecal samples in 2013, 2014, 2015, 2016 and 2017 (MARAN). Given the role of *Shewanella* spp. as natural progenitor of this carbapenemase family (Zong, 2012), these genes were considered of environmental origin and not a public health risk. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2019.

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 (Stolle *et al*, 2013; Pulss *et al*, 2018), Spain (González-Torralba *et al*, 2016), France (Melo, *et al*, 2017) and the UK (Reynolds *et al*, 2019). 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 have not been detected in the Netherlands (MARAN 2017). In 2017, the first case of a bla_{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. The monitoring was continued in 2018.

Faecal samples of cats and dogs were obtained through the VMDC. Because the expected prevalence of CPE remains low and reported CPE are frequently multi-resistant, the inclusion criterion for dog faecal samples was antimicrobial treatment of the animal. Since cats are not frequently treated with antimicrobials, no inclusion criterion was defined and available faecal samples from cats submitted to VMDC were included. In 2018, 117 faecal samples from cats and 159 faecal samples from dogs were screened. From each sample, 0.5 gram feces 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 again inoculated 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 bla_{NDM} (Manchanda *et al*, 2011), bla_{KPC} (Bradford *et al*, 2004), bla_{IMP} (Ellington *et al*, 2007), bla_{VIM} (Ellington *et al*, 2007), $bla_{OXA\text{-}group\text{-}23}$, -24, -51, -58 (Voets *et al*, 2011) and $bla_{OXA\text{-}group\text{-}48}$ (Poirel *et al*, 2004).

All faecal samples from cats that were screened in 2018 were negative for CPE. Two individual dog samples were positive for *E. coli*, harboring $bla_{OXA\text{-}48}$ and $bla_{OXA\text{-}181}$ respectively. Both samples originated from different parts of the Netherlands. Both were sent to the VMDC for parasitology diagnostics. The dog carrying $bla_{OXA\text{-}181}$ was suffering from diarrhea. The dog carrying $bla_{OXA\text{-}48}$ was suspected for lungworm, but was also treated with amoxicillin/clavulanic acid for 10 days, 3 weeks prior to sampling due to suspicion of a urinary tract infection. Molecular analysis of the isolate is ongoing but preliminary analysis suggests that it is transferable and located on a mobile element (J. Hordijk, personal communication).

4.2.3 Monitoring in imported seafood

In 2018, 296 batches of frozen fish and shrimps originating from fish farms in South-East Asia were screened for the presence of CPE. The samples consisted of 100 batches of Pangasius, 102 batches of Tilapia and 94 batches of shrimps. Similar to 2017, two carbapenemase-producing *Enterobacter cloacae* complex isolates were detected in two different batches of frozen shrimps. Both isolates were cultured from frozen shrimps (*Penaeus monodon*) from Vietnam. Molecular analysis of the first isolate (May 2018) revealed the presence of two carbapenemase genes: $bla_{NDM\text{-}5}$ located on an IncX3 plasmid next to $bla_{OXA\text{-}48}$ on a ColE plasmid. (M. Brouwer, personal communication). The second isolate (October 2018) harboured a chromosomally located $bla_{IMI\text{-}1}$ embedded in an insertion element (EcloIMEX) genetically closely related to the earlier described *Enterobacter cloacae* complex isolate obtained from Vietnamese shrimps in 2017 (Brouwer *et al*, 2018).
Consumption of antimicrobials is high in South-East Asia both in humans and in animals, and aquaculture represents an environment with high selective pressure for resistant bacteria, including CPE, and potential for faecal contamination. Therefore, findings of CPE in imported food products from this area is not surprising.

4.3 Colistin resistance

As published in MARAN 2016 a retrospective study revealed the low prevalence of the colistin resistance gene *mcr-1* in *E. coli* from livestock (≤ 1%) and meat (2%), and in *Salmonella* from poultry meat (1%) in the period 2010 – 2015. The fact that no *mcr-1* genes were identified in randomly isolated indicator *E. coli* from faecal samples from 2014 and 2015 suggests a decreasing trend in the occurrence of this gene. Like in former years, no colistin resistant isolates were identified amongst the randomly selected indicator *E. coli* isolated from faecal samples in 2017.

To gain more knowledge on the current spread of *mcr-1* and its allelic variants in livestock, selective monitoring was started in 2016 and continued in 2017 and 2018 as part of the national surveillance program on antibiotic resistance in animals. In order to increase the sensitivity of the test, selective enrichment was started in 2017 by using BPW broth with 2 mg/L colistin. In 2018, purified DNA of pooled PBW cultures (five samples per pool) from a total of 1206 faecal samples of Dutch livestock were tested with conventional PCR for the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* using an in house designed multiplex RT-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). 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, *mcr-1* positive *E. coli* were identified in eleven faecal samples (1.2%) from selective culturing in several animal species: veal calves (n=6, 2.0%), slaughter pigs (n=3, 1.0%) broilers (n=1, 0.3%), and dairy cattle (n=1, 0.3%). Noticeably, *mcr-4* was detected in four white veal calf samples.
For comparison, 205 caecal samples of broilers fattened in Germany, but slaughtered in the Netherlands, were screened for the presence of *mcr*-genes. As a result, *mcr-1* was detected in 50 samples (24.4%) which is a marked difference compared to the prevalence in Dutch broilers (0.3%). The high *mcr-1* prevalence in the German broilers is most probably linked to the relative high usage of colistin in the German poultry production system compared to the Netherlands (ESVAC-2016, GE: 7.9 mg/PCU and NL: 0.3 mg/PCU).
In retail meat four randomly isolated colistin resistant *E. coli* [chicken (n=2) and turkey meat (n=2)] were confirmed as *mcr-1* carriers which is indicative for a higher prevalence in poultry meat than in broilers. *mcr-1* was not identified in *Salmonella*. These results strengthen the idea that fresh retail meat in Dutch supermarkets originating from other EU countries might contain higher concentrations of *mcr-1* due to the differences in use of colistin.
In 2018, *mcr-1* was identified at low-level in *E. coli* from different livestock species using PCR screening. In veal calves, *mcr-4* was detected in four faecal samples. The finding of *mcr-1* positive *E. coli* on poultry meat indicates a higher level in retail meat from chicken and turkey. A significant higher prevalence of *mcr-1* was detected in German broilers. No *mcr* genes were detected in *Salmonella*.

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