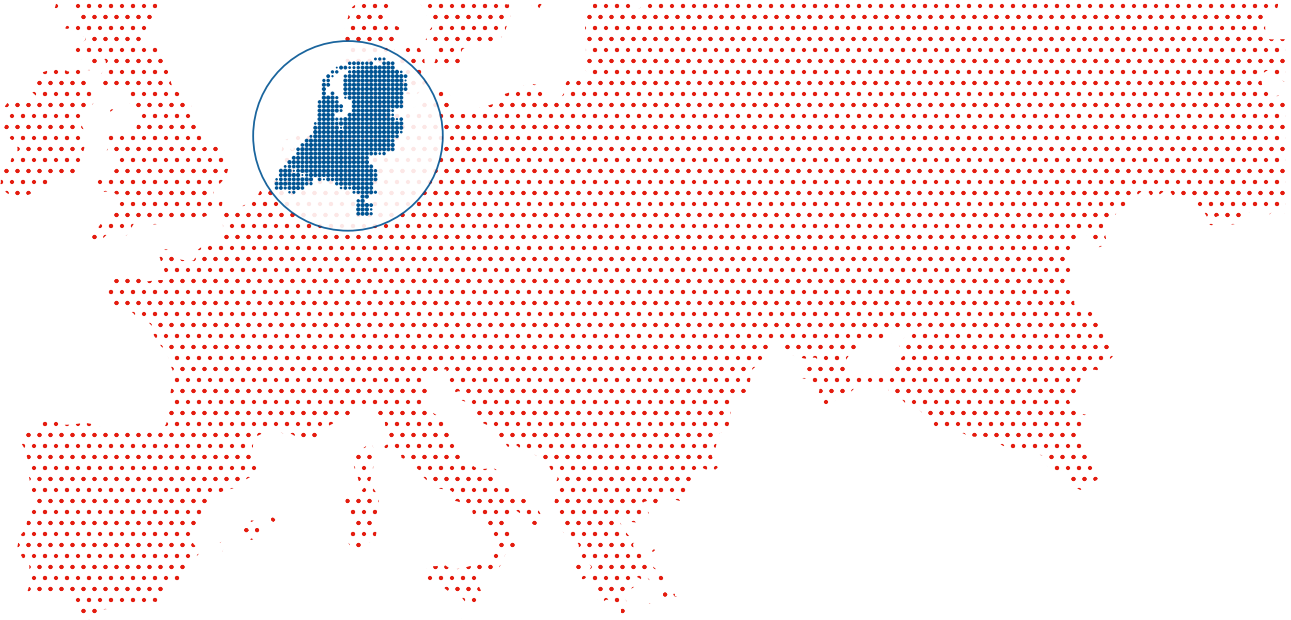
# NethMap 2018

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

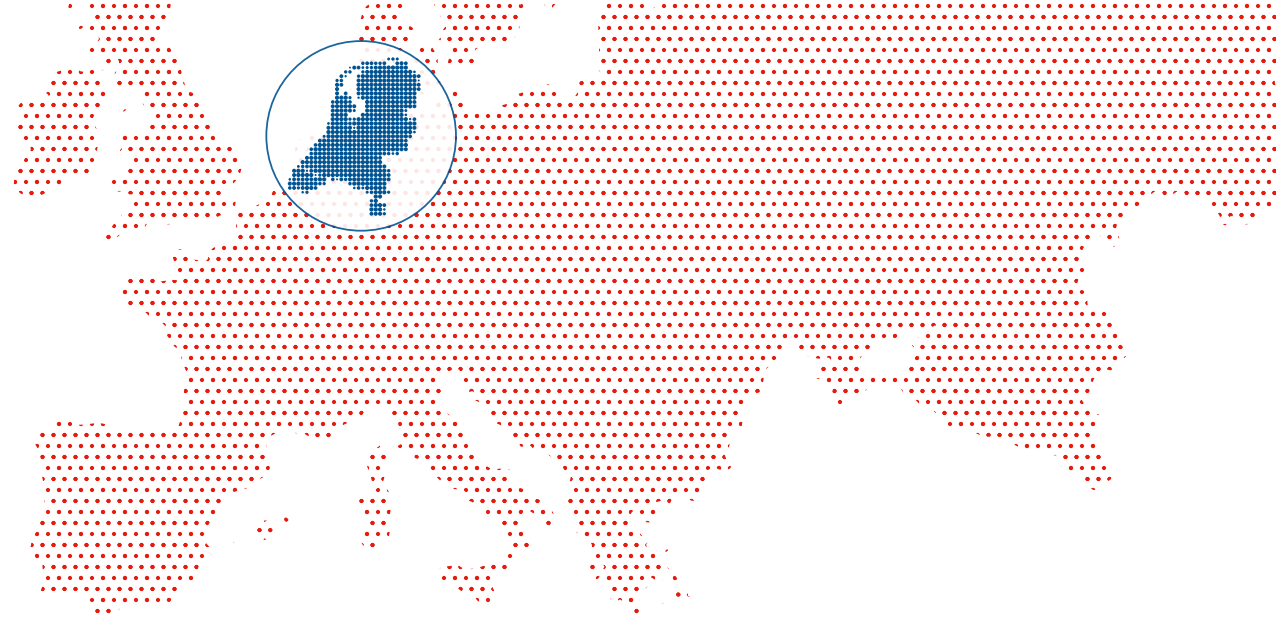


National Institute for Public Health and the Environment
*Ministry of Health, Welfare and Sport*

SWAB

# MARAN 2018

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2017



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

Netherlands Food and Consumer Product Safety Authority
*Ministry of Agriculture, Nature and Food Quality*

WAGENINGEN UNIVERSITY & RESEARCH

Universiteit Utrecht

SDa Autoriteit Diergeneesmiddelen

# NethMap 2018

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2017

June 2018

# Synopsis

## NethMap/MARAN-report

The number of bacteria that are resistant to antibiotics is increasing worldwide. That number has generally remained fairly stable in the Netherlands. Nevertheless, there is cause for concern and caution. Certain resistant bacteria, the so-called ESBL-producing intestinal bacteria, have become more common among patients of GPs and in hospitals during the past 5 years. ESBL are enzymes that can break down commonly used antibiotics such as penicillins. These bacteria can cause harmless infections, such as bladder infections, that are more difficult to treat because of resistance. Moreover, more frequent use must be made of types of antibiotics that are only used as a last resort.

To prevent resistance it is important to use antibiotics properly and only when necessary. In recent years, GPs have been prescribing fewer antibiotics. In hospitals, on the other hand, total antibiotic use increased in 2016 compared to the previous year. On balance, total antibiotic use for animals in 2017 was comparable to 2016. Use declined in some animal sectors, while it increased slightly in other sectors. The antibiotics that are important to humans are only used to a limited extent in the animal sectors. The prevalence of ESBLs has declined further among almost all types of animals used for the food production, with the exception of veal calves where an increase was seen.

This is evident from the annual report NethMap/MARAN 2018, in which various organisations jointly present data on antibiotic use and resistance in the Netherlands, both for humans and animals.

In the past two years, extra measures have been taken in the Netherlands to combat antibiotic resistance. These measures go beyond healthcare. After all, resistant bacteria do not adhere to land borders and also occur in animals, food and in the environment (One Health). To support this approach, 'regional cooperative networks' were set up in 2017. They have the task of stimulating collaboration between different healthcare professionals in preventing and combating antibiotic resistance. In addition, there has been more attention to antibiotic resistance in nursing homes. For example, a new study investigates how many residents carry resistant bacteria. The results are expected at the end of 2018.

**Key words:**
Antibiotic resistance, bacteria, antibiotic use, infection

# Publiekssamenvatting

## NethMap/MARAN-rapport

Wereldwijd neemt het aantal bacteriën die resistent zijn tegen antibiotica toe. In Nederland is dat aantal over het algemeen ongeveer stabiel gebleven. Toch blijft er reden voor zorg en oplettendheid. Zowel bij patiënten van huisartsen als van ziekenhuizen komen bepaalde resistente bacteriën de afgelopen 5 jaar vaker voor, de zogeheten ESBL-producerende darmbacteriën. ESBL zijn enzymen die veelgebruikte antibiotica kunnen afbreken, zoals penicillines. Deze bacteriën kunnen onschuldige infecties zoals een blaasontsteking veroorzaken die door de resistentie moeilijker te behandelen zijn. Ook moet dan vaker gebruik worden gemaakt van soorten antibiotica die alleen als laatste redmiddel worden ingezet.

Om resistentie te voorkomen is het belangrijk om antibiotica op de juiste manier te gebruiken en alleen als het nodig is. In de afgelopen jaren schreven huisartsen minder antibioticakuren voor. In ziekenhuizen daarentegen steeg het totale antibioticagebruik in 2016 ten opzichte van het voorgaande jaar. Het totale antibioticagebruik voor dieren was in 2017 per saldo vergelijkbaar met 2016. In sommige diersectoren daalde het gebruik, terwijl het in andere sectoren licht toenam. Antibiotica die belangrijk zijn om infecties bij de mens te behandelen, zijn de afgelopen jaren nauwelijks meer voor dieren ingezet. Zo is het aantal ESBL's verder afgenomen bij bijna alle soorten dieren die voor de voedselproductie worden gebruikt. Een uitzondering daarop zijn vleeskalveren, waar een lichte toename is gezien.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2018. Hierin presenteren diverse organisaties gezamenlijk de gegevens over het antibioticagebruik en -resistentie in Nederland, zowel voor mensen als voor dieren.

In de afgelopen twee jaar zijn in Nederland extra maatregelen genomen om antibioticaresistentie te bestrijden. Deze maatregelen reiken verder dan de gezondheidszorg. Resistente bacteriën houden zich immers niet aan landgrenzen en komen ook bij dieren, in voeding en in het milieu voor (One Health). Om deze aanpak te ondersteunen zijn in 2017 'regionale zorgnetwerken' opgezet. Zij hebben de taak om de samenwerking tussen verschillende zorgprofessionals te stimuleren bij het voorkomen en bestrijden van antibioticaresistentie. Daarnaast is er meer aandacht voor antibioticaresistentie in verpleeghuizen. Zo is een onderzoek gestart waarin wordt gemeten hoeveel bewoners resistente bacteriën bij zich dragen. De uitkomst hiervan wordt eind 2018 verwacht.

**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 Postbus 39, 5854 ZG Bergen (L) or by email to secretariaat@swab.nl.
NethMap 2018 and earlier versions are also available from the website of the SWAB: www.swab.nl.

**Members of SWAB's working group on surveillance of antimicrobial use**

| | | |
|---|---|---|
| Dr S Natsch (president) | Dr TBY Liem | Drs C Pellicaan |
| Drs PDJ Bollen | Dr PD van der Linden | Drs MMB Roukens |
| Dr EMW van de Garde | Drs M Lourens | Dr AW van der Velden |

**Members of SWAB's working group on antibiotic stewardship**

| | | |
|---|---|---|
| Prof Dr BJ Kullberg (president) | Dr RM van Hest | Prof Dr B Sinha |
| S. Balder | Prof Dr MEJL Hulscher | Prof Dr A Verbon |
| Dr ECH Boel | Dr PD van der Linden | Dr CM Verduin |
| Prof Dr AW Friedrich | Dr. J ten Oever | Prof Dr HFL Wertheim |
| I. Groothuis | Prof Dr JM Prins | Dr TFW Wolfs |

## Acknowledgements

We thank the Foundation for Pharmaceutical Statistics SFK, The Hague, for providing data on community usage of antimicrobial agents and all hospital pharmacists of the centres mentioned below for providing data on hospital usage.
We thank all participants of ISIS-AR, Dr R Willems, Prof Dr MC Vos, the Netherlands Reference Laboratory for Bacterial Meningitis in Amsterdam, GRAS, anaerobic pathogen surveillance, *C. difficile* surveillance, azole resistance surveillance, and the NIVEL for their important contributions; and the staff of the Publishing Department RIVM for preparing this report for printing.

**Centres contributing to the surveillance of antibiotic consumption**

Alkmaar, MC Alkmaar; Almelo/Hengelo, ziekenhuisgroep Twente; Almere, Flevoziekenhuis; Amersfoort, Meander MC; Amsterdam, Antoni van Leeuwenhoek ziekenhuis; Amsterdam, OLVG Oost; Amsterdam, OLVG west; Apeldoorn/Zutphen, Gelre ziekenhuizen; Arnhem, Rijnstate ziekenhuis; Assen, Wilhelmina ziekenhuis; Beverwijk, Rode Kruis ziekenhuis; Boxmeer, Maasziekenhuis Pantein; Breda, Amphia ziekenhuis; Den Bosch, Jeroen Bosch ziekenhuis; Den Haag, HMC; Den Haag, HAGA ziekenhuizen; Deventer, Deventer ziekenhuis; Doetinchem, Slingeland ziekenhuis; Dordrecht, Albert Schweizer ziekenhuis; Drachten, Nij Smellinghe; Ede, Gelderse vallei; Eindhoven, Maxima MC; Emmen, Scheperziekenhuis; Gorinchem, Beatrix ziekenhuis; Gouda, Groene hart ziekenhuis; Groningen, Martini ziekenhuis; Groningen, Refaja ziekenhuis; Groningen, Ommelander ziekenhuis; Groningen, UMCG; Haarlem, Spaarne Gasthuis; Harderwijk; St.Jansdal; Heerenveen, De Tjongerschans; Helmond, Elkerliek; Hoorn, Westfries Gasthuis; Leiden, LUMC; Leeuwarden, Medisch centrum Leeuwarden; Lelystad, MC Zuiderzee; Maastricht, MUMC; Meppel, Diaconessenhuis; Nieuwegein, St. Antonius ziekenhuis; Nijmegen, CWZ; Nijmegen, Radboudumc; Roermond, Laurentius ziekenhuis; Rotterdam, Erasmus MC; Rotterdam, Maasstad ziekenhuis; Rotterdam, St. Franciscus gasthuis; Rotterdam, Ikazia ziekenhuis; Rotterdam-Dirksland, Van Weel Bethesda ziekenhuis; Sittard/Heerlen, Zuyderland, Sneek, Antonius ziekenhuis; Terneuzen, ZorgSaam; Tilburg, Elisabeth TweeSteden ziekenhuis; Tiel, Ziekenhuis Rivierenland; Utrecht, Diakonessenhuis; Utrecht, UMCU; Uden, Ziekenhuis Bernhoven; Venlo, Vie Curi MC; Winterswijk, Streekziekenhuis Koningin Beatrix; Woerden, Zuwe Hofpoort; Zaandam, Zaans MC; Zeeland, ADRZ; Zoetermeer, Lange Land ziekenhuis; Zwolle, Isala.

**Centres contributing to the surveillance of resistance to antimicrobial agents (ISIS-AR)**

Alkmaar, Noordwest Ziekenhuisgroep; Amersfoort, Meander MC; Amsterdam, OLVG / Almere; Amsterdam, Slotervaartziekenhuis / Antoni van Leeuwenhoek; Amsterdam, Streeklaboratorium Amsterdam – GGD; Amsterdam, VUmc; Apeldoorn, Gelre Ziekenhuizen; Bergen op Zoom, Bravis ziekenhuis, locatie Bergen op Zoom; Breda, Amphia Ziekenhuis; Delft, Microbiologisch laboratorium Reinier de Graaf Groep; Deventer, Deventer Ziekenhuis; Doetinchem, Slingeland Ziekenhuis; Dordrecht, Regionaal Laboratorium Medische Microbiologie; Ede, Ziekenhuis Gelderse Vallei; Goes, Admiraal De Ruyter Ziekenhuis; Gouda, Groene Hart Ziekenhuis; Groningen, Certe; Haarlem, Streeklaboratorium voor de Volksgezondheid; Heerlen, Zuyderland MC Heerlen; Hengelo, Laboratorium voor Medische Microbiologie Twente/Achterhoek; Hilversum, Centraal Bacteriologisch en Serologisch Laboratorium; Leeuwarden, Izore, Centrum Infectieziekten Friesland; Leiden, LUMC; Maastricht, Maastricht UMC+; Nieuwegein, St. Antonius Ziekenhuis; Nijmegen, Canisius Wilhelmina Ziekenhuis; Nijmegen, UMC St. Radboud; Roosendaal, Bravis ziekenhuis, locatie Roosendaal; Rotterdam, Erasmus MC; 's-Gravenhage, Alrijne Zorggroep / MMI Bronovo; 's-Gravenhage, HagaZiekenhuis; 's-Gravenhage, MC Haaglanden Westeinde; 's-Hertogenbosch, Jeroen Bosch Ziekenhuis; Sittard-Geleen, Zuyderland MC; Terneuzen, Ziekenhuis ZorgSaam Zeeuws-Vlaanderen; Tilburg, Streeklab v.d. Volksgezondheid; Utrecht, Diakonessenhuis; Utrecht, Saltro; Utrecht, UMC Utrecht; Veldhoven, Stichting PAMM; Velp, Ziekenhuis Rijnstate, loc. Velp; Zwolle, Isala.

# Contents

# 1 Introduction

This is NethMap 2018, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2017 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, the 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. Together these constitute the basis of the surveillance of resistance trends reported in NethMap and are used by CIb to monitor and inform the government about potential national health threats with regard to antimicrobial resistance.

NethMap 2018 extends and updates the information of the annual reports since 2003. Since the introduction of a revised format four 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 third 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 Cooperative Networks were set up to improve regional collaboration to control antimicrobial resistance. They are expected to become more operational in the coming year to contribute in the fight against antimicrobial resistance. 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.

The editors:
Dr Ir SC de Greeff
Prof Dr JW Mouton

# 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 2018) and the veterinary sector (MARAN 2018).

## 2.1 Most important trends in antimicrobial use

**In outpatients**

- In 2017 total antibiotic use in outpatients decreased from 10.44 in 2016 to a level of 10.06 DDD/1000 inhabitant days (DID).
- The decrease in antibiotic use is mainly driven by reductions in the use of tetracyclines (doxycycline), penicillins with extended spectrum (amoxicillin) and penicillins with beta-lactamase-inhibitors (amoxicillin with clavulanic acid).
- The use of nitrofurantoin, ciprofloxacin and azithromycin remained stable in 2017.

**In hospitals**

- The inpatient use of antibiotics in 2016 increased to 84.0 (+6.2) when expressed as DDD/100 patient-days and decreased to 326.1 (-4.0) when expressed as DDD/100 admissions, probably indicating further intensification of the use of antibiotics in hospitals.
- The use of penicillins with extended spectrum increased most and reached a level of 10.9 DDD/100 patient-days (+1.7 DDD/100 patient-days).
- In 2016 the use of beta-lactamase resistant penicillins increased with 0.9 DDD/100 patient-days and is now back again at 8.7 DDD/100 patient-days.
- The use of fluoroquinolones increased with 0.7 to 9.1 DDD/100 patient-days.
- The use of second- and third-generation cephalosporins continued to rise in 2016 (+0.5 and +0.4 DDD/100 patient-days, respectively).
- The use of antimycotics increased with 1.53 DDD/100 patient-days in 2016 and has now reached a level of 14.23 DDD/100 patient-days.

- There are large differences in total antibiotic drug use between Dutch hospitals (range 52-137 DDD/100 patient-days). General hospitals used the least antibiotics (82.3 DDD/100 patient-days), whereas university hospitals reported the most (92.5 DDD/100 patient-days).
- 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.

**In nursing homes**

- Mean use of antibiotics in nursing homes varies from year to year. In 2016, it totalled to a mean of 56.8 DDD/1000 residents, but varied widely between nursing home locations (range 15-128 DDD/1000 residents/day).
- The most frequently used antibiotics remained combinations of penicillins (mainly amoxicillin with clavulanic acid), nitrofurantoin derivatives, and fluoroquinolones with 27%, 16% and 14%, respectively.

## 2.2 Most important trends in antimicrobial resistance

Several surveillance programs have been developed 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 MRSA 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 2018.

**In GPs**

- For most antimicrobials, there are no statistically significant and clinically relevant shifts in resistance levels since 2013.
- 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.
- In *E. coli*, resistance to co-amoxiclav in both age groups and for *K. pneumoniae* in patients aged >12 years has increased. 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.
- The percentage of highly resistant microorganisms (HRMO) and multidrug-resistance was ≤6% in all Enterobacteriaceae.
- Resistance levels for *E. coli* were comparable between the regional cooperative networks for most 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 6% in 2012 to 15% in 2017.

**Table 2.2.1** Overview of surveillance programs in the Netherlands in 2017.

| Surveillance program[1] | Origin of isolates | Availability | Sources 2017 | 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- | 42 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*** | STI centers | 2006- | 90% of STI 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 homes, 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 | Etest |
| ***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 | Central testing | EUCAST methodology |

[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*

### In hospitals

- Compared to 2013, overall resistance rates for many antimicrobials have remained similar, with a few exceptions, for which statistically significant and clinically relevant increasing or decreasing trends were observed:
  - In *E. coli*, resistance to co-amoxiclav in both age groups and for *K. pneumoniae* in patients aged >12 years has increased. 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.
  - Outpatient departments: Comparable to the previous year, in *K. pneumoniae*, a significant increase was seen in resistance to cefotaxime/ceftriaxone and in multidrug resistance.
  - Unselected hospital patient departments: Resistance levels ≥20% were found for trimethoprim in *E. coli*, *K. pneumoniae* and *P. mirabilis*, for amoxicillin/ampicillin and co-trimoxazole in *E. coli* and *P. mirabilis*, and for co-amoxiclav in *E. coli*. A significant increase in resistance was observed for ceftazidime in *K. pneumoniae*. In *P. mirabilis*, resistance to co-amoxiclav decreased to a significant and clinically relevant extent.
  - Intensive Care Units: In *E. coli*, resistance to ceftazidime increased in the last five years. A decreasing trend in resistance was found for gentamicin in *K. pneumoniae*, and in ciprofloxacin, gentamicin, tobramycin, co-trimoxazole, and HRMO for *E. cloacae*.
  - Blood isolates from inpatient departments: In coagulase-negative *Staphylococcus* spp., a decrease in resistance to co-trimoxazole was observed, especially in the last four years. In *P. aeruginosa*, resistance to ceftazidime decreased significantly.
- In 2017, a significant and clinically relevant increasing trend in ESBL in *E. coli* and *K. pneumoniae* with values of 2-6% and 4-9% respectively, was observed for general practitioner patients, in outpatient departments and in hospital inpatient departments excluding Intensive Care Unit (ICU). 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 (0.03% and 0.42%). Of the isolates submitted to the CIb, the most frequently identified carbapenemase encoding genes in Enterobacteriaceae were genes encoding for OXA-48, NDM, VIM and KPC. In pseudomonas this was the $bla_{VIM}$ gene
- The worrisome increase in azole resistance in *Aspergilllus fumigatus* continued and is now 14.7% on average in five academic hospitals.

## 2.3 Antibiotic use and resistance in veterinary sector

**Antibiotic use**

- Sales of antimicrobial veterinary medicinal products (VMP's) in 2017 (181 tonnes) showed an increase of 3% compared to 2016 (176 tonnes). Reasons for the increase of sales in 2017 could be an increase in stock (catching up) and increased use in growing unmonitored sectors.
- The calculation of consumption is based on national conversion factors (DDDA's) of authorized drugs. Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers.
- In most sectors, veal calves, pigs, broilers and turkeys, a further reduction in consumption has been realized. In dairy cows and other cattle a small increase in consumption is noted.
- 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 VMP's 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 VMP's.

**Antimicrobial resistance**

- In 2017, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL-suspected *Salmonella* isolates was 1.8% concerning seven different serovars, isolated from human samples. Cefotaxime resistance was detected in 67.6% of the *Salmonella* isolates obtained from (outside EU) imported poultry products. No cefotaxime resistant isolates were found in fresh meat from Dutch retail (produced within EU). No carbapenemase producing *Salmonella* were found in 2017.
- Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2017, compared to 2016. Resistance to macrolides was rarely detected in isolates from livestock and humans.
- Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with an increase in 2017), which is a concern for public health. Resistance to erythromycin, representing macrolides as a first choice antibiotic in human medicine for campylobacteriosis, remained low.
- There is an increasing tendency in human STEC O157 for resistance since 2009, with proportions of resistance to ampicillin, sulfamethoxazole and trimethoprim isolates of 16.1% for ampicilline, 16.1% for sulfamethoxazole, and 14.5% for trimethoprim in 2017.
- In 2017, resistance proportions of indicator *E. coli* in caecal samples showed a tendency to decrease in broilers, to stabilize in pigs, and showed a slight increase in veal calves. In dairy cattle the resistance proportions remained at a constant low level. As in former years, resistance proportions in *E. coli* from chicken and turkey meat were substantially higher than in pork and beef.
- Within the randomly isolated indicator *E. coli* in caecal samples from broilers a continuous low proportion of ESBL/AmpC-producing *E. coli* was observed in 2017 (1.7%).
- No ESBL/AmpC-producing *E. coli* were detected in faecal samples from pigs, veal calves and dairy cattle.
- For the second year in a row, an increase was observed in white and rosé veal calves carrying ESBL/AmpC-producing *E. coli*.

- The most prevalent ESBL/AmpC gene was $bla_{CTX\text{-}M\text{-}1}$ in all animal species. $bla_{CTX\text{-}M\text{-}15}$ was found frequently in veal calves and dairy cows (30%). $bla_{CMY\text{-}2}$ in broilers (25%), followed by $bla_{SHV\text{-}12}$, $bla_{TEM\text{-}52c}$ and $bla_{CTX\text{-}M\text{-}14}$. A comparable gene distribution was observed in corresponding meat samples.
- The overall prevalence of ESBL/AmpC-producing *E. coli* in meat in 2017 was 9.6%.
- The proportion of human ESBL/AmpC-producing *Salmonella* in 2017 was 1.8%, confirming a continuous low level (≤2%) since 2014. The majority (84%) of these were highly multidrug resistant (5-8 antibiotics).
- No carbapenemase-producing Enterobacteriaceae were detected in active surveillance in livestock. Only $bla_{OXA\text{-}48\text{-}like}$ genes were detected in six samples (three broilers, two slaughter pigs and one dairy cow) and all associated with *Shewanella* spp..
- Colistin resistance gene mcr-1 was identified at a low-level in *E. coli* from livestock (1.2%) and at higher levels in retail meat from chicken (7.7%), but not in *Salmonella*.

- The data on use are to a large extent reflected in the resistance data of 2017. In broilers the continuous reduction in use resulted in an ongoing decrease in proportions of resistant *E. coli* for most antibiotic classes tested. Also the concentration of ESBL/AmpC-producing *E. coli* in broiler faeces and on poultry meat was again lower than in previous years. In contrast to broilers, in 2017 the prevalence of ESBL-carriers again increased in both white and rosé 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 antimicrobial resistance remained stable and ESBL occurrence increased.

## 2.4 Implications for therapy

Overall, no major shifts in resistance rates have occurred in the Netherlands in 2017. The only major exception is susceptibility to co-amoxiclav, but this is primarily due to a change in testing methods. However, 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. 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 dosing regimens should be used with care.
Resistance rates reported are for one isolate per patient, and only the first one, and 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.
- Clindamycin (inducible) resistance in *S. aureus* has risen to more than 10%, a value that should be considered relevant when considering clindamycin therapy without culture.
- For the first time, resistance percentages are now available per region. These indicate that there are clear differences in susceptibility between regions for some antibiotics, and 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.

*Unselected hospital patient departments*

- There was a continuing increase in resistance in *K. pneumoniae*; resistance is now 9% to ceftazidime, above 10% for cefotaxime/ceftriaxone and the %HRMO is now 12%. Patients suspected for *K. pneumoniae* infection are at an increasingly specific risk of non-adequate treatment.
- For other Enterobacteriaceae, resistance to most antimicrobials did not change markedly or was lower. The only major exception was co-amoxiclav as a result of new testing methods. The % resistance in *E. coli* is above 30% and in *K. pneumoniae* close to 20%. This renders the drug unsuitable for empiric therapy, unless it is combined with a second drug.
- For *P. aeruginosa* resistance declined for all antibiotics except for ciprofloxacin, now above 10%. If ciprofloxacin is considered as empiric therapy, combination with a second antipseudomonal should be considered.
- Combination therapy of a beta-lactam with an aminoglycoside are still the best suitable options for empirical treatment in serious infections, unless a quinolone is specifically desired to cover specific pathogens.
- The level of clindamycin inducible resistance in *S. aureus* keeps increasing. The 12% resistance indicates antibiograms are mandatory before starting treatment.

*Intensive care patients*

- Similar to other wards, increase in resistance in *K. pneumoniae* is the main treatment challenge. The %HRMO in this group was 14%. However, the %HRMO in *E. coli* has now also increased to more than 10%. 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, this has significant consequences for therapy. In contrast, resistance in Enterobacteriaceae in general were similar or often even lower than in the previous year(s).
- Local resistance levels vary significantly, including by time. Tailored therapy and culture remain the mainstay of therapy.

*Specific microorganisms*

- The most worrisome development is the continuing increase in azole resistance in *Aspergillus fumigatus* now averaging 14.7%. Monotherapy of azoles is no longer an option for empiric therapy and guidelines for empiric therapy have been renewed following this development.
- A clinical isolate of *C. difficile* PCR ribotype 014 with MIC=8 mg/L to metronidazole was found in December 2017. The emergence of metronidazole resistance needs attention in the near future.

## 2.5 Antimicrobial stewardship

Following the recommendation of the Dutch Health Care Inspectorate (IGZ) in response to the statement of the SWAB to contain antimicrobial resistance, A-teams have been established in an increasing number of hospitals. A survey conducted in 2017 in 80 hospitals, with 64 hospitals responding to the questionnaire, indicated that 60 hospitals had an A-team. This was a considerable increase compared to 2016. A-teams are responsible for the implementation of an antimicrobial stewardship program in hospitals in order to optimize antimicrobial therapy leading to improved patient outcomes, containment of health care costs and reduction of adverse effects including antimicrobial resistance. The time dedicated by the A-team to antimicrobial stewardship-related activities was a mean of 19.8 hours per week. The monitoring of use of restricted antimicrobials was improved and educational sessions on use were organized in the majority of hospitals. Attendance was mandatory in some of these. The quality of antimicrobial use was monitored in a pilot program involving 8 hospitals. This pilot has yielded valuable information on the possibilities of data-extraction for the purpose of surveillance and barriers (and solutions) for its implementation and shows clear progress in implementation of guidelines.

## 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 Enterobacteriaceae (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 2016, 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 for *K. pneumoniae*, which were mostly countries with high resistance percentages to other antibiotic groups. 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, for *K. pneumoniae*, more than one third of the isolates reported to EARS-Net for 2016 were resistant to at least one of the available antibiotic groups and combined resistance to three or more groups was common. In *E. coli*, an increasing EU/EEA trend for combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides between 2013 and 2016 was observed.

In the Netherlands, the prevalence of carbapenem resistance among Enterobacteriaceae remained

rare. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was low (0.03% and 0.42%) and there was no significant increase in the last years. On the other hand, a gradually increasing trend in ESBL-Enterobacteriaceae was observed in all healthcare settings, which was most outspoken for *K. pneumoniae*. Additionally, in hospitals the percentage of HRMO among *K. pneumoniae* was usually ≥10% in all departments. 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 2017, multiple initiatives and projects were further developed. First, the set-up of ten Regional Cooperative Networks concerning antimicrobial resistance was continued. 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. 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 in collaboration with six microbiological labs. The project will be expanded further in the coming year by enrolling more laboratories in the Netherlands. Lastly, a point prevalence study in nursing homes has been set up 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. In April 2018, the Ministry of Health published a letter on the progress of all initiatives.[2]

**Conclusions**

The data presented in NethMap 2018 demonstrate that further implementation of the national approach is needed to combat antibiotic resistance. 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. Timely intervention and control requires good collaboration between all stakeholders.

[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 antibiotic use decreased from 10.44 DID in 2016 to 10.06 DID in 2017 (Table 3.1.1). This decrease is mainly driven by reductions in the use of tetracyclines, penicillins with extended spectrum and penicillins with beta-lactamase-inhibitors (Figure 3.1.1). As in 2016, the use of tetracyclines decreased, resulting in a level of 1.98 DID (-0.12 DID) in 2017. The use of amoxicillin decreased to 1.94 DID (-0.15 DID) and the use of penicillins with beta-lactamase-inhibitors, mainly amoxicillin with clavulanic acid, decreased by 0.10 DID to 1.42 DID (Figure 3.1.2). Since 2009 the use of fosfomycin steadily increased to 0.05 DID in 2017.

**Discussion**

The decrease of total outpatient antibiotic use in the Netherlands in 2016 continued in 2017. Decreased tetracycline prescribing probably still reflects a delayed change caused by adaptation of the national treatment guideline 'acute cough'. Since 2012, amoxicillin is the preferred antibiotic for this indication, because of increasing resistance of *S. pneumoniae* for doxycycline. However, the decrease in use of doxycycline 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 prescriptions have increased in recent years.

**Table 3.1.1** 10-years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2008-2017 (source: SFK).

| ATC Group* | Therapeutic group | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| J01AA | Tetracyclines | 2.66 | 2.67 | 2.67 | 2.60 | 2.49 | 2.33 | 2.23 | 2.25 | 2.10 | 1.98 |
| J01CA | Penicillins with extended spectrum | 1.91 | 1.89 | 1.81 | 1.91 | 1.94 | 1.99 | 1.94 | 2.13 | 2.08 | 1.94 |
| J01CE | Beta-lactamase sensitive penicillins | 0.42 | 0.39 | 0.37 | 0.35 | 0.33 | 0.31 | 0.30 | 0.23 | 0.24 | 0.22 |
| J01CF | Beta-lactamase resistant penicillins | 0.36 | 0.38 | 0.38 | 0.39 | 0.41 | 0.41 | 0.44 | 0.43 | 0.46 | 0.46 |
| J01CR | Penicillins + beta-lactamase-inhibitors | 1.71 | 1.74 | 1.80 | 1.82 | 1.82 | 1.67 | 1.55 | 1.56 | 1.52 | 1.42 |
| J01D | Cephalosporins & carbapenems | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.03 | 0.03 |
| J01EA | Trimethoprim and derivatives | 0.21 | 0.21 | 0.20 | 0.20 | 0.19 | 0.17 | 0.16 | 0.14 | 0.14 | 0.13 |
| J01EE | Sulphonamides + trimethoprim | 0.36 | 0.35 | 0.35 | 0.34 | 0.33 | 0.29 | 0.28 | 0.28 | 0.28 | 0.29 |
| J01FA | Macrolides | 1.36 | 1.33 | 1.31 | 1.34 | 1.34 | 1.22 | 1.18 | 1.20 | 1.17 | 1.17 |
| J01FF | Lincosamides | 0.11 | 0.12 | 0.14 | 0.15 | 0.16 | 0.17 | 0.18 | 0.19 | 0.20 | 0.21 |
| J01GB | Aminoglycosides | 0.03 | 0.03 | 0.03 | 0.03 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 |
| J01MA | Fluoroquinolones | 0.89 | 0.86 | 0.85 | 0.82 | 0.80 | 0.76 | 0.79 | 0.77 | 0.75 | 0.73 |
| J01XE | Nitrofuran derivatives | 1.13 | 1.17 | 1.23 | 1.31 | 1.38 | 1.37 | 1.40 | 1.40 | 1.39 | 1.36 |
| J01XX01 | Fosfomycin | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.05 |
| | other | 0.03 | 0.04 | 0.05 | 0.05 | 0.05 | 0.04 | 0.04 | 0.04 | 0.02 | 0.05 |
| **J01** | **Antibiotics for systemic use (total)** | **11.24** | **11.21** | **11.23** | **11.37** | **11.34** | **10.83** | **10.58** | **10.72** | **10.44** | **10.06** |

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

**Figure 3.1.1** Use of antibiotics for systemic use (J01) in outpatients at ATC4 level, 2008-2017 (source: SFK).

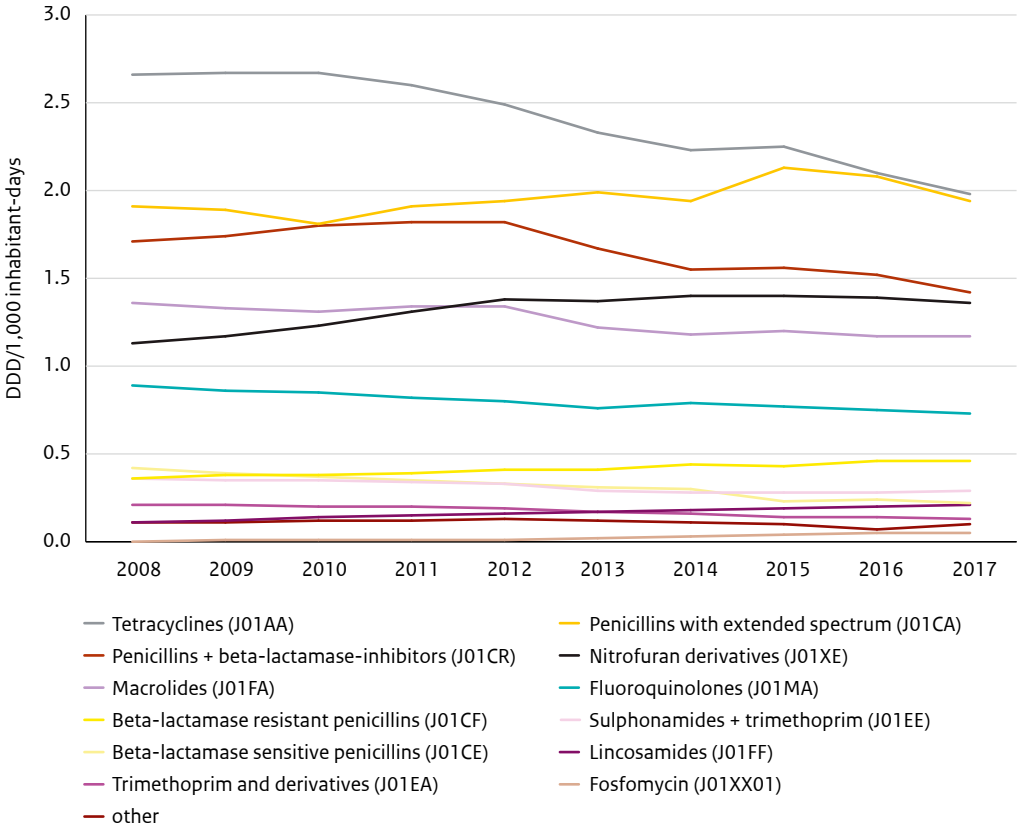
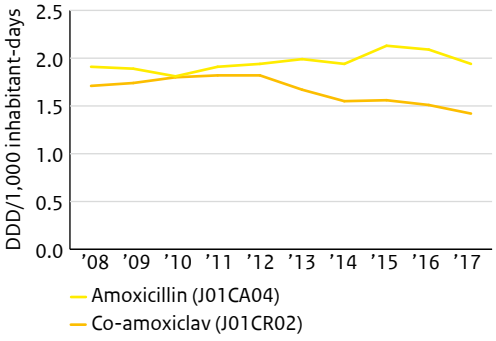
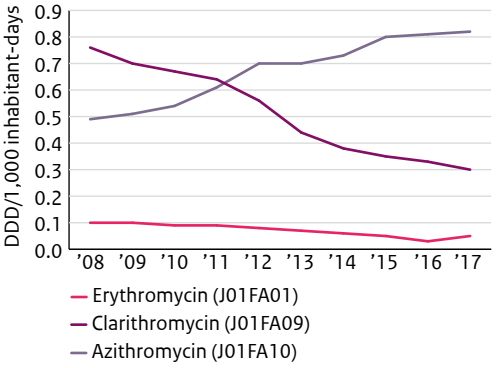
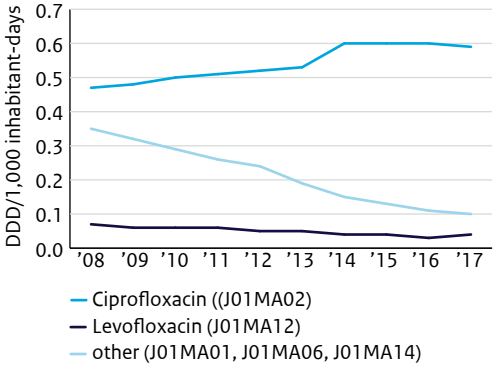
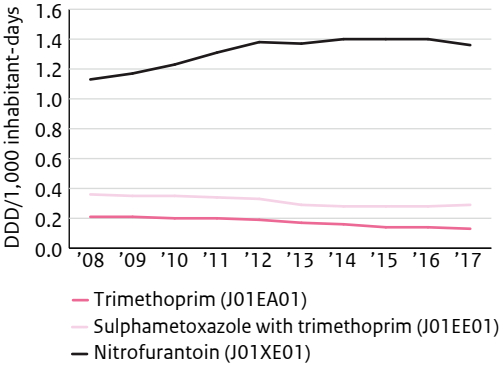


**Figure 3.1.2 A-D** Use of antibiotics for systemic use in outpatients at ATC5level, 2008-2017 (source: SFK).



## 3.2 Hospital care

### 3.2.1 Hospital antibiotic use in DDD

**Methods**

Data on the use of antibiotics in Dutch hospitals in 2016 were collected by means of a questionnaire distributed to all Dutch hospital pharmacists. Data were entered in the ABC-calculator (www.escmid.org) for conversion into DDDs, using the ATC/DDD classification from the WHO.[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). Dutch hospitals furthermore collected detailed data on antibiotic usage (according to the methodology proposed by the ECDC), combined with the PREZIES prevalence study on healthcare associated infections. 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 2016 were received from 62 hospitals, together with the annual number of bed-days and admissions. The inpatient use of antibiotics increased to 84.0 DDD/100 patient-days in 2016 (+6.2 DDD/100 patient-days). However, total inpatient use of antibiotics, when calculated as DDD/100 admissions, decreased with -4.0 from 330.1 to 326.1 (Table 3.2.1.1 and 3.2.1.2).

The use of penicillins and cephalosporins increased in 2016. The use of penicillins with extended spectrum in particular increased and reached a level of 10.9 DDD/100 patient days (+1.7 DDD/100 patient-days). Although in 2015 a decrease was seen in the use of beta-lactamase resistant penicillins, in 2016 the use was back at 8.7 DDD/100 patient-days (+0.9 DDD/100 patient-days). Another notable increase (+0.7 DDD/100 patient-days) was seen in the use of fluoroquinolones (Figure 3.2.1.1).
In addition, the use of second- and third-generation cephalosporins increased with 0.5 and 0.4 DDD/100 patient-days, respectively (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 2016, general hospitals used the lowest amount of antibiotics (82.3 DDD/100 patient-days), whereas university hospitals reported the highest overall antibiotic use (92.5 DDD/100 patient-days).

The use of combinations of penicillins with a beta-lactamase inhibitor, mainly amoxicillin with clavulanic acid, is still the highest in general hospitals, with 22.3% versus 15.5% and 12.3% in large teaching hospitals and university hospitals, respectively. Carbapenems, third generation cephalosporins and glycopeptides are primarily used in university hospitals, whereas most of the use of combinations of penicillins, penicillins with extended spectrum and nitrofuran derivates comes from general hospitals (Figure 3.2.1.5). The increase in use of fluoroquinolones in 2016 is caused by an increase in use in general and large teaching hospitals, whereas the use of fluoroquinolones in university hospitals remained stable (Figure 3.2.1.6).

In table 3.2.1.3 use of antimycotics (J02), antimycobacterials (J04) and antivirals (J05) in university hospitals is provided from the years 2007 to 2016, expressed in DDD/100 patient-days. In particular the use of antimycotics has increased in 2016, and has now reached a level of 14.23 DDD/100 patient-days (+1.53 DDD/100 patient-days).

In 2017, PREZIES data were received from 38 hospitals, including 8700 patients of which 2868 received antibiotics, with a total of 3752 prescriptions. Antibiotic use divided by surgical versus medical prophylaxis and hospital versus community acquired infections is depicted in Figure 3.2.1.8. For surgical prophylaxis, cefazolin was used in 61% of cases in 2017 as compared to 54% of cases in 2016. Use for medical prophylaxis was more diverse. Antibiotic use for hospital and community acquired infections in 2017 is comparable to the distribution in 2016.

**Discussion**

In 2016, antibiotic use in hospitals increased when expressed as DDD/100 patient-days and decreased when expressed as DDD/100 admissions. This could indicate further intensification of the use of antibiotics in hospitals, which may be the result of the increasing number of interventions, or the implementation of new and higher antibiotic dosing strategies in Dutch hospitals. Moreover, there is a large variation in total antibiotic use between Dutch hospitals and significant shifts are observed between different subgroups of antibiotics, e.g. in use of fluoroquinolones. In addition, the use of cephalosporins continued to rise in 2016.

**Table 3.2.1.1** Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2007-2016 (source: SWAB).

| ATC Group* | Therapeutic group | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| J01AA | Tetracyclines | 1.4 | 1.7 | 1.6 | 1.7 | 1.8 | 1.7 | 1.7 | 1.9 | 1.9 | 2.0 |
| J01CA | Penicillins with extended spectrum | 7.3 | 6.5 | 7.6 | 7.3 | 7.3 | 7.6 | 8.0 | 8.4 | 9.2 | 10.9 |
| J01CE | Beta-lactamase sensitive penicillins | 1.2 | 1.3 | 1.6 | 1.5 | 1.5 | 1.7 | 1.9 | 2.4 | 2.4 | 2.5 |
| J01CF | Beta-lactamase resistant penicillins | 5.7 | 6.4 | 6.6 | 6.8 | 6.7 | 7.1 | 8.1 | 8.7 | 7.7 | 8.7 |
| J01CR | Combinations of penicillins, incl. beta-lactamase-inhibitors | 14.5 | 16.2 | 16.5 | 16.0 | 15.8 | 15.0 | 14.8 | 14.5 | 14.3 | 14.6 |
| J01DB | First-generation cephalosporins | 2.6 | 2.6 | 3.0 | 3.0 | 3.5 | 3.6 | 3.7 | 4.4 | 4.6 | 4.6 |
| J01DC | Second-generation cephalosporins | 2.8 | 3.0 | 3.6 | 3.4 | 3.7 | 4.1 | 4.7 | 5.0 | 5.3 | 5.8 |
| J01DD | Third-generation cephalosporins | 3.0 | 3.2 | 3.5 | 3.7 | 3.9 | 4.4 | 5.0 | 5.7 | 5.5 | 5.9 |
| J01DH | Carbapenems | 0.8 | 1.0 | 1.1 | 1.2 | 1.4 | 1.5 | 1.7 | 1.6 | 1.7 | 1.8 |
| J01EA | Trimethoprim and derivatives | 0.5 | 0.4 | 0.4 | 0.5 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 |
| J01EE | Combinations of sulfonamides and trimethoprim, including derivatives | 2.3 | 2.4 | 2.0 | 2.0 | 1.9 | 1.8 | 1.9 | 1.9 | 1.8 | 2.1 |
| J01FA | Macrolides | 2.8 | 2.7 | 2.6 | 2.7 | 2.9 | 2.8 | 2.6 | 2.9 | 2.7 | 3.0 |
| J01FF | Lincosamides | 2.1 | 2.1 | 2.4 | 2.3 | 2.3 | 2.2 | 2.3 | 2.3 | 2.4 | 2.4 |
| J01GB | Aminoglycosides | 2.6 | 3.9 | 4.2 | 4.1 | 3.9 | 3.3 | 3.5 | 3.6 | 3.7 | 3.7 |
| J01MA | Fluoroquinolones | 7.6 | 8.8 | 9.3 | 9.0 | 9.2 | 8.9 | 8.6 | 9.0 | 8.4 | 9.1 |
| J01XA | Glycopeptides | 1.0 | 1.1 | 1.3 | 1.3 | 1.3 | 1.4 | 1.5 | 1.6 | 1.6 | 1.6 |
| J01XB | Polymyxins | 0.1 | 0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| J01XD | Imidazole derivatives | 1.8 | 1.7 | 1.8 | 1.9 | 2.2 | 2.3 | 2.6 | 2.6 | 2.6 | 2.8 |
| J01XE | Nitrofuran derivatives | 1.1 | 1.2 | 1.1 | 1.2 | 1.2 | 1.2 | 1.3 | 1.6 | 1.4 | 1.7 |
| J01XX08 | Linezolid | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| | other | 0.2 | 0.2 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **J01** | **Antibiotics for systemic use (total)** | **61.6** | **66.8** | **70.9** | **70.2** | **71.3** | **71.3** | **74.7** | **78.5** | **77.8** | **84.0** |
| | *expressed in DDD/100 admissions:* | | | | | | | | | | |
| **J01** | **Antibiotics for systemic use (total)** | **337.5** | **344.7** | **321.3** | **315.9** | **306.4** | **295.7** | **307.8** | **326.0** | **330.1** | **326.1** |

* *From the 2016 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 ATC4 level, 2007-2016 (source: SWAB).

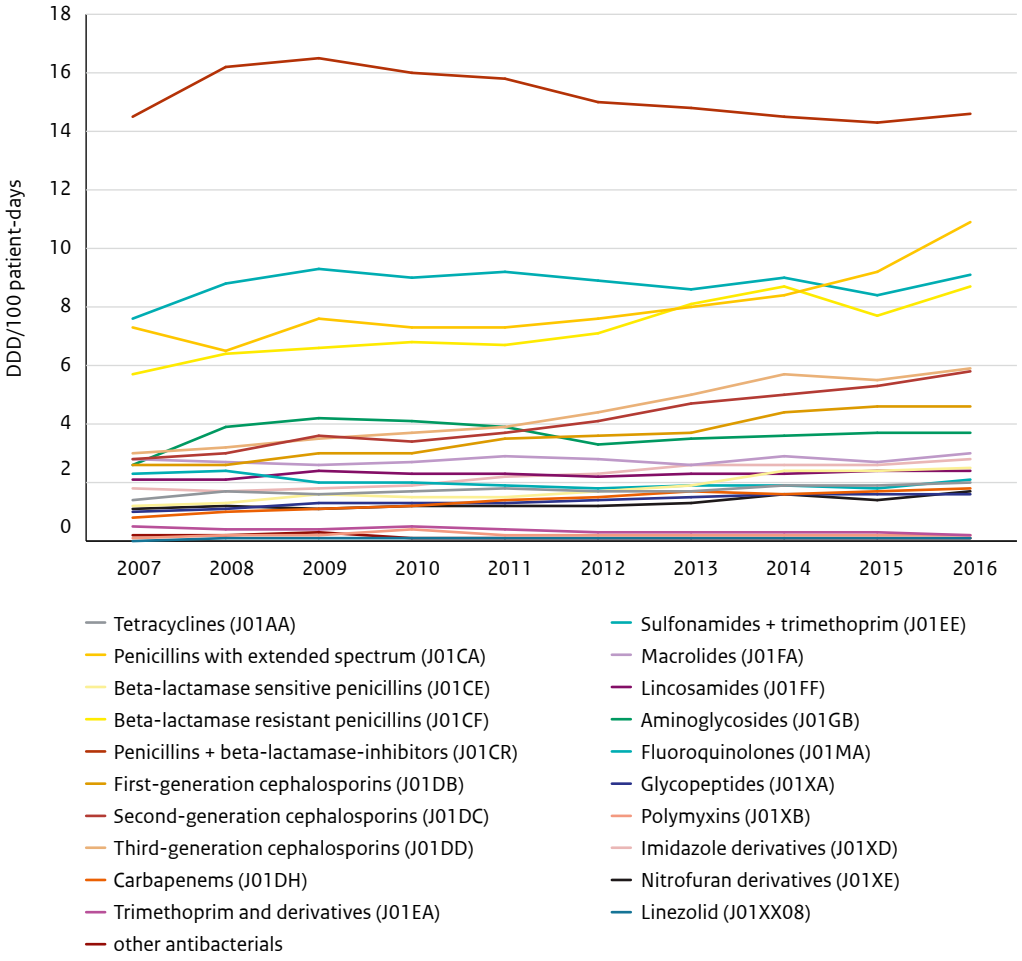


**Table 3.2.1.2** 10-years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1,000 inhabitant-days), 2007-2016 (source: SWAB).

| ATC Group* | Therapeutic group | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| J01AA | Tetracyclines | 0.025 | 0.023 | 0.025 | 0.027 | 0.026 | 0.024 | 0.022 | 0.023 | 0.025 | 0.022 |
| J01CA | Penicillins with extended spectrum | 0.110 | 0.101 | 0.111 | 0.110 | 0.103 | 0.100 | 0.099 | 0.101 | 0.118 | 0.125 |
| J01CE | Beta-lactamase sensitive penicillins | 0.020 | 0.019 | 0.023 | 0.023 | 0.020 | 0.023 | 0.023 | 0.028 | 0.028 | 0.029 |
| J01CF | Beta-lactamase resistant penicillins | 0.087 | 0.086 | 0.093 | 0.097 | 0.089 | 0.093 | 0.100 | 0.105 | 0.097 | 0.102 |
| J01CR | Penicillins + beta-lactamase-inhibitors | 0.233 | 0.229 | 0.241 | 0.256 | 0.223 | 0.211 | 0.199 | 0.187 | 0.186 | 0.171 |
| J01DB | First-generation cephalosporins | 0.035 | 0.034 | 0.040 | 0.042 | 0.045 | 0.049 | 0.047 | 0.052 | 0.055 | 0.053 |
| J01DC | Second-generation cephalosporins | 0.051 | 0.045 | 0.051 | 0.055 | 0.050 | 0.052 | 0.055 | 0.058 | 0.065 | 0.066 |
| J01DD | Third-generation cephalosporins | 0.037 | 0.040 | 0.047 | 0.050 | 0.050 | 0.057 | 0.062 | 0.066 | 0.067 | 0.068 |
| J01DH | Carbapenems | 0.010 | 0.011 | 0.014 | 0.015 | 0.018 | 0.019 | 0.020 | 0.019 | 0.021 | 0.020 |
| J01EA | Trimethoprim and derivatives | 0.009 | 0.007 | 0.007 | 0.009 | 0.006 | 0.005 | 0.004 | 0.003 | 0.003 | 0.003 |
| J01EE | Sulphonamides + trimethoprim | 0.033 | 0.029 | 0.030 | 0.030 | 0.026 | 0.024 | 0.024 | 0.022 | 0.021 | 0.024 |
| J01FA | Macrolides | 0.040 | 0.037 | 0.039 | 0.041 | 0.037 | 0.038 | 0.034 | 0.034 | 0.034 | 0.034 |
| J01FF | Lincosamides | 0.031 | 0.029 | 0.033 | 0.035 | 0.032 | 0.031 | 0.032 | 0.028 | 0.030 | 0.028 |
| J01GB | Aminoglycosides | 0.041 | 0.048 | 0.055 | 0.058 | 0.054 | 0.044 | 0.045 | 0.044 | 0.046 | 0.043 |
| J01MA | Fluoroquinolones | 0.124 | 0.139 | 0.129 | 0.138 | 0.127 | 0.124 | 0.116 | 0.112 | 0.112 | 0.106 |
| J01XA | Glycopeptide antibacterials | 0.011 | 0.012 | 0.015 | 0.016 | 0.017 | 0.017 | 0.018 | 0.018 | 0.019 | 0.019 |
| J01XB | Polymyxins | 0.006 | 0.008 | 0.009 | 0.006 | 0.003 | 0.002 | 0.003 | 0.002 | 0.003 | 0.002 |
| J01XD | Imidazole derivatives | 0.027 | 0.025 | 0.026 | 0.030 | 0.027 | 0.029 | 0.030 | 0.030 | 0.032 | 0.032 |
| J01XE | Nitrofuran derivatives | 0.018 | 0.016 | 0.017 | 0.018 | 0.015 | 0.018 | 0.016 | 0.018 | 0.018 | 0.018 |
| J01XX08 | Linezolid | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 |
|  | other antibiotics | 0.002 | 0.003 | 0.003 | 0.003 | 0.002 | 0.003 | 0.001 | 0.001 | 0.001 | 0.000 |
| **J01** | **Antibiotics for systemic use (total)** | **0.952** | **0.941** | **1.008** | **1.061** | **0.971** | **0.963** | **0.950** | **0.953** | **0.982** | **0.967** |

** From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

**Figure 3.2.1.2** Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hopitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC5 level, 2007-2016 (source: SWAB).

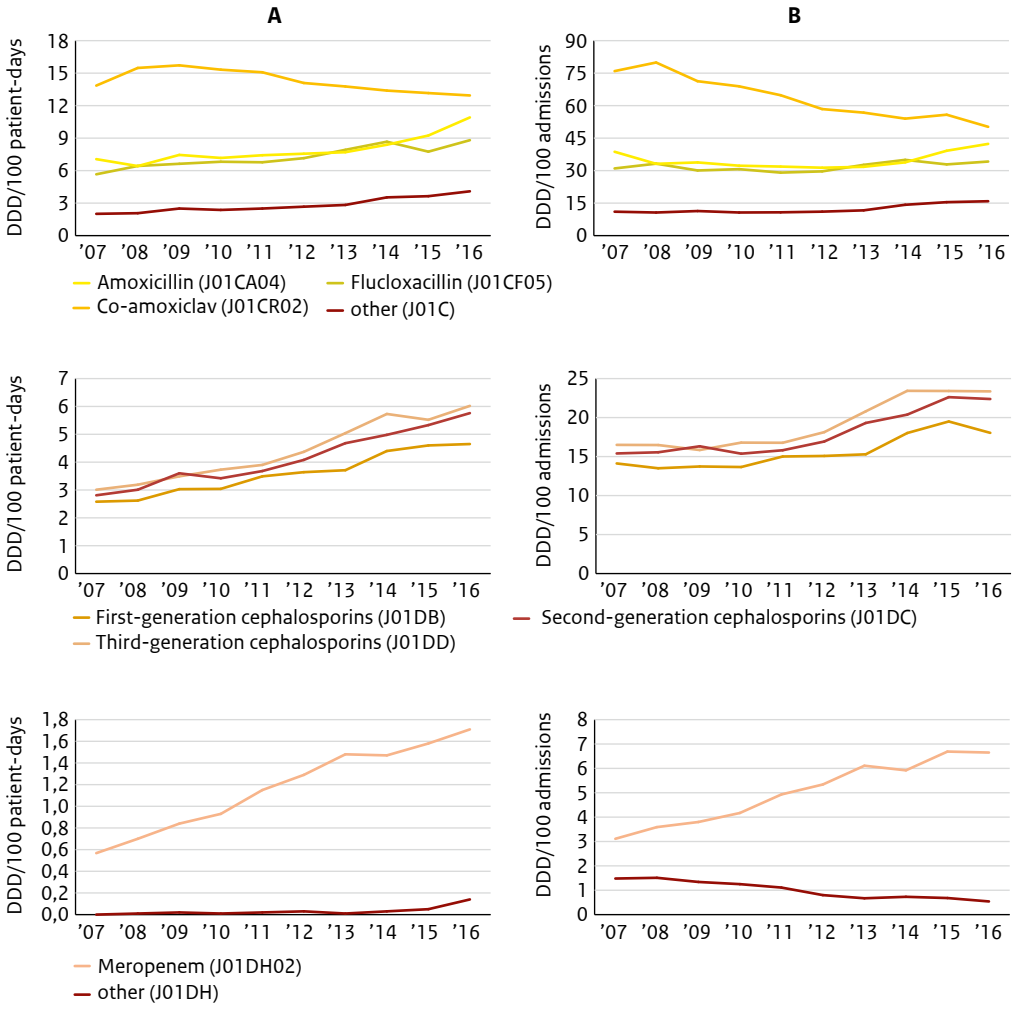


**Figure 3.2.1.2 (continued)** Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hopitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC5 level, 2007-2016 (source: SWAB).

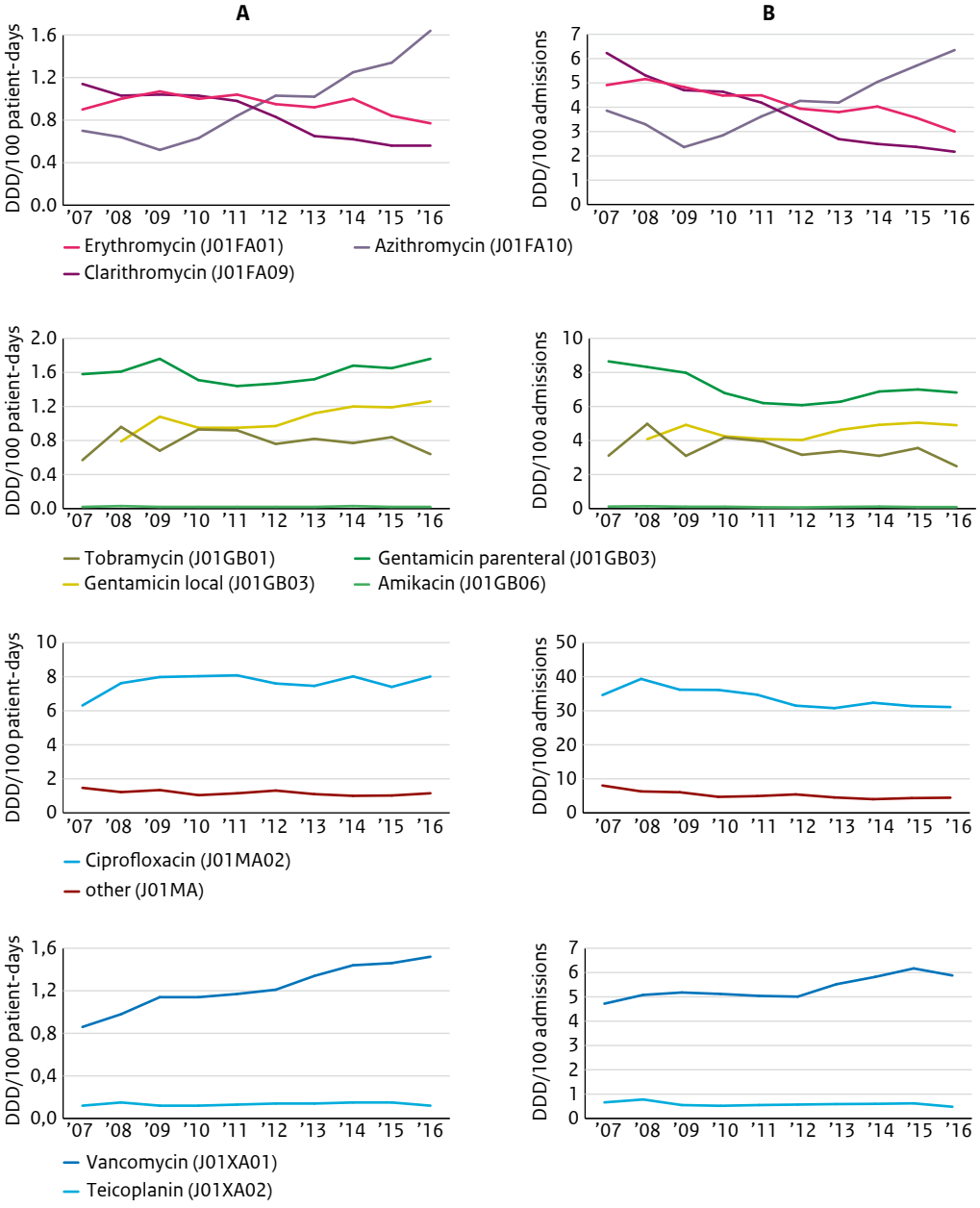


**Figure 3.2.1.3** Total systemic antibiotic use and comparison across university, large teaching and general hospitals in 2016 (source: SWAB).

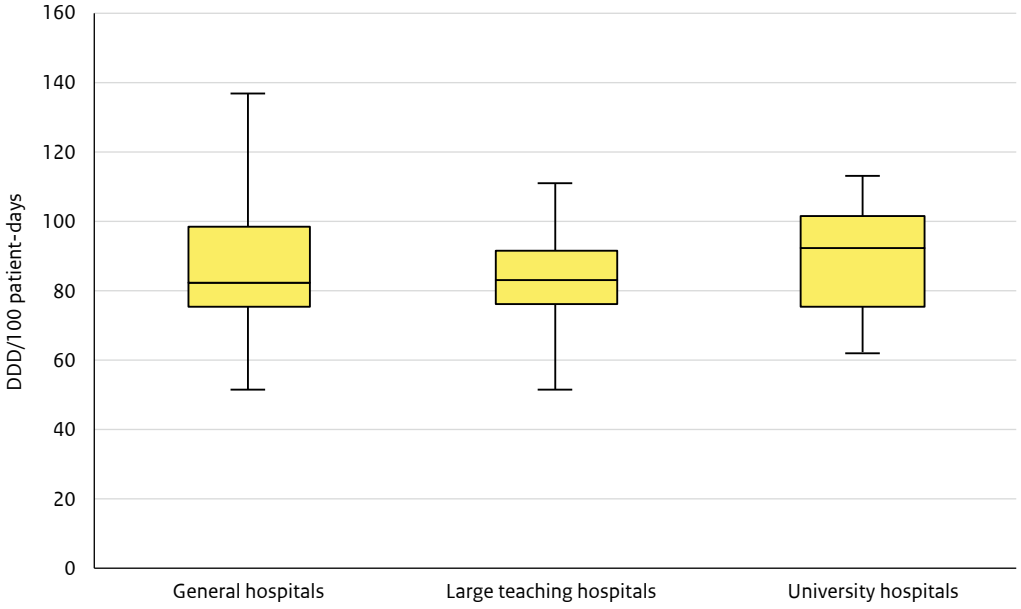


**Table 3.2.1.3** Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2007-2016 (source: SWAB).

| ATC Group* | Therapeutic group | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| J02AA01 | Antibiotics (amphotericin B) | 4.44 | 1.12 | 1.35 | 1.65 | 1.77 | 2.43 | 3.01 | 3.46 | 4.17 | 4.34 |
| J02AB02 | Imidazole derivatives (ketoconazole) | 0.12 | 0.11 | 0.08 | 0.15 | 0.09 | 0.10 | 0.06 | 0.24 | 0.34 | 0.04 |
| J02AC | Triazole derivatives** | 5.18 | 6.36 | 6.72 | 6.31 | 5.83 | 6.25 | 6.29 | 7.15 | 7.55 | 9.22 |
| J02AX | Other antimycotics for systemic use (mainly echinocandines) | 0.19 | 0.40 | 0.61 | 0.56 | 0.57 | 0.55 | 0.71 | 0.61 | 0.64 | 0.64 |
| **J02** | **Antimycotics for systemic use (total)** | **9.93** | **7.98** | **8.77** | **8.66** | **8.26** | **9.33** | **10.06** | **11.47** | **12.70** | **14.23** |
| 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) | 1.44 | 1.34 | 1.27 | 1.41 | 1.56 | 1.24 | 1.43 | 1.39 | 1.33 | 1.13 |
| J04AC | Hydrazides (mainly isoniazide) | 0.39 | 0.29 | 0.40 | 0.34 | 0.30 | 0.40 | 0.57 | 0.56 | 0.35 | 0.30 |
| J04AD | Thiocarbamide derivatives | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.12 | 0.14 |
| J04AK | Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol) | 0.38 | 0.31 | 0.34 | 0.37 | 0.26 | 0.31 | 0.16 | 0.28 | 0.19 | 0.15 |
| J04AM | Combinations of drugs for tuberculosis | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 | 0.04 | 0.07 | 0.11 |
| J04BA | Drug for treatment of leprosy (dapson) | 0.53 | 0.39 | 0.33 | 0.45 | 0.49 | 0.62 | 0.70 | 0.60 | 0.70 | 0.71 |
| **J04** | **Antimycobacterials for systemic use (total)** | **2.74** | **2.33** | **2.35** | **2.58** | **2.62** | **2.57** | **2.88** | **2.87** | **2.76** | **2.55** |
| J05AB | Nucleosides excl. Reverse transcriptase inhibitors (J05AB) | 1.72 | 2.00 | 2.22 | 2.02 | 2.18 | 2.24 | 2.33 | 2.71 | 2.76 | 2.97 |
| J05AD | Phosphonic acid derivatives (J05AD) | 0.06 | 0.11 | 0.13 | 0.10 | 0.10 | 0.15 | 0.12 | 0.16 | 0.14 | 0.20 |
| J05AE | Protease inhibitors (J05AE) | 0.70 | 0.92 | 0.75 | 0.78 | 0.55 | 0.81 | 0.63 | 0.40 | 0.33 | 0.30 |
| J05AF | Nucleoside reverse transcriptase inhibitors (J05AF) | 0.83 | 0.74 | 0.64 | 0.67 | 0.63 | 0.69 | 0.54 | 0.59 | 0.71 | 0.52 |
| J05AG | Non-nucleoside reverse transcriptase inhibitors (J05AG) | 0.20 | 0.25 | 0.23 | 0.22 | 0.14 | 0.18 | 0.16 | 0.18 | 0.23 | 0.22 |
| J05AH | Neuraminidase inhibitors (J05AH) | 0.02 | 0.05 | n.a.# | 0.21 | 0.42 | 0.19 | 0.49 | 0.16 | 0.30 | 0.43 |
| J05AR | Antivirals for the treatment of HIV, combinations (J05AR) | 0.33 | 0.52 | 0.55 | 0.76 | 0.69 | 0.91 | 0.89 | 0.94 | 0.95 | 0.99 |
| J05AX | Other antivirals (J05AX) | 0.00 | 0.06 | 0.06 | 0.15 | 0.17 | 0.24 | 0.29 | 0.22 | 0.33 | 0.46 |
| **J05** | **Antivirals for systemic use (total)** | **3.86** | **4.65** | **4.59** | **4.91** | **4.89** | **5.41** | **5.47** | **5.37** | **5.75** | **6.09** |

* *from the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *DDD alteration for posaconazole from 0.8 towards 0.3 DDD, recalculated since 2016*

\# *Total use not to be assesed because of alternative distribution during the pandemic*

**Figure 3.2.1.4** Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2016 (source: SWAB).

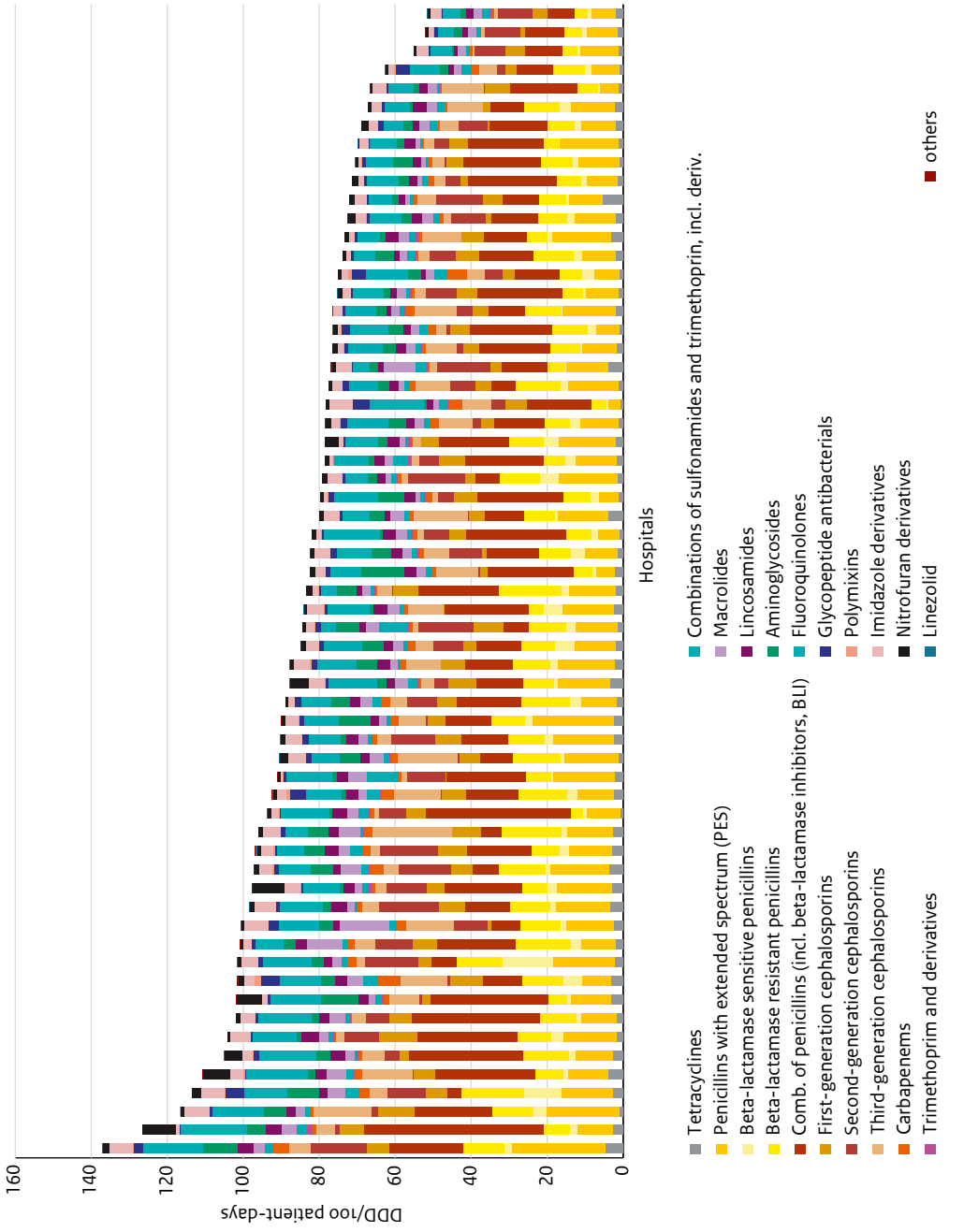


**Figure 3.2.1.5** Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2016 (source: SWAB).

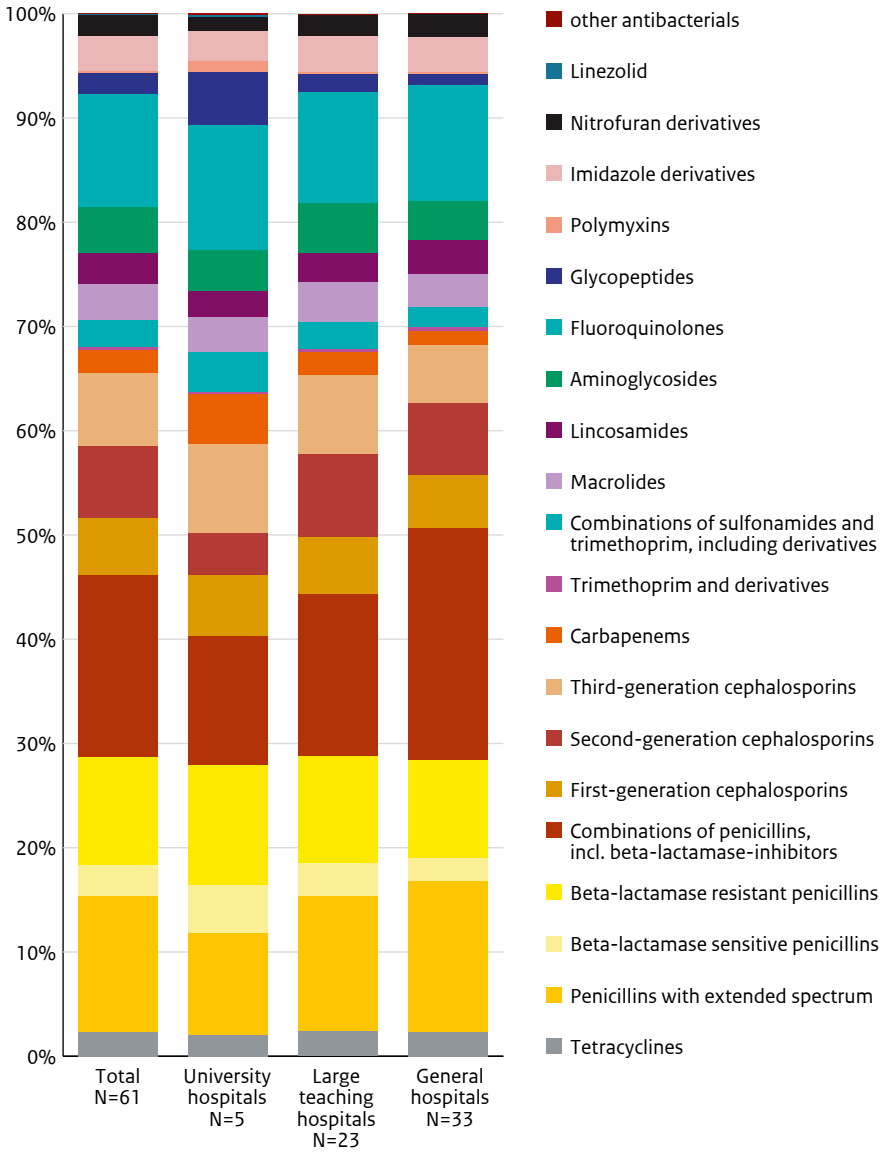


**Figure 3.2.1.6** Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2007-2016 (source: SWAB).

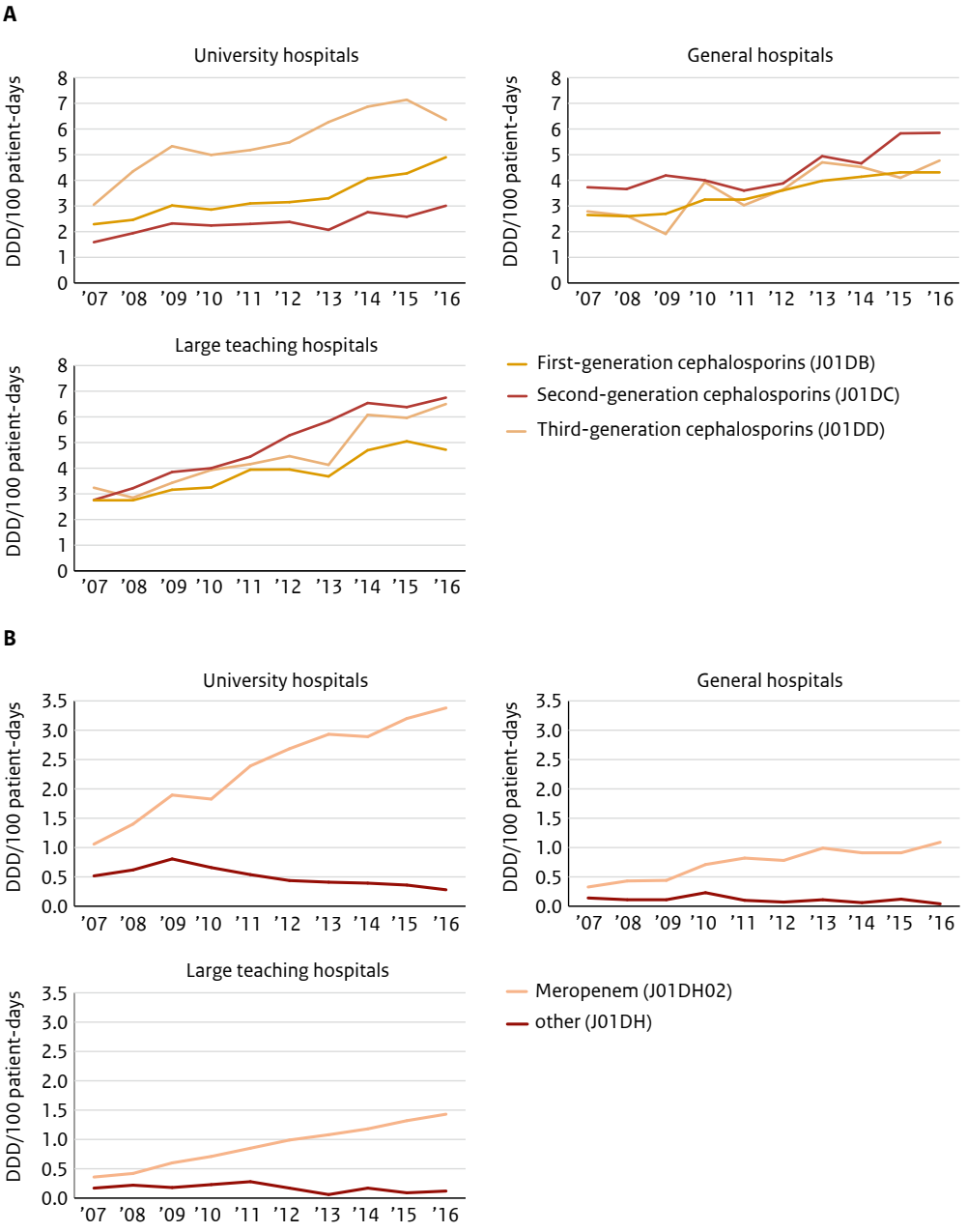
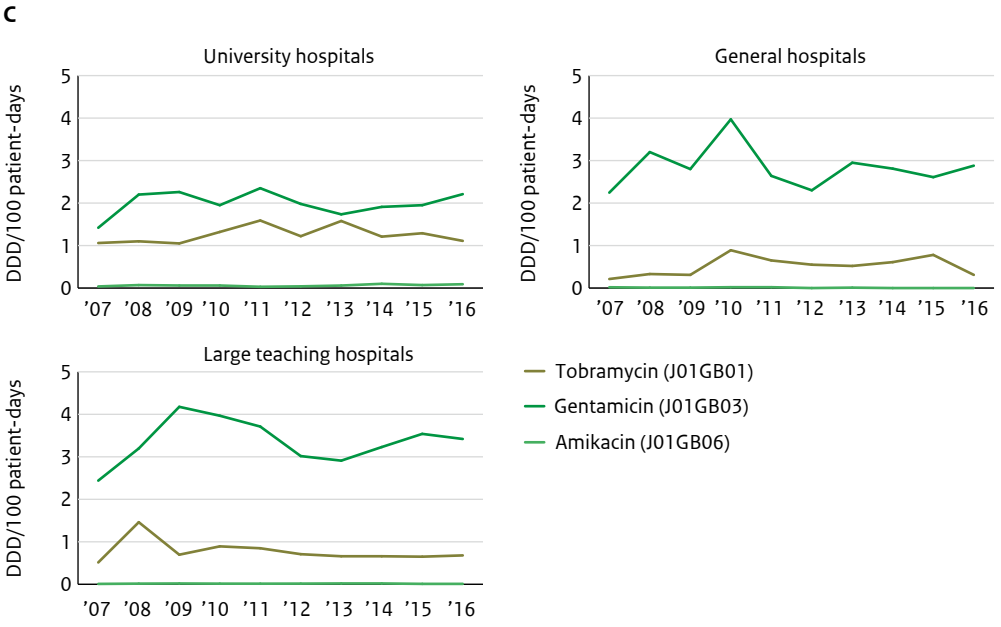


**Figure 3.2.1.6 (continued)** Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2007-2016 (source: SWAB).



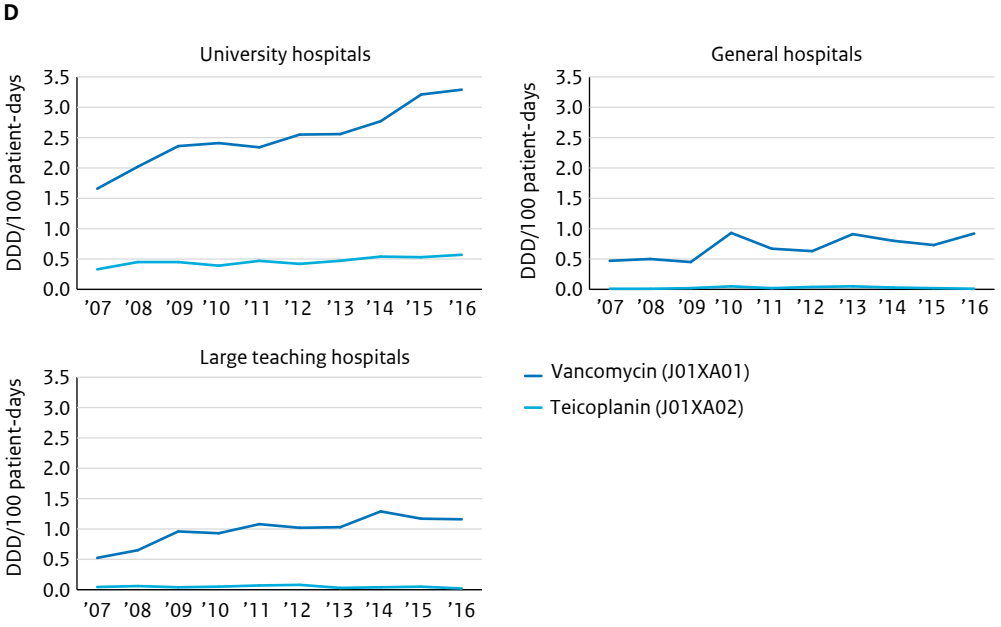



**Figure 3.2.1.6 (continued)** Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2007-2016 (source: SWAB).

**E**

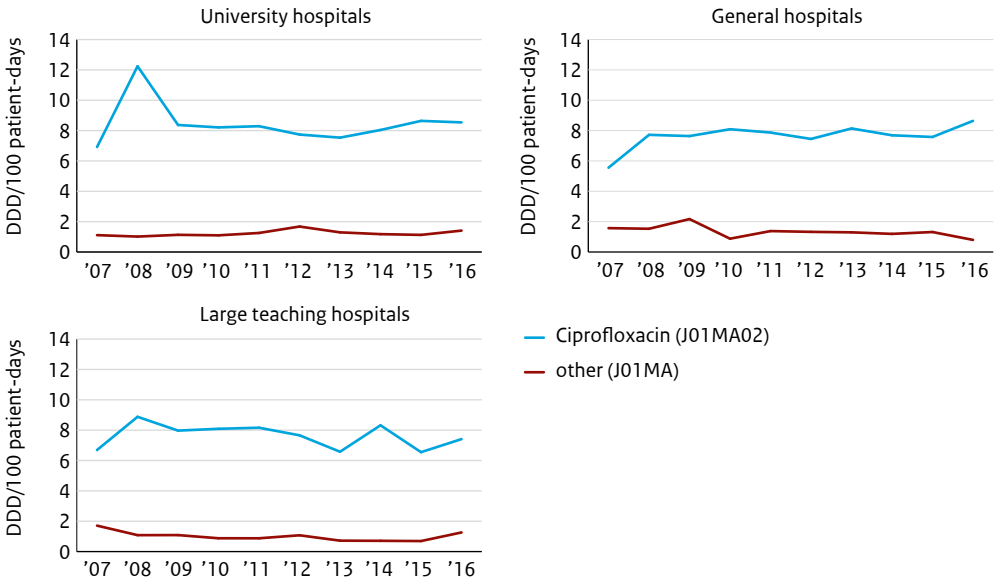


**Figure 3.2.1.7** Use of 1st, 2nd and 3rd generation cephalosporins in university, large teaching and general hospitals at ATC5 level in 2010-2016 (source: SWAB).

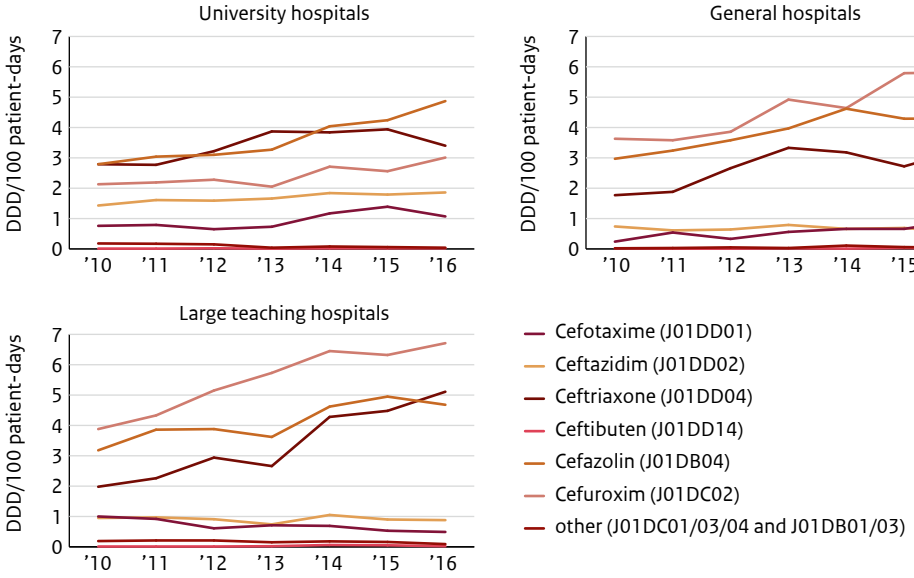
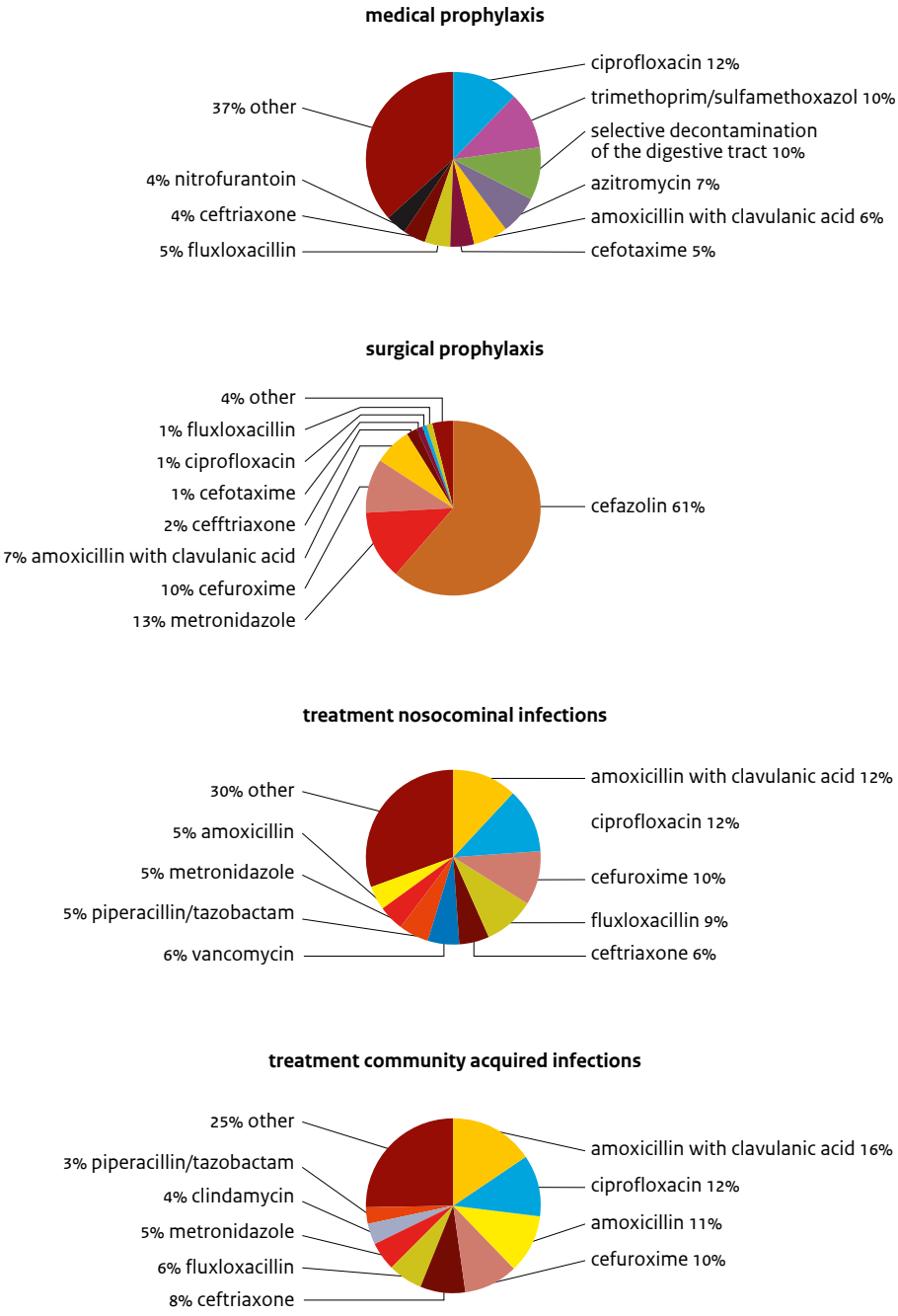


**Figure 3.2.1.8** Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2017 (source: PREZIES).



## 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 2016. From these data the number of DOT was calculated and expressed as DOT/100 patient-days. The method for calculation of the number of patient-days is described in chapter 3.2.1.

### Results

Data over 2016 was evaluated for 26 hospitals and number of DOT/100 patient-days for antibiotics mainly restricted to in-hospital use is shown in table 3.2.2.1. Especially the use of carbapenems, aminoglycosides and glycopeptides expressed as DOT/100 patient-days was lower compared to their use expressed as DDD/100 patient-days. In contrast, the number of DOT/100 patient-days for first-generation cephalosporins exceeded the number of DDD/100-patient days.

### 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 carbapenems, aminoglycosides and cephalosporins probably higher doses, that exceed the actual DDD, are given to individual patients. The lower DDD/100 patient-days as compared to the number of DOT/100 patient-days observed for first-generation cephalosporins could be explained by cefazolin which is mainly given as single dose for surgical prophylaxis. 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 compared to DDD/100 patient-days in 2016 for selected antibiotics.

| ATC Group* | Therapeutic group | DDD/100 patient-days | DOT/100 patient-days |
|---|---|---|---|
| J01DB | First-generation cephalosporins | 4.63 | 5.55 |
| J01DC | Second-generation cephalosporins | 5.75 | 5.10 |
| J01DD | Third-generation cephalosporins | 5.95 | 4.41 |
| J01DH | Carbapenems | 1.83 | 0.88 |
| J01GB | Aminoglycosides | 3.70 | 1.43 |
| J01XA | Glycopeptides | 1.62 | 0.97 |

** From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

## 3.3 Care in nursing homes

### Methods

All hospital pharmacists participating in the surveillance of antibiotic use in hospitals were asked to provide the antibiotic consumption data from nursing homes their pharmacy is serving. For each nursing home the amount of DDD/1,000 residents/day was calculated, while assuming occupancy of 100%, and their weighed mean was calculated.
In nursing homes of the SNIV network of RIVM, in 2017 a prevalence study was performed comparable to the intramural methods described above (3.2.1, PREZIES). Dutch nursing homes 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 nursing home on the registration date, were included. Only systemic antibacterials (ATC-code J01) were included, with a maximum of four concomitant substances per patient.

### Results

The antibiotic use of 9189 residents of nursing homes was included in data analysis for 2016. The size of nursing homes varied from 39 to 1700 residents per home, with a mean of 475 residents. The mean antibiotic use in nursing homes decreased by 8.5 DDD/1,000 residents/day to 56.8 DDD/1,000 residents/day. The use varied highly with a minimum of 15 and a maximum of 128 DDD/1,000 residents/day. Especially, the use of combinations of penicillins including beta-lactamase-inhibitors decreased and reached a level of 15.3 DDD/1,000 residents/day in 2016 (-4.4 DDD/1,000 residents/day). Also the use of nitrofuran derivatives decreased by -2.7 DDD/1,000 residents/day to 9.0 DDD/1,000 residents/day. The use of tetracyclines increased again to 4.9 DDD/1,000 residents/day (Table 3.3.1). Figure 3.3.1 depicts antibiotics used in the prevalence study in 78 nursing homes in 2017. A total of 6228 residents were participating, of which 393 patients on antibiotics, with a total of 457 prescriptions. Nitrofurantoin is used the most (23% of total antibiotic use) and mainly represents prophylactic use and use in revalidation and psychogeriatric care (Figure 3.3.1A and B).

### Discussion

Although the antibiotic use in nursing homes decreased in 2016, compared with previous years, more or less the same pattern of usage is seen. Amoxicillin with clavulanic acid, nitrofuran derivatives and fluoroquinolones are still the most widely used antibiotics in nursing homes. Nevertheless, the observed decline in the use of combinations of penicillins with beta-lactamase-inhibitors in 2016 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 broad range of use suggests that there is considerable variation in antimicrobial use in nursing homes across the Netherlands. However, details about differences in characteristics of residents and care provided (rehabilitation, palliative care) are still lacking. As nursing home patients are frequently transferred to acute care hospitals, and vice versa, more information should be available in order to optimise antimicrobial use and limit the development of antimicrobial resistance. The results of the point prevalence study (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.

## References


[1] WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2011. WHO Collaborating Centre; Oslo, Norway, 2012.

[2] Kwint HM, Van der Linden PD, Roukens MMB et al. Intensification of antibiotic use within acute care hospitals in the Netherlands, J of antimicrob chemother 2012: 2283-2288.


**Table 3.3.1** Distribution of the use of antibiotics (J01) in nursing homes, expressed as DDD/1,000 residents/day, 2011-2016 (source: SWAB).

| ATC Group* | Therapeutic group | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|---|---|---|---|---|---|---|---|
| J01AA | Tetracyclines | 5.4 | 6.8 | 7.2 | 4.7 | 3.9 | 4.9 |
| J01CA | Penicillins with extended spectrum | 4.9 | 6.6 | 5.0 | 5.0 | 5.5 | 5.8 |
| J01CE | Beta-lactamase sensitive penicillins | 0.3 | 0.2 | 0.4 | 0.4 | 0.7 | 0.3 |
| J01CF | Beta-lactamase resistant penicillins | 2.5 | 3.7 | 1.6 | 1.3 | 2.7 | 1.8 |
| J01CR | Combinations of penicillins, incl. beta-lactamase-inhibitors | 18.6 | 18.1 | 18.9 | 17.7 | 19.6 | 15.3 |
| J01DB | First-generation cephalosporins | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 |
| J01DC | Second-generation cephalosporins | 0.1 | 0.0 | 0.1 | 0.1 | 0.4 | 0.2 |
| J01DD | Third-generation cephalosporins | 0.5 | 1.2 | 1.0 | 0.5 | 1.0 | 0.5 |
| J01DH | Carbapenems | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| J01EA | Trimethoprim and derivatives | 2.3 | 2.0 | 2.7 | 2.2 | 1.4 | 1.7 |
| J01EE | Combinations of sulfonamides and trimethoprim, including derivatives | 3.5 | 2.7 | 1.3 | 1.5 | 1.8 | 1.4 |
| J01FA | Macrolides | 2.1 | 2.4 | 2.4 | 2.1 | 2.2 | 2.6 |
| J01FF | Lincosamides | 3.7 | 4.5 | 2.2 | 1.9 | 2.6 | 3.9 |
| J01GB | Aminoglycosides | 0.1 | 0.1 | 0.0 | 0.1 | 0.4 | 0.1 |
| J01MA | Fluoroquinolones | 10.5 | 11.2 | 7.9 | 8.6 | 9.5 | 8.1 |
| J01XA | Glycopeptides | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 |
| J01XB | Polymyxins | 0.4 | 0.4 | 0.0 | 0.0 | 0.1 | 0.2 |
| J01XD | Imidazole derivatives | 0.1 | 0.1 | 0.0 | 0.1 | 0.2 | 0.1 |
| J01XE | Nitrofuran derivatives | 10.8 | 12.8 | 13.7 | 10.6 | 11.7 | 9.0 |
| J01XX08 | Linezolid | 0.1 | 0.1 | 0.0 | 0.0 | 0.4 | 0.0 |
| | other antibacterials | 0.7 | 0.7 | 0.1 | 0.0 | 0.7 | 0.7 |
| **J01** | **Antibiotics for systemic use (total)** | **67.0** | **73.8** | **64.7** | **57.1** | **65.3** | **56.8** |

** From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

**Figure 3.3.1A** Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2017 (source: SNIV).

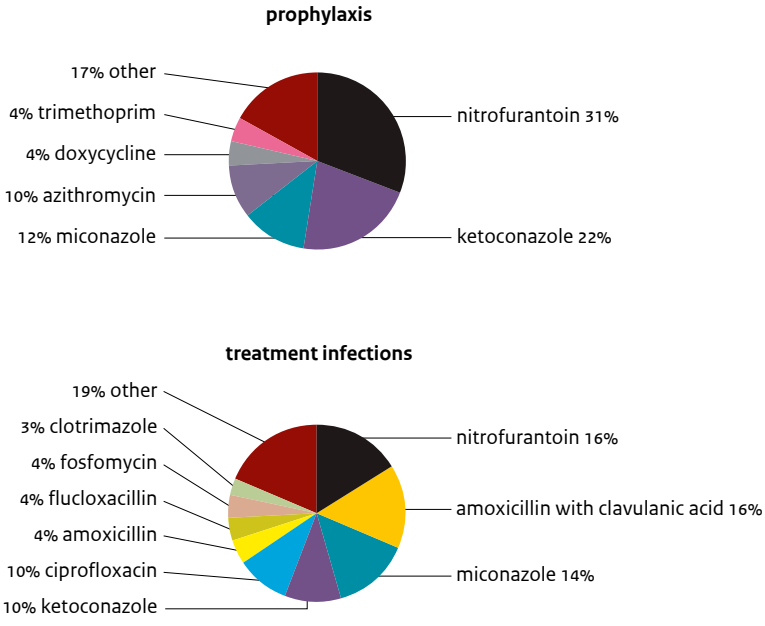
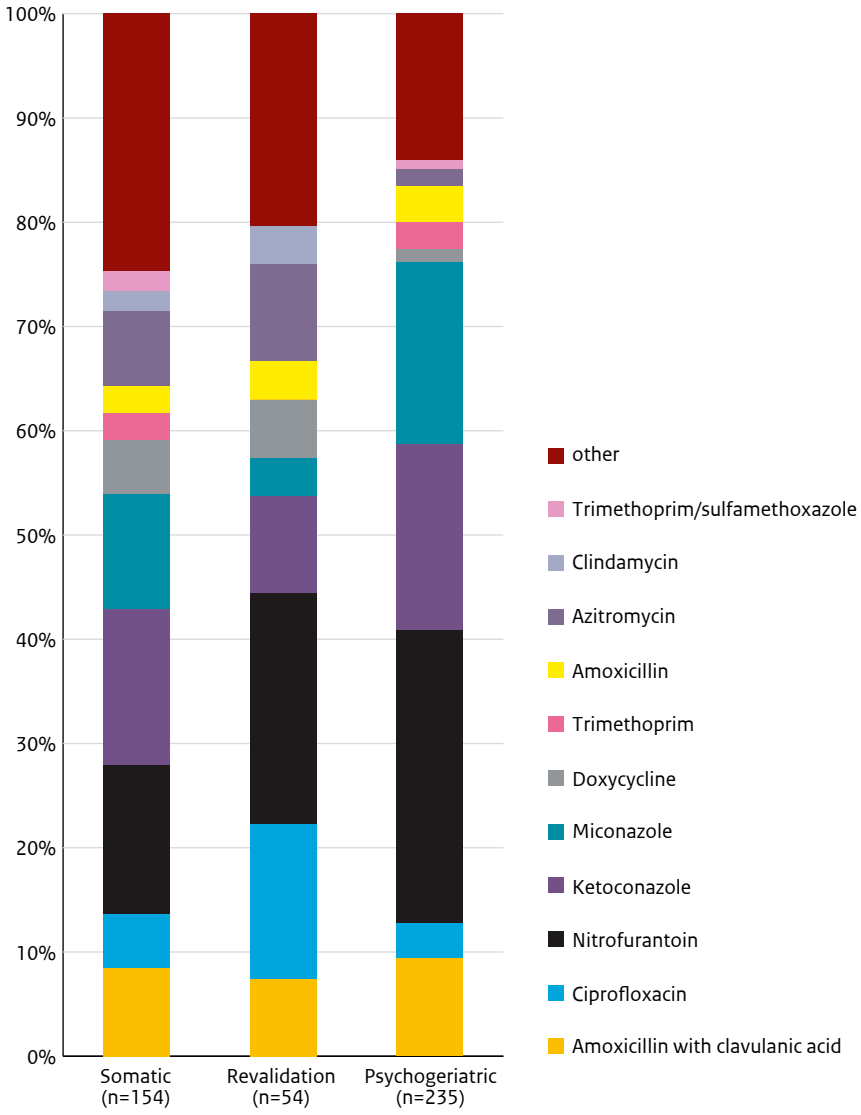


**Figure 3.3.1B** Comparison of the distribution of antibiotic usage (J01) in nursing homes in somatic, revalidation and psychogeriatic care in 2017 (source: SNIV).



# 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 2017, 42 laboratories were connected to ISIS-AR, of which 29 laboratories provided complete data over the five most recent years (2013 to 2017). Four of these laboratories exclusively served university hospitals, 24 laboratories served non-university hospitals, general practitioners and long-term care facilities and one laboratory only served general practitioners and long-term care facilities. To avoid bias in time trends due to incomplete data we selected data from these 29 laboratories for most analyses in the current report. We calculated resistance percentages and linear time trends over the five most recent years (2013 to 2017) for the most prevalent pathogens in combination with their main antimicrobial treatment options. For calculation of resistance percentages for pathogens for which no time trends were calculated (for details see below) we used data from 34 laboratories for which at least complete data for the year 2017 were available, and that were known to use EUCAST antibiotic susceptibility testing (AST) guidelines (six serving university hospitals, 26 serving non-university hospitals, general practitioners, and long-term care facilities, and 2 serving general practitioners and long-term care facilities only). For *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* isolates from general practitioners' patients an extra analysis was conducted to calculate resistance to a selection of antibiotics in 2017 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 28 non-university laboratories for which at least complete data for the year 2017 were available.

### Selection of isolates

Resistance levels and, if applicable, time trends were calculated by site; i.e. general practice, outpatient departments, inpatient departments (excl. intensive care units), intensive care units, urology departments, and long-term care facilities. 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 wound/pus 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), resistance levels were calculated based on antimicrobial susceptibility results in isolates from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound/pus, except for resistance to nitrofurantoin among *E. faecalis* in inpatient departments (excl. intensive care units), which was based on urinary isolates only. 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, for the analysis on respiratory pathogens (*Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, chapter 4.3.6) we selected isolates from blood, cerebrospinal fluid, higher respiratory tract, and lower respiratory tract.
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 samples that were taken for screening and inventory purposes. 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. Finally, for sufficient representativeness of the results, the resistance level and time trend of each pathogen-agent combination was only calculated if at least 50% of laboratories could be included, and data on at least 100 isolates were available for analysis.

### Calculation of resistance levels

The percentage of resistant isolates ('R') was calculated. To avoid bias due to differences in breakpoint guidelines and expert rules used in the participating laboratories, these calculations were conducted using reinterpreted MIC values from automated susceptibility testing systems or gradient tests according to EUCAST breakpoints, version 7.1. However, reinterpretation of MIC-values does not take into account differences in testing methods that result in higher or lower MIC-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 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 organism. 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*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus aureus*, and coagulase-negative *Staphylococcus* spp. including *Staphylococcus epidermidis*) at least 75% of the reported MICs were reinterpretable according to EUCAST clinical breakpoints version 7.1. When reinterpretation could not be achieved, this was because of missing crude data or MICs that were not compatible with EUCAST breakpoints version 7.1. For *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, *Haemophilus influenzae*,and *Moraxella catarrhalis* less than 75% of MICs could be reinterpreted. Therefore, for these pathogens calculation of resistance percentages was based on S/I/R interpretations as reported by laboratories known to have used EUCAST AST guidelines in 2017.
Because data on inducible clindamycin resistance tests was often not available in ISIS-AR, resistance levels for clindamycin including inducible resistance are 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, resistance to flucloxacillin in *S. aureus* and coagulase-negative *Staphylococcus* spp. was estimated 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, resistance and non-susceptibility percentages were estimated based on laboratory S/I/R interpretation for oxacillin, or, if the isolate was intermediate or resistant to oxacillin, on the 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, 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, 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, resistance was defined as resistance to both agents.
For *S. aureus* and coagulase-negative *Staphylococcus* spp. resistance to ciprofloxacin was calculated 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). Enterobacteriaceae except *Enterobacter cloacae* were considered an HRMO if they were resistant to cefotaxime/ceftriaxone and/or ceftazidime as indicator agents for the production of Extended-spectrum beta-lactamase (ESBL), or resistant to both fluoroquinolones and aminoglycosides. *E. cloacae* was considered an HRMO if resistant to both fluoroquinolones and aminoglycosides. *P. aeruginosa* was considered an HRMO if resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin-tazobactam. Finally, for *Acinetobacter* spp. HRMO was defined as resistance to imipenem or meropenem or resistance to

both fluoroquinolones and aminoglycosides.
In addition, for Enterobacteriaceae isolates from general practices, outpatient departments, urology departments, and long-term care facilities, multidrug resistance was calculated, defined as resistance to the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole combined.

**Calculation of time trends**
In addition to resistance levels in 2017, we calculated time trends over the five most recent years (2013 to 2017), 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 on resistance levels that were based on reinterpretation of crude MIC-values (for criteria see 'Calculation of resistance levels'-section above), except for trends in resistance for flucloxacillin and clindamycin including inducible resistance in *S. aureus*. We do not expect spurious time trends in resistance for these two pathogen-drug combinations, even though resistance percentages were based on laboratory S/I/R interpretation for cefoxitin or oxacillin/flucloxacillin and clindamycin respectively. In EUCAST AST guidelines, breakpoints for these combinations were not changed between 2013 and 2017 and use of different versions of the guideline will therefore not cause a biased time trend. However, for coagulase-negative *Staphylococcus* spp. breakpoints for cefoxitin were changed in 2017, and a time trend for flucloxacillin resistance was not calculated.
Two sided p-values <0.05 were considered statistically significant. If resistance in 2017 was below 10%, a change of ≥2.5% in the last 5 years was considered clinically relevant. If resistance in 2017 was 10% or higher, a change of ≥5% was considered clinically relevant. To assess clinical relevance the predicted resistance levels from the logistic model were used. 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 2013 to 2017 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 geographical distribution of laboratories is presented by connection status and status of inclusion in the analyses in chapter 4.2 to 4.4 (see methods section for inclusion criteria). In figure 4.1.2.2 the smoothed distribution of isolates over the country, based on percentage of residents for whom at least one isolate was included in the analyses in chapters 4.2 to 4.4, is shown by 4-digit postal code area. In table 4.1.2.1 descriptive characteristics are presented for connected laboratories, with data from included and excluded laboratories separately. In table 4.1.2.2 descriptive characteristics of isolates from included laboratories only are listed by pathogen.

**Figure 4.1.2.1** Geographical distribution of laboratories by connection status and status of inclusion in the analyses in chapter 4.2 to 4.4, within regional cooperative networks.

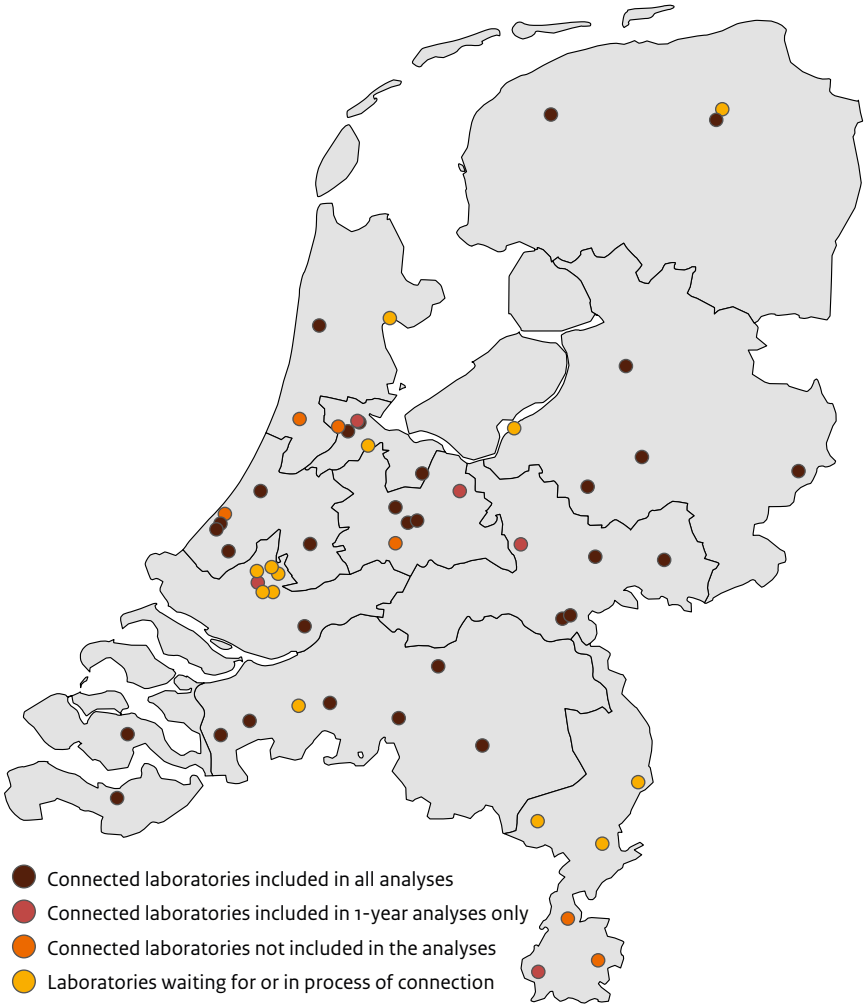


**Figure 4.1.2.2** Smoothed geographical distribution of isolates, based on percentage of residents for whom at least one isolate was included in the analyses in chapters 4.2 to 4.4, by 4-digit postal code area, within regional cooperative networks.

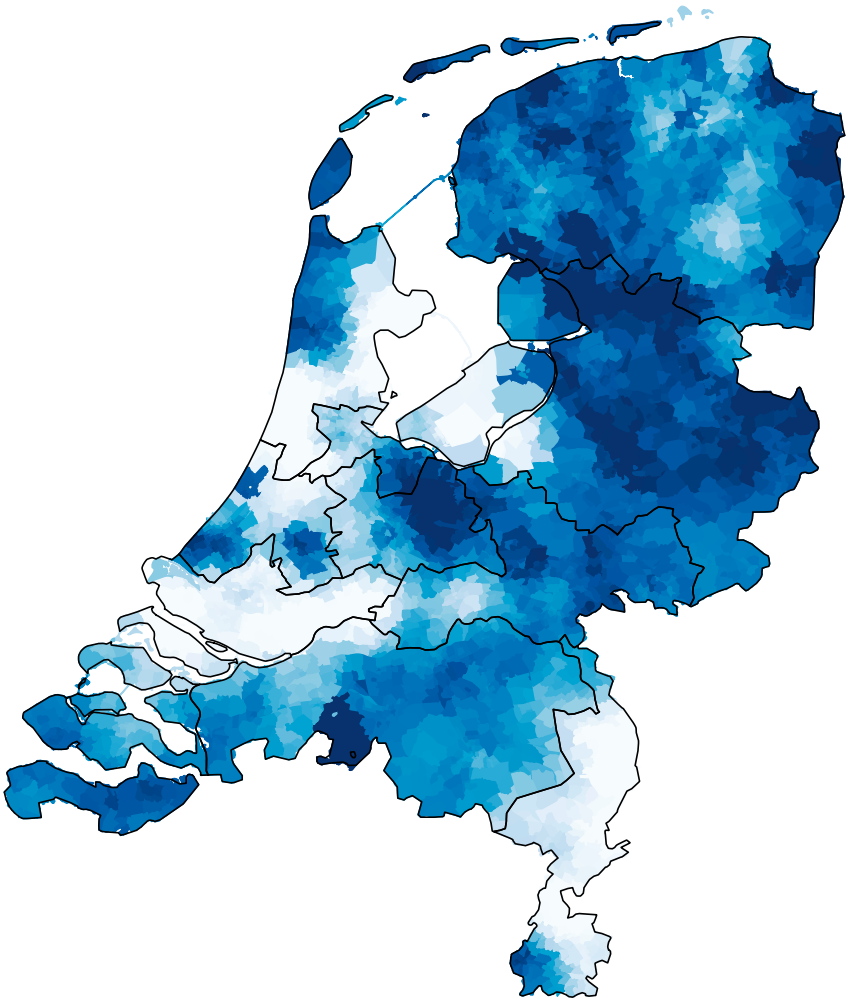


**Table 4.1.2.1** Characteristics of isolates in 2017 from 34 laboratories for which data were included in the analyses in chapters 4.2 through 4.4 and 8 laboratories for which data were excluded.

| | Included | Excluded |
|---|---|---|
| Total number of isolates | 440,297 | 42,241 |
| Mean number of isolates per laboratory | 12,950 | 8,448 |
| | | |
| **Pathogen (%)** | | |
| *E. coli* | 35 | 29 |
| *K. pneumoniae* | 5 | 5 |
| *E. cloacae* | 2 | 2 |
| *P. mirabilis* | 5 | 4 |
| Other Enterobacteriaceae[1] | 9 | 8 |
| *P. aeruginosa* | 5 | 5 |
| *Acinetobacter* spp. | 1 | 1 |
| *M. catarrhalis* | 1 | 1 |
| Other non-fermenters[2] | 1 | 1 |
| *H. influenzae* | 2 | 3 |
| Other Gram-negatives[3] | 1 | 1 |
| *E. faecalis* | 6 | 8 |
| *E. faecium* | 1 | 2 |
| *S. aureus* | 12 | 12 |
| CNS | 5 | 7 |
| *S. pneumoniae* | 1 | 2 |
| Other Gram-positives[4] | 8 | 9 |
| | | |
| **Sex of patient (%)** | | |
| Male | 40 | 43 |
| Female | 60 | 57 |
| | | |
| **Type of care (%)** | | |
| General practitioners | 42 | 26 |
| Outpatient departments | 22 | 30 |
| Inpatient departments (excl. Intensive Care Units) | 28 | 37 |
| Intensive Care Units | 4 | 4 |
| Long-term care facilities | 4 | 3 |
| | | |
| **Age category of patient in years (%)** | | |
| 0-4 | 4 | 3 |
| 5-18 | 5 | 3 |
| 19-64 | 37 | 35 |
| >65 | 55 | 58 |

**Table 4.1.2.1 (continued)** Characteristics of isolates in 2017 from 34 laboratories for which data were included in the analyses in chapters 4.2 through 4.4 and 8 laboratories for which data were excluded.

| | Included | Excluded |
|---|---|---|
| **Isolate source (%)** | | |
| Blood | 6 | 7 |
| Lower respiratory tract | 8 | 10 |
| Urine | 59 | 54 |
| Wound/Pus | 14 | 13 |
| Other | 14 | 15 |
| | | |
| **Type of hospital (hospital isolates only, %)** | | |
| General | 31 | 53 |
| Top clinical | 47 | 47 |
| University hospital | 21 | 0 |

*CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis*
*GP = general practitioners*
*LTCF = long-term care facilities*
*Only the first diagnostic isolate per patient was included*
[1] *Klebsiella spp. (non-pneumoniae), Citrobacter spp., Serratia spp., Morganella spp., Enterobacter spp. (non-cloacae), Proteus spp. (non-mirabilis), Salmonella spp., Providencia spp., Pantoea spp., Hafnia spp., Shigella spp., Yersinia spp., Escherichia spp. (non-coli), Cronobacter spp.*
[2] *S. maltophilia, Pseudomonas spp. (non-aeruginosa)*
[3] *C. jejuni, C. lari, B. fragilis, H. pylori, N. meningitidis*
[4] *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. (non-faecalis, non-faecium), M. tuberculosis, Staphylococcus spp. (non-aureus, non-CNS), L. monocytogenes*

**Table 4.1.2.2** Characteristics of 368,644 isolates included in the analyses in chapters 4.2 through 4.4, by pathogen.

| | *E. coli* | *K. pneumoniae* | *E. cloacae* | *P. mirabilis* | *P. aeruginosa* | *Acinetobacter* spp. | *E. faecalis* | *E. faecium* | *S. aureus* | CNS | *S. pneumoniae* | *H. influenzae* | *M. catarrhalis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of isolates | 159,619 | 24,983 | 8,899 | 21,072 | 22,030 | 4,574 | 25,379 | 5,373 | 53,931 | 23,998 | 5,150 | 10,585 | 3,051 |
| **Sex of patient (%)** | | | | | | | | | | | | | |
| Male | 28 | 34 | 54 | 41 | 53 | 51 | 53 | 52 | 52 | 51 | 55 | 52 | 52 |
| Female | 72 | 66 | 46 | 59 | 47 | 49 | 47 | 48 | 48 | 49 | 45 | 48 | 48 |
| **Type of care (%)** | | | | | | | | | | | | | |
| General practitioners | 57 | 46 | 29 | 46 | 30 | 47 | 37 | 9 | 24 | 17 | 8 | 12 | 12 |
| Outpatient departments | 15 | 20 | 24 | 19 | 30 | 25 | 23 | 10 | 40 | 13 | 27 | 40 | 38 |
| Inpatient departments (excl. Intensive Care Units) | 21 | 26 | 37 | 24 | 31 | 22 | 32 | 59 | 30 | 56 | 55 | 40 | 43 |
| Intensive Care Units | 2 | 3 | 6 | 2 | 4 | 4 | 3 | 20 | 4 | 13 | 10 | 6 | 6 |
| Long-term care facilities | 4 | 6 | 3 | 10 | 5 | 2 | 5 | 3 | 2 | 1 | 0 | 1 | 2 |
| **Age category of patient in years (%)** | | | | | | | | | | | | | |
| 0-4 | 3 | 2 | 4 | 3 | 2 | 5 | 3 | 1 | 5 | 5 | 7 | 8 | 10 |
| 5-18 | 6 | 2 | 2 | 2 | 5 | 5 | 2 | 1 | 7 | 5 | 3 | 4 | 3 |
| 19-64 | 35 | 28 | 30 | 22 | 30 | 32 | 28 | 31 | 44 | 42 | 34 | 35 | 30 |
| >65 | 56 | 69 | 64 | 73 | 63 | 59 | 66 | 67 | 44 | 48 | 55 | 52 | 57 |
| **Isolate source (%)** | | | | | | | | | | | | | |
| Blood | 3 | 4 | 4 | 1 | 2 | 3 | 3 | 12 | 4 | 42 | 27 | 2 | 1 |
| Lower respiratory tract | 2 | 4 | 10 | 3 | 18 | 8 | 0 | 1 | 9 | 0 | 56 | 83 | 87 |
| Urine | 88 | 82 | 52 | 80 | 42 | 56 | 82 | 52 | 12 | 27 | 1 | 0 | 0 |
| Wound/Pus | 4 | 5 | 23 | 10 | 19 | 20 | 11 | 28 | 41 | 22 | 8 | 5 | 4 |
| Other | 4 | 4 | 11 | 6 | 19 | 12 | 4 | 7 | 33 | 9 | 8 | 11 | 8 |
| **Type of hospital (hospital isolates only, %)** | | | | | | | | | | | | | |
| General | 36 | 32 | 32 | 34 | 29 | 30 | 32 | 20 | 31 | 26 | 35 | 31 | 31 |
| Top clinical | 47 | 47 | 48 | 48 | 46 | 48 | 48 | 50 | 47 | 44 | 50 | 50 | 51 |
| University hospital | 17 | 21 | 20 | 18 | 26 | 23 | 20 | 30 | 22 | 30 | 15 | 19 | 18 |

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

**Key results**

- Included laboratories were well distributed throughout the country, although the proportion of laboratories with complete data in the regions 'Noord-Holland West', 'Zuidwest Nederland' and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' was relatively low.
- The distribution of included laboratories was reflected in the geographical distribution of isolates (Figure 4.1.2.2). The coverage was relatively high in the regions 'Euregio-Zwolle', 'Noord Nederland', 'Gelders Antibioticaresistentie & Infectiepreventie Netwerk' (GAIN)', 'Utrecht', and 'Noord-Brabant'. In the other regions the coverage was less dense.
- Although the mean number of isolates per laboratory was lower in excluded laboratories (12950 in included laboratories versus 8448 in excluded laboratories), isolate characteristics were largely comparable between included and excluded laboratories. However, included laboratories had a higher proportion of *E. coli* isolates (35% versus 29%), a higher proportion of isolates from general practitioners (42% versus 26%), a lower proportion from outpatient departments (22% versus 30%) and inpatient departments excluding intensive care units (28% versus 37%). The proportion of isolates from general hospitals was lower among included laboratories (31% versus 53%), but the proportion of isolates from university hospitals was higher (21% versus 0%). All these differences were mainly caused by the fact that both connected laboratories serving general practitioners and long-term care facilities only, and all connected laboratories serving university hospitals were among the included laboratories. However, because the ISIS-AR database contains large numbers of data, it is expected that inclusion of these laboratories did not influence the overall resistance percentages towards a deviation from the true mean for the Netherlands.
- Enterobacteriaceae were more often isolated from female patients (e.g. 72% of *E. coli* and 66% of *K. pneumoniae*), likely because women are more prone to urinary tract infections. For the other pathogens, the percentage of male and female patients was similar.
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *S. aureus* were most often isolated from patients from general practitioners and outpatient departments (combined 53%-72%, depending on the pathogen), whereas a large part of *E. faecium* (79%), coagulase-negative *Staphylococcus* spp. (69%), and *S. pneumoniae* (65%) 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. faecium*, and *E. faecalis* were mainly isolated from urine (42-88%, depending on the pathogen), whereas *S. aureus* was mainly isolated from wound or pus (41%), coagulase-negative *Staphylococcus* spp. from blood, and *H. influenzae*, *S. pneumoniae*, *M. catarrhalis* from the lower respiratory tract (56-87%).

## 4.2 Primary care

The distribution of pathogens in diagnostic urine and wound or pus samples from general practitioners' (GP) patients is presented in table 4.2.1. The resistance levels in 2017 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 or pus 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 infection, 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, and pus samples for culture and susceptibility testing in case of antimicrobial therapy failure and (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 and pus 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 age), and diagnostic wound and pus samples from selected general practitioners' patients, ISIS-AR 2017.

| | Urine | | Wound or pus |
|---|---|---|---|
| | Age≤12 | Age>12 | |
| Pathogen | N (%) | N (%) | N (%) |
| *E. coli* | 7,372 (73) | 73,385 (57) | 343 (4) |
| *K. pneumoniae* | 162 (2) | 9,733 (8) | 111 (1) |
| *P. mirabilis* | 547 (5) | 7,456 (6) | 262 (3) |
| Other Enterobacteriaceae[1] | 433 (4) | 12,290 (9) | 802 (10) |
| *P. aeruginosa* | 149 (1) | 3,362 (3) | 546 (7) |
| Other non-fermenters[2] | 130 (1) | 2,194 (2) | 427 (6) |
| Other Gram-negatives[3] | 5 (0) | 7 (0) | 163 (2) |
| *S. aureus* | 104 (1) | 2,428 (2) | 3,716 (48) |
| Other Gram-positives[4] | 1,213 (12) | 18,804 (15) | 1,364 (18) |

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

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

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

[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.*

**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 2017.

| | *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 | 66 | 5 | 74 | 3 | 75 | 4 | 79 |
| **Antibiotic** | | | | | | | | |
| amoxicillin/ampicillin | 34 | 38 | - | - | 18 | 21 | - | - |
| co-amoxiclav[1] - non-uuti | 28 | 31 | 15 | 14 | 4 | 6 | - | - |
| cefuroxime | 4 | 8 | 5 | 15 | 0 | 1 | - | - |
| cefotaxime/ceftriaxone | 2 | 4 | 4 | 5 | 0 | 1 | - | - |
| ceftazidime | 2 | 3 | 2 | 4 | 0 | 0 | 1 | 1 |
| ciprofloxacin | 5 | 11 | 5 | 14 | 7 | 11 | 1 | 10 |
| gentamicin | 3 | 4 | 1 | 2 | 5 | 5 | 2 | 3 |
| tobramycin | 3 | 4 | 4 | 3 | 4 | 4 | 0 | 0 |
| fosfomycin | 1 | 1 | 16 | 27 | 6 | 15 | - | - |
| trimethoprim | 21 | 24 | 11 | 23 | 26 | 34 | - | - |
| co-trimoxazole | 19 | 22 | 9 | 11 | 22 | 27 | - | - |
| nitrofurantoin | 0 | 2 | - | - | - | - | - | - |
| **Multidrug resistance** | | | | | | | | |
| HRMO[2] | 3 | 5 | 5 | 6 | 2 | 4 | - | - |
| multidrug resistance[3] - non-uuti | 2 | 4 | 2 | 3 | 1 | 1 | - | - |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides.*

[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 2013 to 2017) 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.

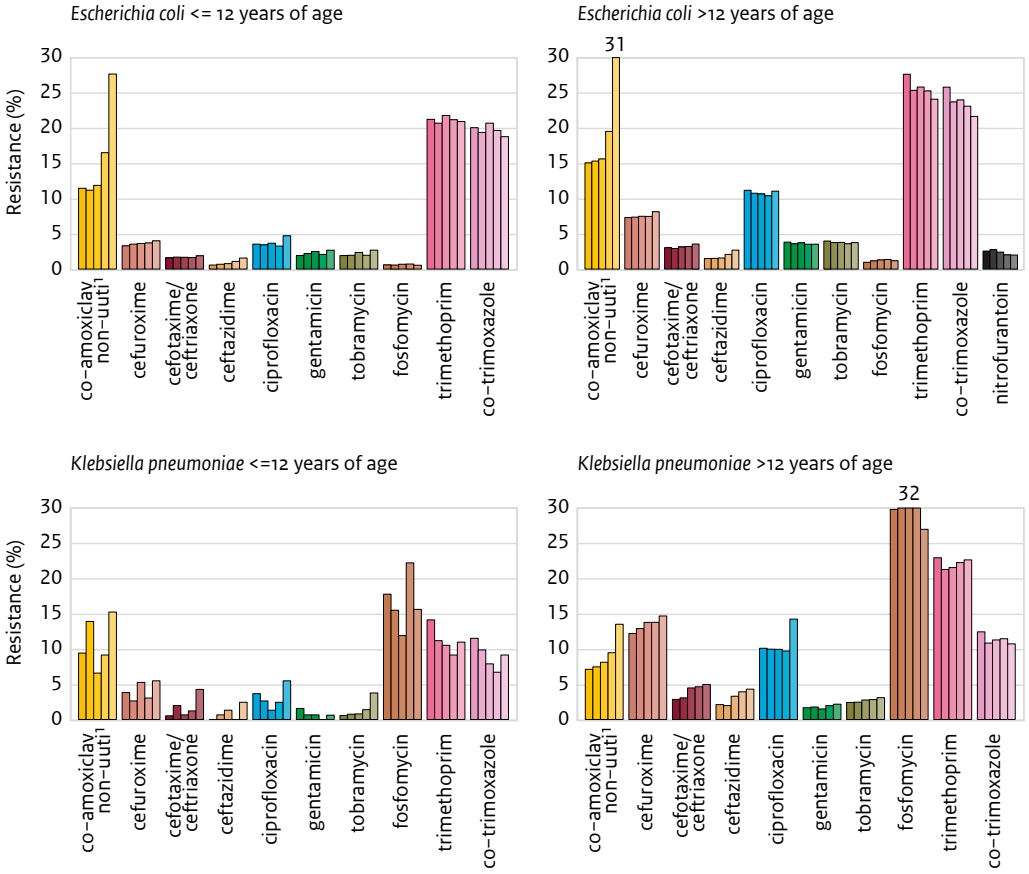
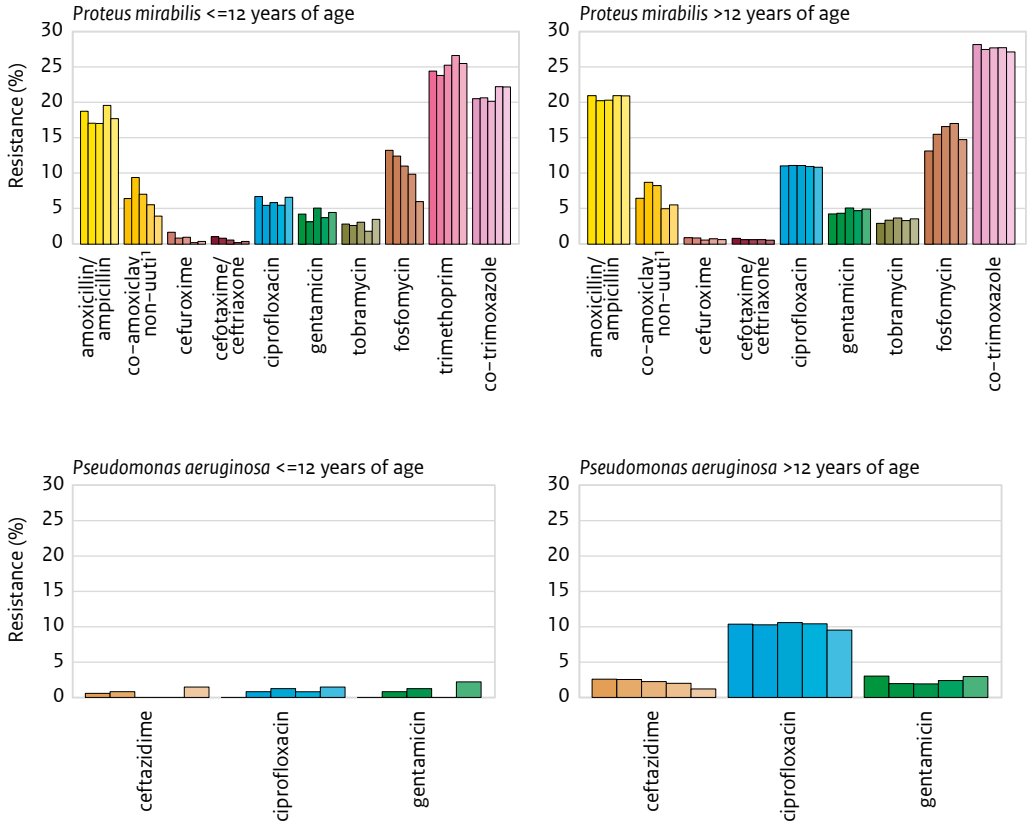


**Figure 4.2.1 (continued)** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category.




*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 diagnostic urinary *E. coli* isolates from selected general practitioners' patients (based on percentage of residents for whom at least one isolate was included in the analysis) and their resistance levels (%) for nitrofurantoin, fosfomycin, and cefotaxime/ceftriaxone/ceftazidime, by regional cooperative network, ISIS-AR 2017.

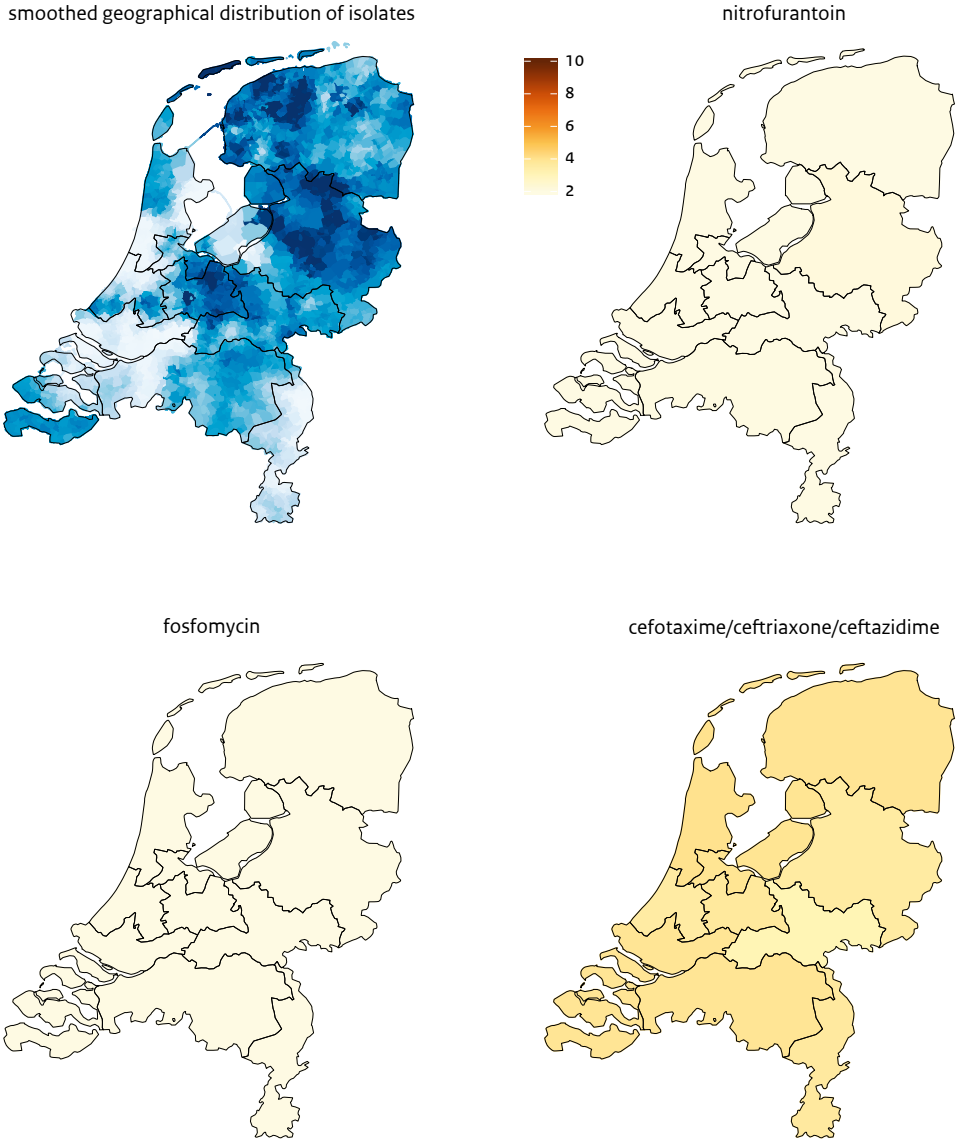
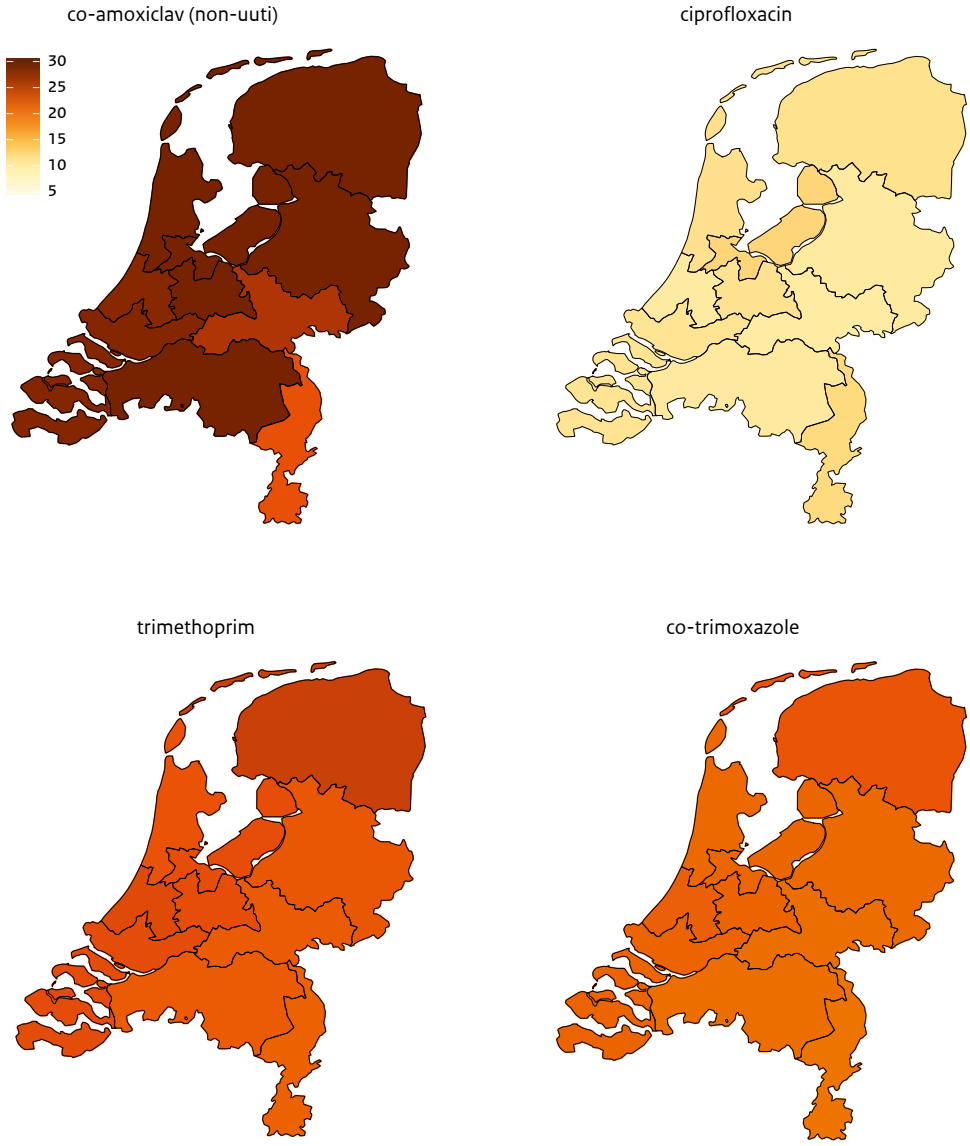


**Figure 4.2.2B** Resistance levels (%) for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole among diagnostic urinary *E. coli* isolates from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2017.




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

**Figure 4.2.3A** Smoothed geographical distribution of diagnostic urinary *K. pneumoniae* isolates from selected general practitioners' patients (based on percentage of residents for whom at least one isolate was included in the analysis) and their resistance levels (%) for cefotaxime/ceftriaxone/ceftazidime, by regional cooperative network, ISIS-AR 2017.

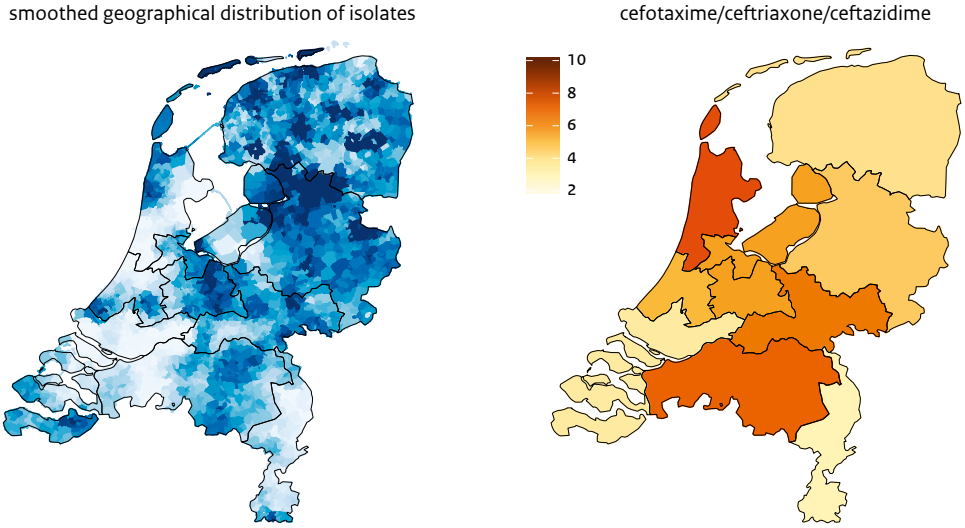



**Figure 4.2.3B** Resistance levels (%) for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole among diagnostic urinary *K. pneumoniae* isolates from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2017.

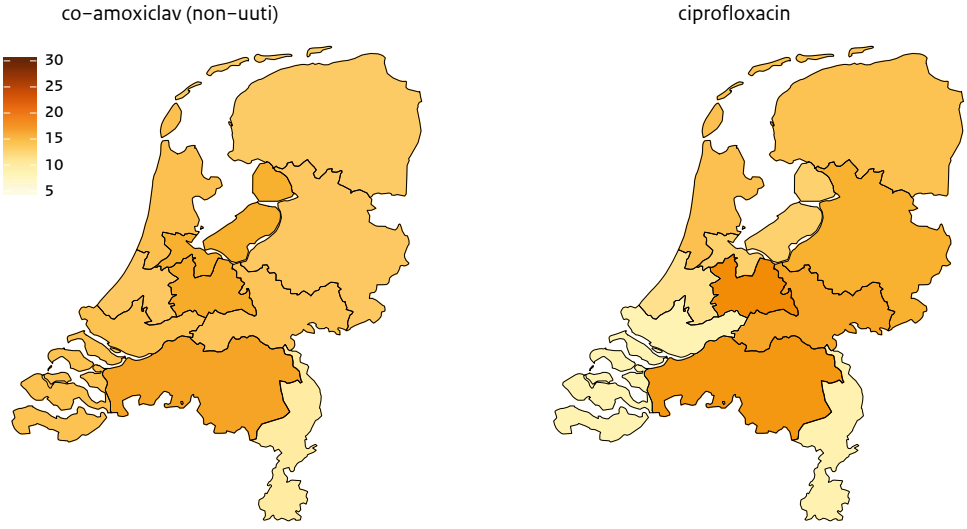
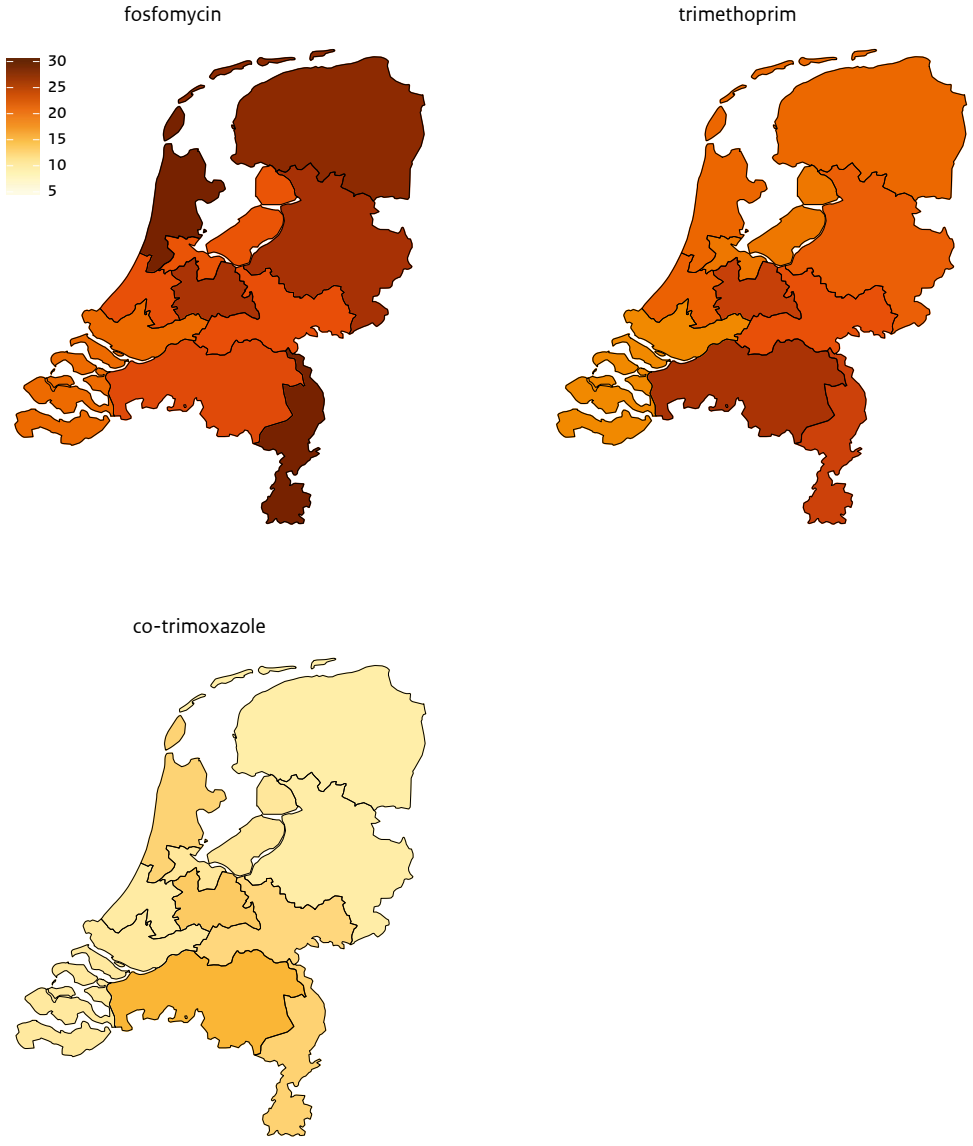


**Figure 4.2.3B (continued)** Resistance levels (%) for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole among diagnostic urinary *K. pneumoniae* isolates from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2017.




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

**Table 4.2.3** Resistance levels (%) among diagnostic wound and pus isolates of *S. aureus* from selected general practitioners' patients, ISIS-AR 2017.

| | *S. aureus* |
|---|---|
| **Antibiotic** | |
| flucloxacillin[1] | 3 |
| ciprofloxacin[2] | 5 |
| erythromycin | 11 |
| clindamycin including inducible resistance[3] | 10 |
| doxycycline/tetracycline | 4 |
| fusidic acid | 17 |
| co-trimoxazole | 4 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |

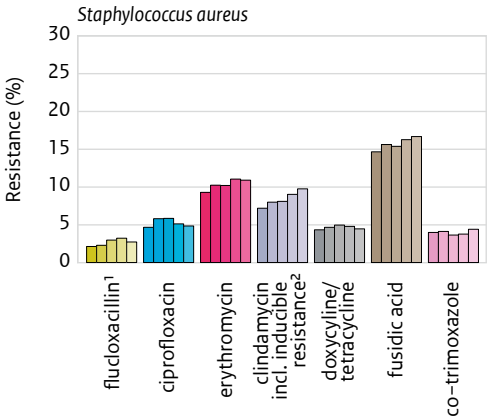
(For the definition of a clinically relevant trend see the methods section)

[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).*

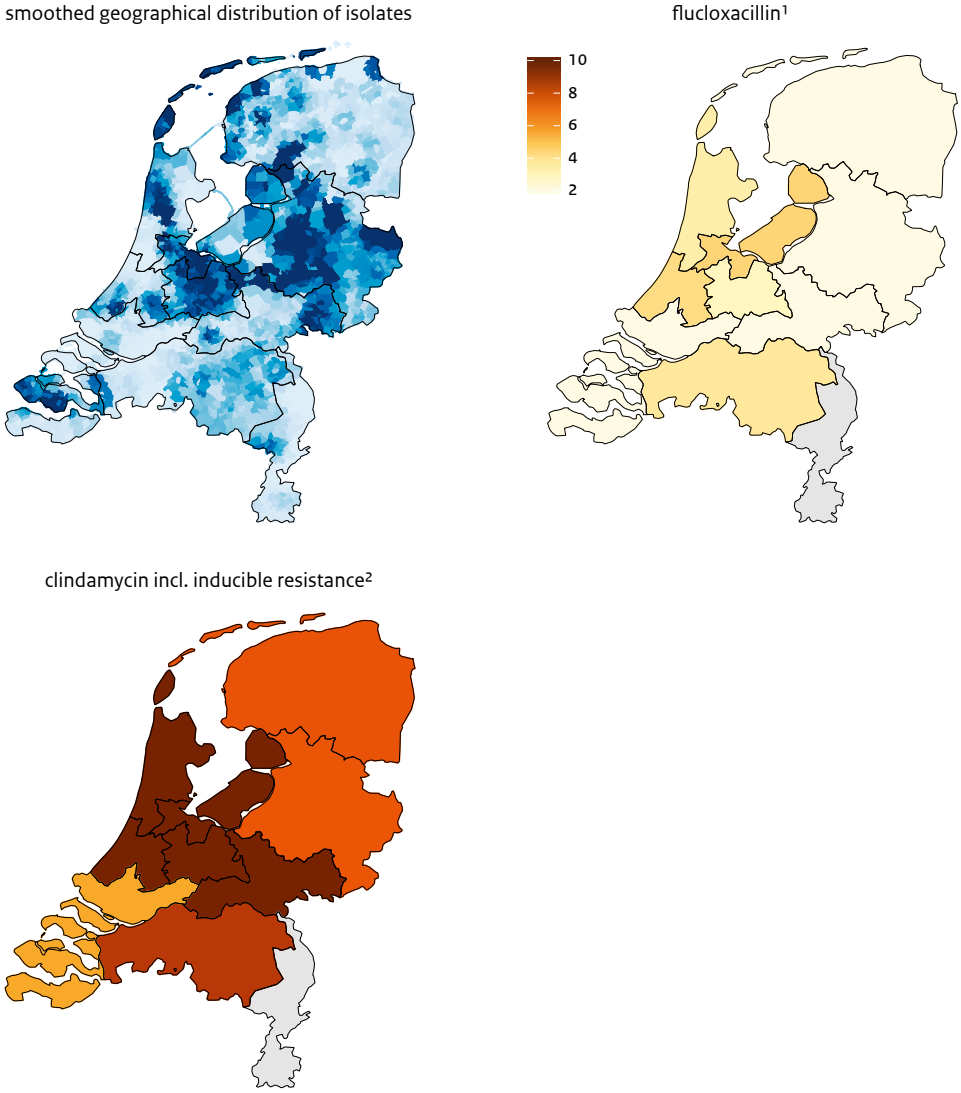
**Figure 4.2.4** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic wound and pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR.




[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 diagnostic wound or pus *S. aureus* isolates from selected general practitioners' patients (based on percentage of residents for whom at least one isolate was included in the analysis), and their resistance levels (%) for flucloxacillin and clindamycin including inducible resistance, by regional cooperative network, ISIS-AR 2017.




[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**

- In urine, resistance levels in selected GP patients aged >12 years were generally higher than in patients aged ≤12 years.

***Enterobacteriaceae***

- For all Enterobacteriaceae resistance levels for cefuroxime (≤8), cefotaxime/ceftriaxone (≤5), ceftazidime (≤4), gentamicin (≤5), and tobramycin (≤4) were ≤10%, except for cefuroxime in *K. pneumoniae* in patients aged >12 years (15%). Resistance levels for ciprofloxacin (≤7%) were ≤10% in Enterobacteriaceae from patients aged ≤12 years only. Additionally, resistance levels ≤10% were found for fosfomycin (1%) and nitrofurantoin (≤2%) in *E. coli*, co-trimoxazole in *K. pneumoniae* (9%) from patients aged ≤12 years, and for co-amoxiclav (≤6%) and fosfomycin (patients aged ≤12 years only, 6%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥34%), co-amoxiclav (≥28%) trimethoprim (≥21%), and co-trimoxazole (patients aged >12 years only, 22%) in *E. coli*, for fosfomycin (27%) and trimethoprim (23%) in *K. pneumoniae* in patients aged >12 years, and in amoxicillin/ampicillin (patients >12 years only, 21%), trimethoprim (≥26%) and co-trimoxazole (≥22%) in *P. mirabilis*.
- There was a significant and clinically relevant increase in resistance to co-amoxiclav for *E. coli* in both age groups (from 11% in 2013 to 28% in 2017 for patients aged ≤12 years, and from 15% to 31% for patients aged >12 years) and for *K. pneumoniae* from patients aged >12 years (from 7% to 14%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). Significant and clinically relevant increases in resistance were also seen for *K. pneumoniae*; for cefotaxime/ceftriaxone and tobramycin in patients aged ≤12 years (both from 1% to 4%), and for ceftazidime in patients aged >12 years (from 2% to 4%). In *P. mirabilis*, statistically significant and clinically relevant decreasing trends between 2013 and 2017 were observed for resistance to co-amoxiclav (from 6% to 4%) and fosfomycin (from 13% to 6%) in patients aged ≤12 years.
- The percentage of HRMO and multidrug resistance was ≤6% in all Enterobacteriaceae.
- For both *E. coli* and *K. pneumoniae* coverage in the regional cooperative networks 'Zuidwest-Nederland' and 'LINK' was low compared to other regional networks and resistance levels may be influenced by suboptimal representativeness.
- Resistance levels for *E. coli* were similar between all regional cooperative networks for all selected agents (range for nitrofurantoin, fosfomycin, and cefotaxime/ceftriaxone/ceftazidime ≤0.9%, range for ciprofloxacin, trimethoprim, and co-trimoxazole ≤3.9%), except for co-amoxiclav, for which resistance levels were similar between most regional networks (median=30.4%; interquartile range=29.1%-31.2%), but slightly lower in 'LINK' (23.7%).

- For *K. pneumoniae* regional differences in resistance were more pronounced; for cefotaxime/ceftriaxone/ceftazidime (median=5.2%; interquartile range=4.1%-6.3%) resistance was slightly lower in 'Zuidwest-Nederland' (3.5%) and 'LINK' (3.1%) and slightly higher in 'Noord-Holland West' (8.2%) and 'Noord-Brabant' (7.3%). For co-amoxiclav (13.4%; 12.9%-14.7%) resistance was lower in 'LINK' (9.7%) and higher in 'Noord-Brabant' (15.7%). For ciprofloxacin (13.6%; 11.5%-15.4%) resistance was lower in 'Holland West' (11.2%), 'Zuidwest-Nederland' (8.5%), and 'LINK' (8.7%), and higher in 'Utrecht' (17.3%), 'GAIN' (15.7%), and 'Noord-Brabant' (16.6%). For fosfomycin (25.9%; 23.4%-28.4%) resistance was lower in 'Zuidwest-Nederland' (20.5%) and higher in 'Noord-Holland West' (30.4%) and 'LINK' (30.3%). For trimethoprim (22.0%; 21.0%-25.1%) resistance was lower in 'Zuidwest-Nederland' (17.6%) and higher in 'Utrecht' (25.7%), 'Noord-Brabant' (27.4%), and 'LINK' (25.5%). Lastly, for co-trimoxazole (11.0%; 9.7%-12.2%) resistance was lower in 'Noord-Nederland' (9.3%) and higher in 'Utrecht' (12.9%) and 'Noord-Brabant' (14.6%).

***P. aeruginosa***

- Resistance levels ≤10% were found for each of the selected agents.

***S. aureus***

- Resistance to each of the selected agents was ≤10%, except for erythromycin (11%) and fusidic acid (17%).
- Coverage in most regional cooperative networks was low, and resistance levels may be influenced by suboptimal representativeness.
- Resistance percentages in regional cooperative networks varied for flucloxacillin (median=2.9%; interquartile range=1.5%-3.7%) and clindamycin including inducible resistance (10.8%; 7.9%-11.4%).

## 4.3 Hospital departments

In chapters 4.3.1, 4.3.2, and 4.3.3 resistance levels among isolates from patients in outpatient departments, inpatient departments (excluding intensive care units), and intensive care units, respectively, 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 or pus) from patients attending outpatient departments is presented in table 4.3.1.1. The resistance levels for pathogens isolated from these patients in 2017 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*) for the respective pathogens.

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 2017.

| Pathogen | Lower respiratory tract N (%) | Urine N (%) | Wound or pus N (%) |
|---|---|---|---|
| *E. coli* | 478 (5) | 19,535 (45) | 1,294 (6) |
| *K. pneumoniae* | 235 (2) | 3,722 (9) | 278 (1) |
| *P. mirabilis* | 139 (1) | 2,205 (5) | 765 (4) |
| Other Enterobacteriaceae[1] | 925 (9) | 5,473 (13) | 2,106 (10) |
| *P. aeruginosa* | 1,315 (12) | 1,632 (4) | 1,498 (7) |
| Other non-fermenters[2] | 1,545 (15) | 719 (2) | 570 (3) |
| Other Gram-negatives[3] | 3,256 (31) | 13 (0) | 488 (2) |
| *S. aureus* | 1,427 (14) | 1,508 (3) | 9,102 (44) |
| Other Gram-positives[4] | 1,201 (11) | 8,661 (20) | 4,552 (22) |

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

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

[3] *H. influenzae, 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, 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 2017.

| | *E. coli* | *K. pneumoniae* | *P. mirabilis* | *P. aeruginosa* |
|---|---|---|---|---|
| **Antibiotic** | | | | |
| amoxicillin/ampicillin | 44 | - | 22 | - |
| co-amoxiclav[1] - non-uuti | 36 | 18 | 8 | - |
| piperacillin-tazobactam | 5 | 7 | 0 | 6 |
| cefuroxime | 12 | 16 | 1 | - |
| cefotaxime/ceftriaxone | 6 | 9 | 1 | - |
| ceftazidime | 4 | 8 | 0 | 3 |
| meropenem/imipenem | 0 | 0 | 0 | - |
| meropenem | - | - | - | 1 |
| imipenem | - | - | - | 4 |
| ciprofloxacin | 18 | 16 | 14 | 12 |
| gentamicin | 5 | 4 | 6 | 4 |
| tobramycin | 6 | 6 | 4 | 1 |
| fosfomycin | 2 | 23 | 13 | - |
| trimethoprim | 29 | 25 | 34 | - |
| co-trimoxazole | 26 | 16 | 28 | - |
| nitrofurantoin | 3 | - | - | - |
| **Empiric therapy combinations** | | | | |
| gentamicin + amoxicillin/ampicillin | 5 | - | 5 | - |
| gentamicin + co-amoxiclav - non-uuti | 4 | 4 | 2 | - |
| gentamicin + cefuroxime | 2 | 3 | 0 | - |
| gentamicin + cefotaxime/ceftriaxone | 1 | 3 | 0 | - |
| gentamicin + ceftazidime | 1 | 3 | 0 | 0 |
| **Multidrug resistance** | | | | |
| HRMO[2] | 9 | 11 | 5 | 2 |
| multidrug resistance[3] - non-uuti | 7 | 6 | 2 | - |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |
| | (For the definition of a clinically relevant trend see the methods section) |

*- = Resistance not calculated.*

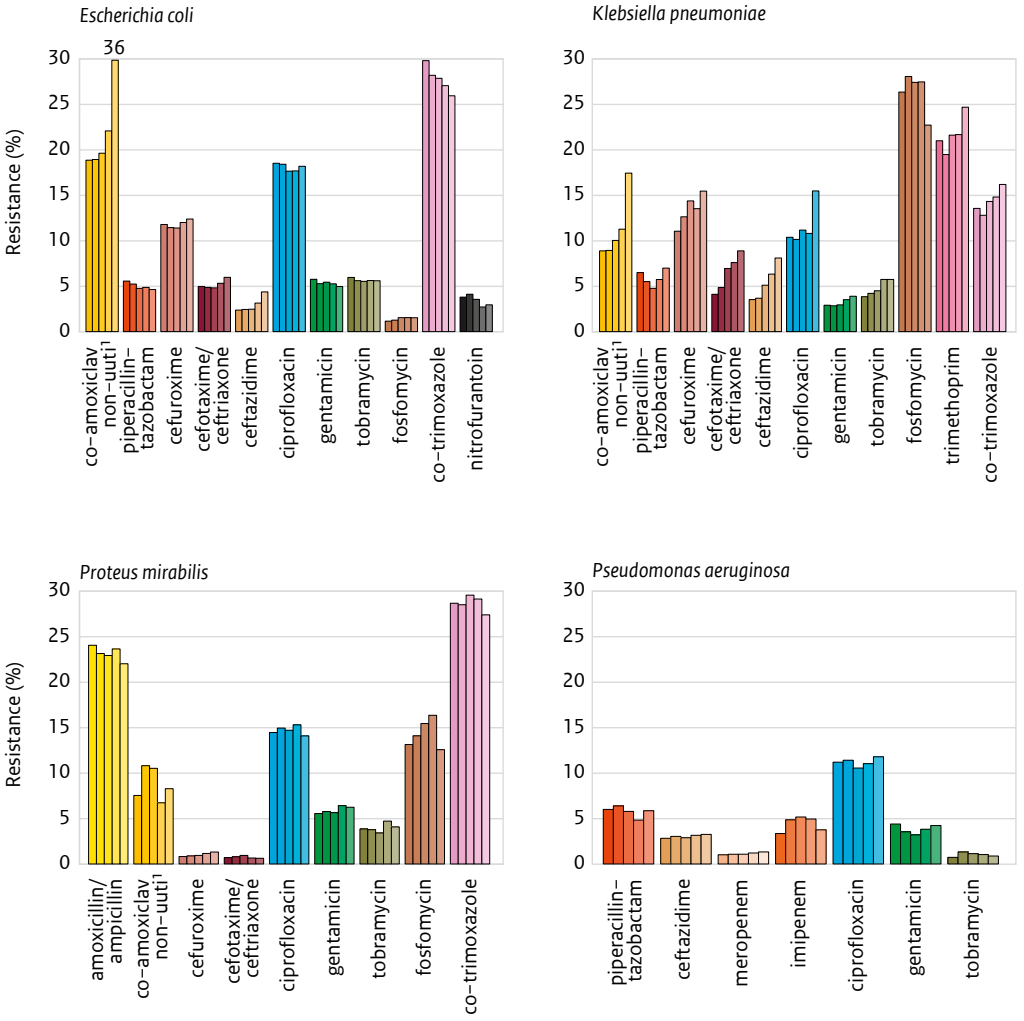
*non-uuti = according to breakpoint for non-uncomplicated urinary tract infection*

[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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, 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 2013 to 2017) 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 2017.

| | *S. aureus* |
|---|---|
| **Antibiotic** | |
| flucloxacillin[1] | 2 |
| ciprofloxacin[2] | 8 |
| gentamicin | 1 |
| erythromycin | 13 |
| clindamycin including inducible resistance[3] | 13 |
| doxycycline/tetracycline | 4 |
| fusidic acid | 8 |
| linezolid | 0 |
| co-trimoxazole | 2 |
| rifampicin | 0 |

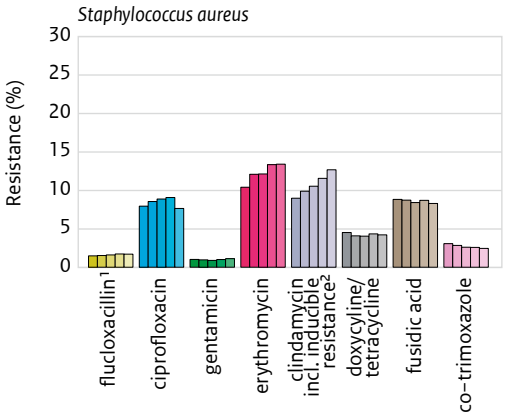
| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |
| | (For the definition of a clinically relevant trend see the methods section) |

[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 2013 to 2017) 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 (≤7%), cefotaxime/ceftriaxone (≤9%), ceftazidime (≤8%), meropenem/imipenem (0%), gentamicin (≤6%), and tobramycin (≤6%). 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 amoxicillin/ampicillin (≥22%) and trimethoprim (≥25%) in all Enterobacteriaceae, for co-trimoxazole in *E. coli* and *P. mirabilis* (≥26%), for co-amoxiclav in *E. coli* (36%), and fosfomycin in *K. pneumoniae* (23%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 19% in 2013 to 36% in 2017) and in *K. pneumoniae* (from 9% to 18%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). Furthermore, in *K. pneumoniae*, significant and clinically relevant increasing trends were observed for cefotaxime/ceftriaxone (from 4% in 2013 to 9% in 2017) and ceftazidime (from 4% to 8%).
- Resistance to empiric therapy combinations was ≤5% for all Enterobacteriaceae.
- The percentage HRMO was ≤9% (except for *K. pneumoniae*, 11%) and the percentage of multidrug resistance was ≤7% for all Enterobacteriaceae. The percentage of multidrug resistance in *K. pneumoniae* increased to a statistically significant and clinically relevant extent (from 2% in 2013 to 6% in 2017).

***P. aeruginosa***

- Resistance to each of the selected agents was ≤6%, except for ciprofloxacin (12%).

***S. aureus***

- Resistance to each of the selected agents was ≤10%, except for erythromycin and clindamycin including inducible resistance (both 13%).

## 4.3.2 Inpatient hospital departments (excl. ICU)

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to inpatient hospital departments (excl. ICU) is presented in table 4.3.2.1. The resistance levels for pathogens isolated from these patients in 2017 are presented in tables 4.3.2.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *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*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp.) and 4.3.2.2 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

In inpatient hospital departments in the Netherlands, a sample is taken from the majority of 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 2017.

| | Blood or cerebrospinal fluid | Lower respiratory tract | Urine | Wound or pus |
|---|---|---|---|---|
| Pathogen | N (%) | N (%) | N (%) | N (%) |
| *E. coli* | 5,061 (24) | 1,207 (9) | 20,945 (45) | 3,882 (14) |
| *K. pneumoniae* | 879 (4) | 515 (4) | 3,780 (8) | 794 (3) |
| *E. cloacae* | 289 (1) | 439 (3) | 1,114 (2) | 1,010 (4) |
| *P. mirabilis* | 322 (2) | 238 (2) | 3,053 (7) | 889 (3) |
| Other Enterobacteriaceae[1] | 1,015 (5) | 1,534 (11) | 4,439 (9) | 2,518 (9) |
| *P. aeruginosa* | 457 (2) | 1,508 (11) | 2,353 (5) | 1,568 (6) |
| *Acinetobacter* spp. | 83 (0) | 110 (1) | 297 (1) | 292 (1) |
| Other non-fermenters[2] | 68 (0) | 1,617 (11) | 201 (0) | 345 (1) |
| Other Gram-negatives[3] | 491 (2) | 3,539 (25) | 20 (0) | 757 (3) |
| *E. faecalis* | 640 (3) | 35 (0) | 4,873 (10) | 1,720 (6) |
| *E. faecium* | 391 (2) | 18 (0) | 1,376 (3) | 963 (3) |
| *S. aureus* | 2,044 (10) | 1,790 (13) | 1,531 (3) | 7,410 (26) |
| CNS | 6,548 (31) | 19 (0) | 1,001 (2) | 3,073 (11) |
| Other Gram-positives[4] | 2,581 (12) | 1,566 (11) | 1,768 (4) | 3,197 (11) |

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

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

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

[3] *H. influenzae, B. fragilis, N. meningitidis, C. jejuni, 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, L. monocytogenes, Staphylococcus spp. (non-aureus, non-CNS).*

**Table 4.3.2.2** Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2017.

| | *E. coli* | *K. pneumoniae* | *E. cloacae* | *P. mirabilis* | *P. aeruginosa* | *Acinetobacter* spp. |
|---|---|---|---|---|---|---|
| **Antibiotic** | | | | | | |
| amoxicillin/ampicillin | 44 | - | - | 22 | - | - |
| co-amoxiclav[1] - non-uuti | 36 | 19 | - | 8 | - | - |
| piperacillin-tazobactam | 5 | 8 | - | 0 | 6 | - |
| cefuroxime | 13 | 16 | - | 1 | - | - |
| cefotaxime/ceftriaxone | 6 | 10 | - | 1 | - | - |
| ceftazidime | 5 | 9 | - | 0 | 3 | - |
| meropenem/imipenem | 0 | 0 | 0 | 0 | - | 2 |
| meropenem | - | - | - | - | 1 | - |
| imipenem | - | - | - | - | 4 | - |
| ciprofloxacin | 14 | 13 | 5 | 12 | 11 | 7 |
| gentamicin | 5 | 5 | 3 | 6 | 3 | 5 |
| tobramycin | 5 | 7 | 3 | 4 | 1 | 5 |
| fosfomycin | 1 | 19 | - | 12 | - | - |
| trimethoprim | 25 | 20 | 7 | 33 | - | - |
| co-trimoxazole | 23 | 15 | 6 | 27 | - | 3 |
| nitrofurantoin | 2 | - | - | - | - | - |
| **Empiric therapy combinations** | | | | | | |
| gentamicin + amoxicillin/ampicillin | 4 | - | - | 5 | - | - |
| gentamicin + co-amoxiclav - non-uuti | 4 | 4 | - | 2 | - | - |
| gentamicin + piperacillin-tazobactam | 1 | 2 | - | 0 | 1 | - |
| gentamicin + cefuroxime | 2 | 4 | - | 0 | - | - |
| gentamicin + cefotaxime/ceftriaxone | 1 | 4 | - | 0 | - | - |
| gentamicin + ceftazidime | 1 | 3 | - | 0 | 0 | - |
| tobramycin + ceftazidime | - | - | - | - | 0 | - |
| tobramycin + ciprofloxacin | - | - | - | - | 0 | - |
| **Multidrug resistance** | | | | | | |
| HRMO[2] | 9 | 12 | 2 | 4 | 2 | 4 |

10 Significant and clinically relevant increasing trend since 2013
10 Significant and clinically relevant decreasing trend since 2013
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 Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for E. cloacae as resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as resistant to imipenem or meropenem or resistant to both fluoroquinolones and aminoglycosides.*

**Figure 4.3.2.1** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.

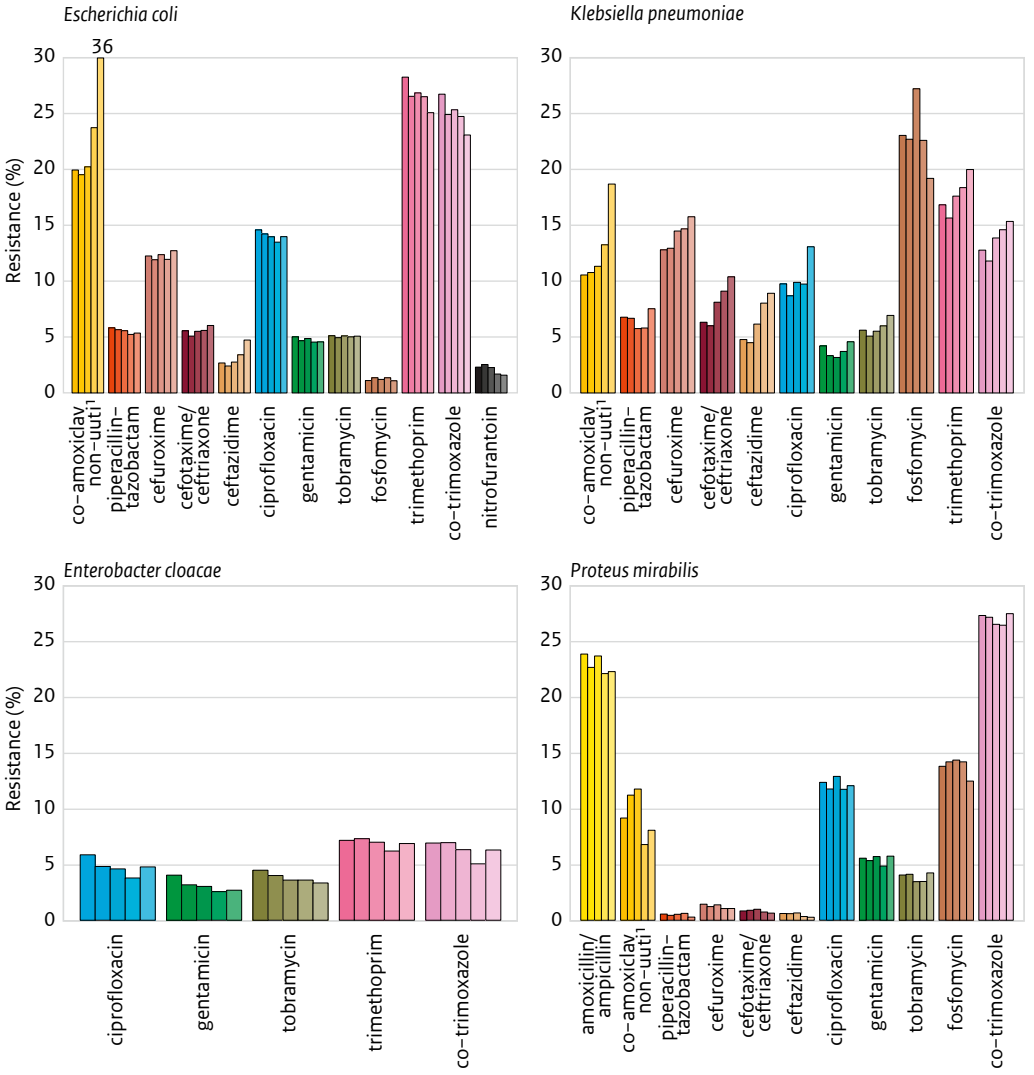
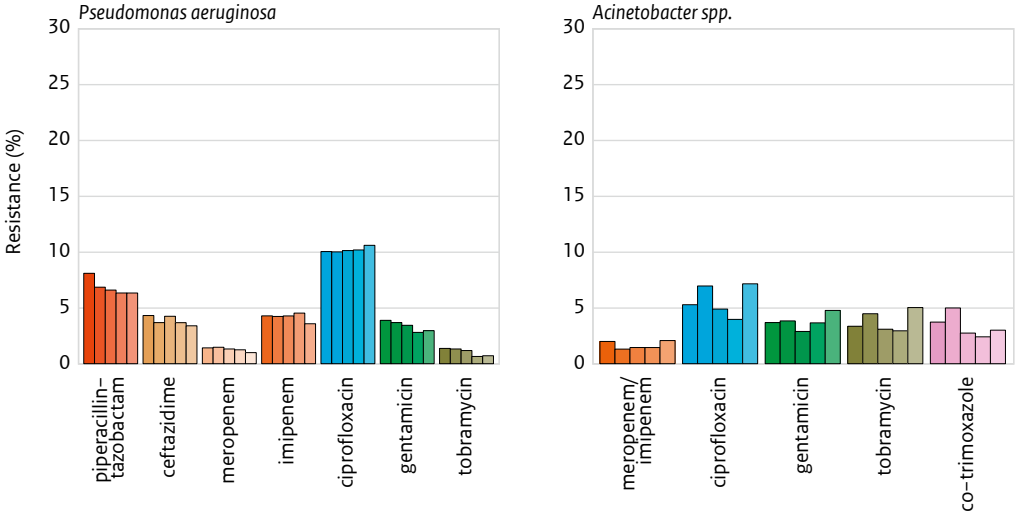


**Figure 4.3.2.1 (continued)** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) 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 2017.

| | *E. faecalis* | *E. faecium* |
|---|---|---|
| **Antibiotic** | | |
| amoxicillin/ampicillin | - | 87 |
| vancomycin | 0 | 1 |
| nitrofurantoin[1] | 0 | - |

*- = Resistance not calculated.*

[1] *Resistance based on isolates from urine only.*

**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 2017.

| | *S. aureus* | CNS |
|---|---|---|
| **Antibiotic** | | |
| flucloxacillin[1] | 2 | 41 |
| ciprofloxacin[2] | 8 | 30 |
| gentamicin | 1 | 25 |
| erythromycin | 13 | 44 |
| clindamycin including inducible resistance[3] | 12 | 30 |
| doxycycline/tetracycline | 3 | 17 |
| fusidic acid | 7 | 45 |
| linezolid | 0 | 0 |
| co-trimoxazole | 3 | 19 |
| rifampicin | 0 | 3 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |
| | (For the definition of a clinically relevant trend see the methods section) |

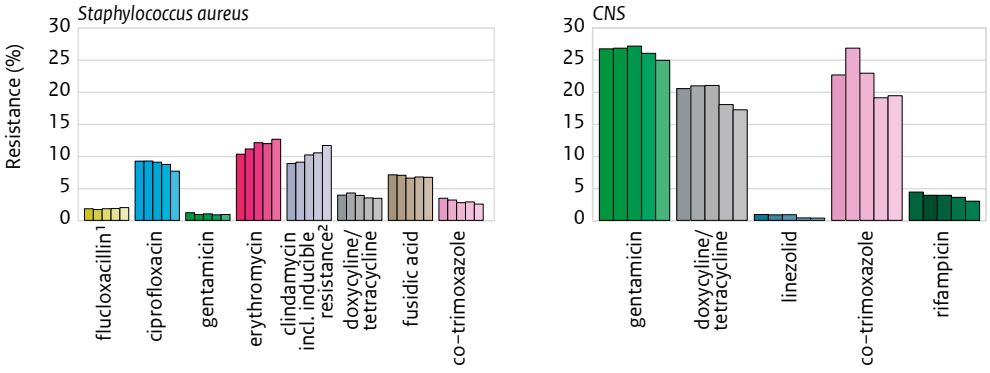
*CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.*

[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 4.3.2.2** Trends in antibiotic resistance (from left to right 2013 to 2017) 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 (≤8%), cefotaxime/ceftriaxone (≤10%), ceftazidime (≤9%), meropenem/imipenem (0%), gentamicin (≤6%), and tobramycin (≤7%). Resistance levels ≤10% were also found for fosfomycin (1%) and nitrofurantoin (2%) in *E. coli*, ciprofloxacin (5%), trimethoprim (7%), and co-trimoxazole (6%) in *E. cloacae*, and co-amoxiclav (8%) and cefuroxime (1%) in *P. mirabilis*.
- Resistance levels ≥20% were found for trimethoprim (≥20%) in *E. coli*, *K. pneumoniae* and *P. mirabilis*, for amoxicillin/ampicillin (≥22%), and co-trimoxazole (≥23%) in *E. coli* and *P. mirabilis*, and for co-amoxiclav in *E. coli* (36%).
- A significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2013 to 36% in 2017) and *K. pneumoniae* (from 11% to 19%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see methods section). Furthermore, in *K. pneumoniae*, resistance to ceftazidime increased from 5% in 2013 to 9% in 2017. In *P. mirabilis* resistance to co-amoxiclav decreased to a significant and clinically relevant extent, especially in the last 3 years (from 12% in 2015 to 8% in 2017).
- For empiric therapy combinations, resistance was ≤5%.
- The percentage of HRMO was ≤9%, except for *K. pneumoniae* (12%).

***P. aeruginosa***

- Resistance to each of the selected agents, empiric therapy combinations, and the percentage HRMO, was ≤6% in 2017, except for ciprofloxacin (11%).

***Acinetobacter spp.***

- Resistance to each of the selected agents, and the percentage HRMO, was ≤7% in 2017.

***E. faecalis and E. faecium***

- Vancomycin resistance (≤1%), and nitrofurantoin resistance (0%, calculated for *E. faecalis* only) was rare in 2017.
- In *E. faecium*, resistance to amoxicillin/ampicillin was 87%.

***S. aureus***

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

***Coagulase-negative Staphylococcus spp.***

- Apart from doxycycline/tetracycline (17%), linezolid (0%), co-trimoxazole (19%) 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, especially in the last four years (from 27% in 2014 to 19% in 2017).

## 4.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to intensive care units is presented in table 4.3.3.1. The resistance levels for pathogens isolated from these patients in 2017 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *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*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp.) and 4.3.3.2 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

In intensive care units in the Netherlands, a sample is taken from almost all 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 2017.

| | Blood or cerebrospinal fluid | Lower respiratory tract | Urine | Wound or pus |
|---|---|---|---|---|
| Pathogen | N (%) | N (%) | N (%) | N (%) |
| *E. coli* | 350 (11) | 532 (11) | 775 (38) | 509 (17) |
| *K. pneumoniae* | 88 (3) | 221 (5) | 150 (7) | 107 (4) |
| *E. cloacae* | 46 (1) | 229 (5) | 42 (2) | 142 (5) |
| *P. mirabilis* | 25 (1) | 119 (3) | 134 (7) | 75 (2) |
| Other Enterobacteriaceae[1] | 143 (4) | 752 (16) | 193 (10) | 328 (11) |
| *P. aeruginosa* | 65 (2) | 363 (8) | 111 (6) | 216 (7) |
| *Acinetobacter* spp. | 16 (0) | 67 (1) | 8 (0) | 37 (1) |
| Other non-fermenters[2] | 14 (0) | 330 (7) | 6 (0) | 30 (1) |
| Other Gram-negatives[3] | 61 (2) | 559 (12) | 0 (0) | 75 (2) |
| *E. faecalis* | 102 (3) | 42 (1) | 242 (12) | 255 (8) |
| *E. faecium* | 202 (6) | 62 (1) | 169 (8) | 363 (12) |
| *S. aureus* | 258 (8) | 908 (20) | 72 (4) | 308 (10) |
| CNS | 1,676 (51) | 31 (1) | 80 (4) | 339 (11) |
| Other Gram-positives[4] | 229 (7) | 441 (9) | 33 (2) | 245 (8) |

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

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

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

[3] *H. influenzae, 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), L. monocytogenes, M. tuberculosis, Staphylococcus spp. (non-aureus, non-CNS).*

**Table 4.3.3.2** Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to intensive care units, ISIS-AR 2017.

| | *E. coli* | *K. pneumoniae* | *E. cloacae* | *P. mirabilis* | *P. aeruginosa* | *Acinetobacter* spp. |
|---|---|---|---|---|---|---|
| **Antibiotic** | | | | | | |
| amoxicillin/ampicillin | 46 | - | - | 21 | - | - |
| co-amoxiclav[1] - non-uuti | 37 | 19 | - | 9 | - | - |
| piperacillin-tazobactam | 8 | 10 | - | 1 | 10 | - |
| cefuroxime | 16 | 19 | - | 2 | - | - |
| cefotaxime/ceftriaxone | 9 | 13 | - | 1 | - | - |
| ceftazidime | 7 | 12 | - | 1 | 6 | - |
| meropenem/imipenem | 0 | 1 | 0 | 0 | - | 10 |
| meropenem | - | - | - | - | 1 | - |
| imipenem | - | - | - | - | 4 | - |
| ciprofloxacin | 15 | 13 | 4 | 10 | 12 | 11 |
| gentamicin | 5 | 5 | 3 | 5 | 4 | 7 |
| tobramycin | 6 | 9 | 5 | 5 | 2 | 7 |
| co-trimoxazole | 22 | 16 | 5 | 24 | - | 10 |
| **Empiric therapy combinations** | | | | | | |
| gentamicin + amoxicillin/ampicillin | 5 | - | - | 4 | - | - |
| gentamicin + co-amoxiclav - non-uuti | 4 | 5 | - | 3 | - | - |
| gentamicin + piperacillin-tazobactam | 1 | 3 | - | 0 | 1 | - |
| gentamicin + cefuroxime | 2 | 5 | - | 0 | - | - |
| gentamicin + cefotaxime/ceftriaxone | 2 | 5 | - | 0 | - | - |
| gentamicin + ceftazidime | 1 | 5 | - | 0 | 1 | - |
| tobramycin + ceftazidime | - | - | - | - | 1 | - |
| tobramycin + ciprofloxacin | - | - | - | - | 2 | - |
| **Multidrug resistance** | | | | | | |
| HRMO[2] | 12 | 14 | 2 | 4 | 3 | 10 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for E. cloacae as resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as resistant to imipenem or meropenem or resistant to both fluoroquinolones and aminoglycosides.*

**Figure 4.3.3.1** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR.

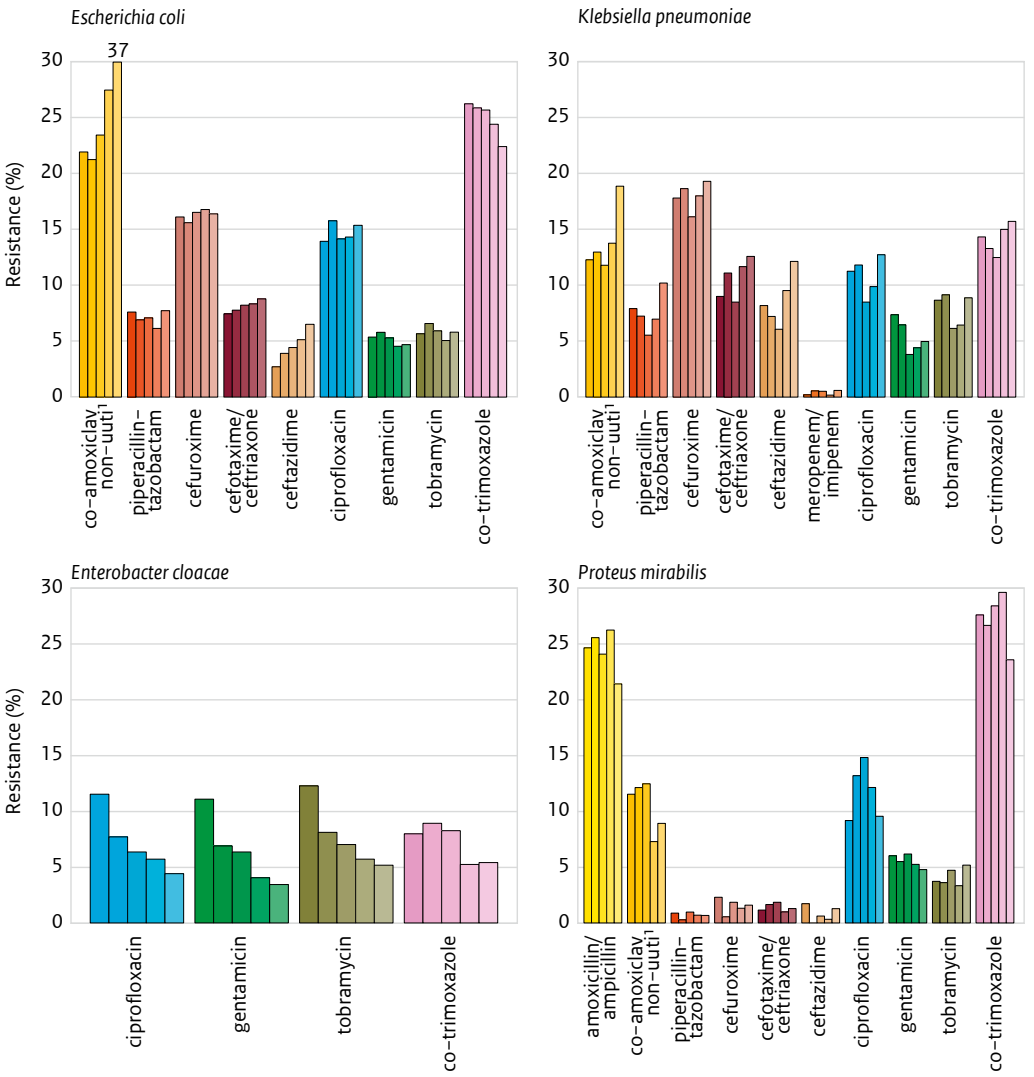
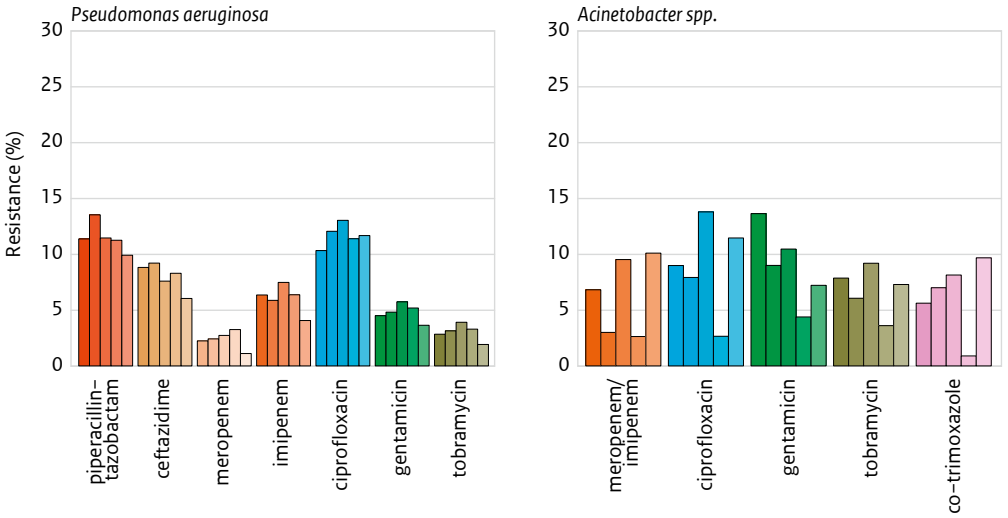


**Figure 4.3.3.1 (continued)** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) 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 4.3.3.3** Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to intensive care units, ISIS-AR 2017.

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

*- = Resistance not calculated.*

**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 2017.

| | *S. aureus* | *CNS* |
|---|---|---|
| **Antibiotic** | | |
| flucloxacillin[1] | 2 | 65 |
| ciprofloxacin[2] | 5 | 54 |
| gentamicin | 1 | 49 |
| erythromycin | 13 | 61 |
| clindamycin including inducible resistance[3] | 10 | 50 |
| doxycycline/tetracycline | 3 | 19 |
| linezolid | 0 | 0 |
| co-trimoxazole | 2 | 32 |
| rifampicin | 0 | 7 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |
| | (For the definition of a clinically relevant trend see the methods section) |

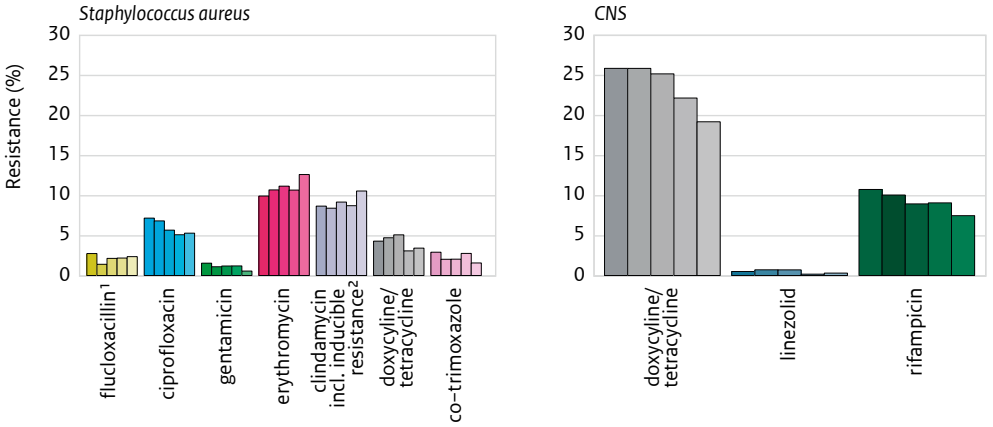
*CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.*

[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 4.3.3.2** Trends in antibiotic resistance (from left to right 2013 to 2017) 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 piperacillin-tazobactam (≤10%), meropenem/imipenem (≤1%), gentamicin (≤5%), and tobramycin (≤9%). Resistance levels ≤10% were also found for cefotaxime/ceftriaxone (≤9%) and ceftazidime (≤7%) in *E. coli* and *P. mirabilis*, for ciprofloxacin in *E. cloacae* and *P. mirabilis* (≤10%), for co-trimoxazole in *E. cloacae* (5%), and for co-amoxiclav (9%) and cefuroxime (2%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥21%) and co-trimoxazole (≥22%) in *E. coli* and *P. mirabilis*, and for co-amoxiclav in *E. coli* (37%).
- A significant and clinically relevant increasing trend in resistance was observed for co-amoxiclav in *E. coli* (from 22% in 2013 to 37% in 2017) and *K. pneumoniae* (from 12% to 19%), 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 3% to 7%). In *K. pneumoniae*, a decreasing trend in resistance was found for gentamicin (from 7% to 5%). In *E. cloacae*, significant and clinically relevant decreases in resistance levels between 2013 and 2017 were found for ciprofloxacin (from 12% to 4%), gentamicin (from 11% to 3%), tobramycin (from 12% to 5%), and co-trimoxazole (from 8% to 5%).
- Resistance to the empiric therapy combinations was ≤5%.
- The percentage HRMO was ≤10%, except for *E. coli* (12%) and *K. pneumoniae* (14%). In *E. cloacae*, the percentage of HRMO decreased significantly and to a clinically relevant extent, from 8% in 2013 to 2% in 2017.

***P. aeruginosa***

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

***Acinetobacter spp.***

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

***E. faecalis and E. faecium***

- Resistance to vancomycin was rare (≤1%).
- In *E. faecium*, resistance to amoxicillin/ampicillin was 88%.

***S. aureus***

- Resistance to each of the selected agents was 10% or lower, except for erythromycin (13%).

***Coagulase-negative Staphylococcus spp.***

- Apart from linezolid (0%) and rifampicin (7%), resistance to each of the selected agents was ≥20%.
- Significant and clinically relevant decreases in resistance were found for ciprofloxacin (from 60% in 2013 to 54% in 2017), erythromycin (from 68% to 61%), clindamycin including inducible resistance (from 58% to 50%), doxycycline/tetracycline (from 26% to 19%), co-trimoxazole (from 41% to 32%), and rifampicin (from 11% to 7%).

## 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 these pathogens in 2017 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, 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*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.4.2 (*S. aureus* and coagulase-negative *Staphylococcus* spp.).

In most hospitals blood samples are taken from all patients with a body temperature of >38.5 °C 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 2017.

| Pathogen | Non-ICU N (%) | ICU N (%) |
|---|---|---|
| *E. coli* | 5,056 (25) | 349 (11) |
| *K. pneumoniae* | 877 (4) | 87 (3) |
| *E. cloacae* | 283 (1) | 43 (1) |
| *P. mirabilis* | 320 (2) | 25 (1) |
| Other Enterobacteriaceae[1] | 1,011 (5) | 139 (4) |
| *P. aeruginosa* | 455 (2) | 63 (2) |
| *Acinetobacter* spp. | 78 (0) | 12 (0) |
| Other non-fermenters[2] | 67 (0) | 14 (0) |
| Other Gram-negatives[3] | 476 (2) | 57 (2) |
| *E. faecalis* | 631 (3) | 100 (3) |
| *E. faecium* | 386 (2) | 199 (6) |
| *S. aureus* | 2,030 (10) | 256 (8) |
| CNS | 6,415 (31) | 1,634 (51) |
| Other Gram-positives[4] | 2,546 (12) | 207 (6) |

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

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

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

[3] *B. fragilis, H. influenzae, N. meningitidis, C. jejuni.*

[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*, *E. cloacae*, *P. mirabilis* and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2017.

| | *E. coli* | *K. pneumoniae* | *E. cloacae* | *P. mirabilis* | *P. aeruginosa* |
|---|---|---|---|---|---|
| **Antibiotic** | | | | | |
| amoxicillin/ampicillin | 45 | - | - | 21 | - |
| co-amoxiclav[1] - non-uuti | 37 | 17 | - | 8 | - |
| piperacillin-tazobactam | 5 | 7 | - | 1 | 5 |
| cefuroxime | 12 | 14 | - | 1 | - |
| cefotaxime/ceftriaxone | 6 | 10 | - | 1 | - |
| ceftazidime | 4 | 9 | - | 0 | 2 |
| meropenem/imipenem | 0 | 0 | 0 | 0 | - |
| meropenem | - | - | - | - | 1 |
| imipenem | - | - | - | - | 3 |
| ciprofloxacin | 14 | 14 | 5 | 11 | 9 |
| gentamicin | 4 | 5 | 3 | 5 | 2 |
| tobramycin | 5 | 7 | 4 | 4 | 1 |
| co-trimoxazole | 24 | 15 | 5 | 24 | - |
| **Empiric therapy combinations** | | | | | |
| gentamicin + amoxicillin/ampicillin | 4 | - | - | 4 | - |
| gentamicin + co-amoxiclav - non-uuti | 3 | 4 | - | 2 | - |
| gentamicin + piperacillin-tazobactam | 1 | 2 | - | 0 | 0 |
| gentamicin + cefuroxime | 2 | 4 | - | 0 | - |
| gentamicin + cefotaxime/ceftriaxone | 1 | 4 | - | 0 | - |
| gentamicin + ceftazidime | 1 | 3 | - | 0 | 0 |
| tobramycin + ceftazidime | - | - | - | - | 0 |
| tobramycin + ciprofloxacin | - | - | - | - | 1 |
| **Multidrug resistance** | | | | | |
| HRMO[2] | 8 | 11 | 2 | 4 | 1 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for E. cloacae as resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

**Figure 4.3.4.1** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.

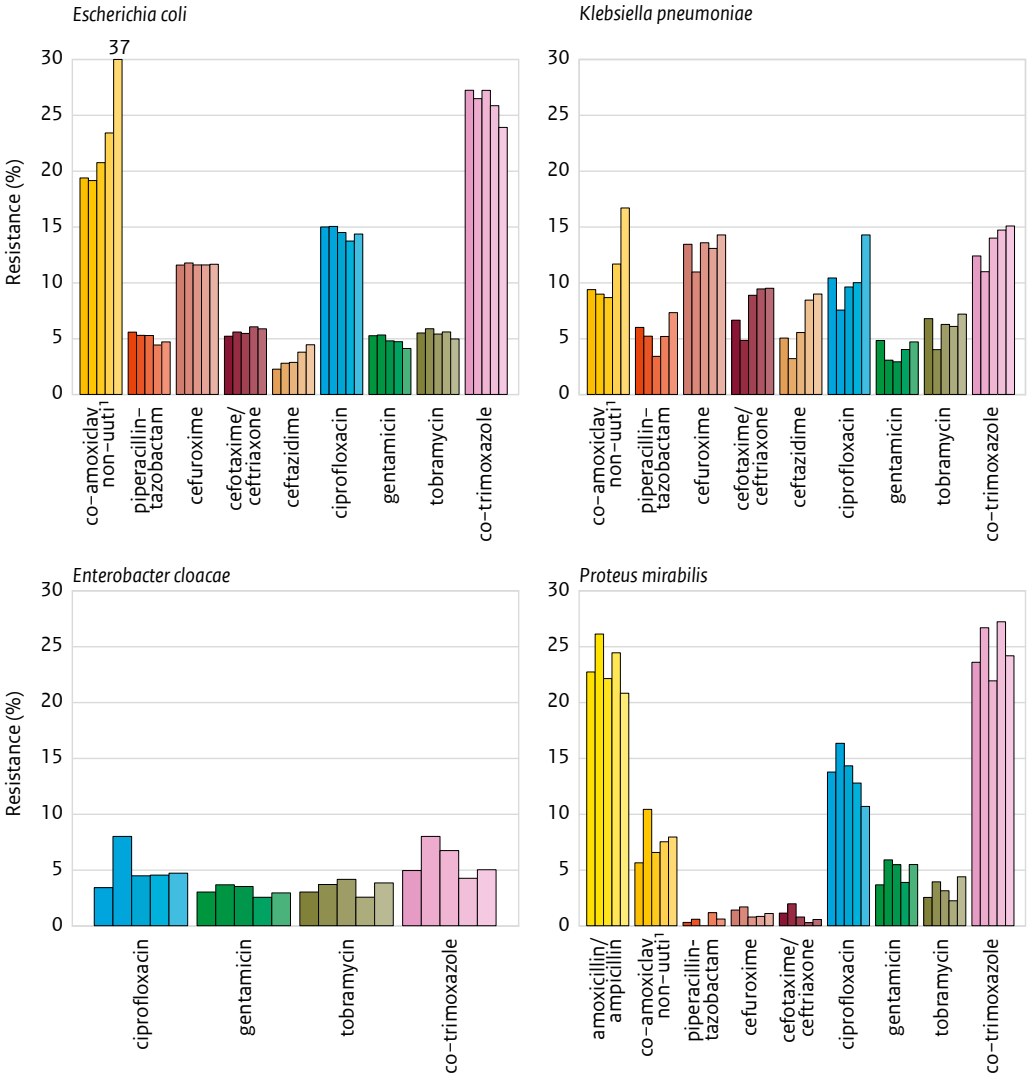
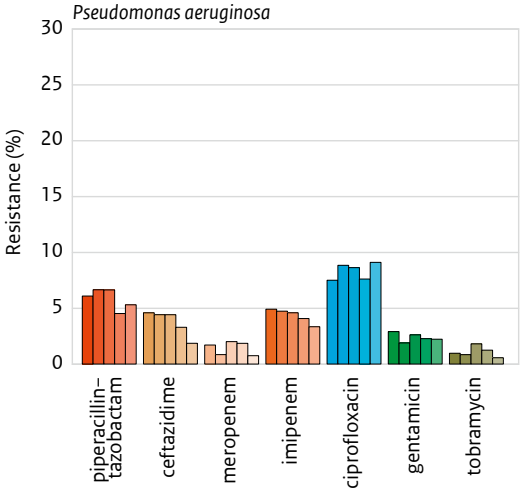


**Figure 4.3.4.1 (continued)** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.




*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 2017.

| | *E. faecalis* | *E. faecium* |
|---|---|---|
| **Antibiotic** | | |
| amoxicillin/ampicillin | - | 86 |
| vancomycin | 0 | 1 |

*- = Resistance not calculated.*

**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 2017.

| | *S. aureus* | CNS |
|---|---|---|
| **Antibiotic** | | |
| flucloxacillin[1] | 1 | 44 |
| ciprofloxacin[2] | 6 | 31 |
| gentamicin | 0 | 26 |
| erythromycin | 10 | 46 |
| clindamycin including inducible resistance[3] | 9 | 32 |
| doxycycline/tetracycline | 3 | 19 |
| linezolid | 0 | 0 |
| co-trimoxazole | 1 | 19 |
| rifampicin | 0 | 3 |

| | |
|---|---|
| 10 | Significant and clinically relevant increasing trend since 2013 |
| 10 | Significant and clinically relevant decreasing trend since 2013 |
| 10 | No significant and clinically relevant time trend |
| | (For the definition of a clinically relevant trend see the methods section) |

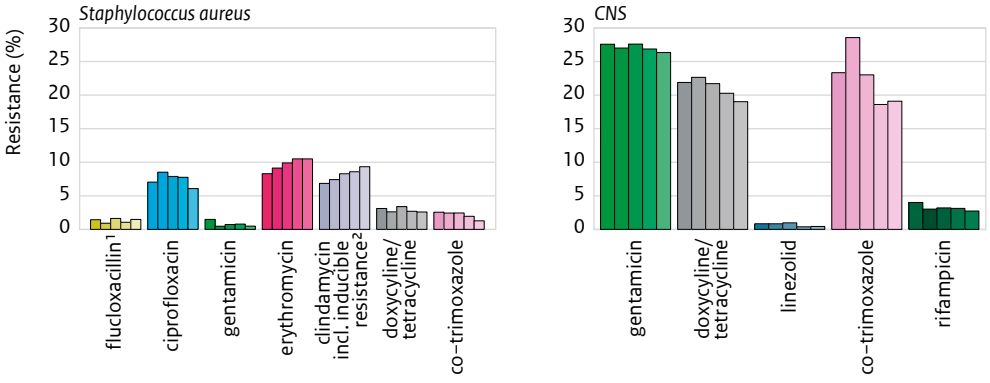
*CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.*

[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 4.3.4.2** Trends in antibiotic resistance (from left to right 2013 to 2017) 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 (or slightly lower than; *S. aureus*) resistance in non-ICU departments in all diagnostic samples combined (chapter 4.3.2).
- Significant and clinically relevant increasing trends in resistance were observed for co-amoxiclav in *E. coli* (from 19% in 2013 to 37% in 2017) and *K. pneumoniae* (from 9% to 17%), 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 *K. pneumoniae* resistance to ceftazidime increased as well (from 5% in 2013 to 9% in 2017). In coagulase-negative *Staphylococcus* spp., a decrease in resistance to co-trimoxazole was observed, especially in the last four years (from 29% in 2014 to 19% in 2017). All these trends were similar to those in non-ICU departments in all diagnostic samples combined. In addition, in *P. aeruginosa*, resistance to ceftazidime decreased to a significant and clinically relevant extent, from 5% in 2013 to 2% in 2017, and in *P. mirabilis*, resistance to ciprofloxacin decreased in the last four years (from 16% in 2014 to 11% in 2017, p=0.02), although the trend in all five years was not significant.

## 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 pathogens isolated from these patients in 2017, 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 for the Enterobacteriaceae and *P. aeruginosa* are shown in figure 4.3.5.1.

**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 2017.

| Pathogen | OPD N (%) | IPD N (%) |
|---|---|---|
| *E. coli* | 10,502 (42) | 1,973 (35) |
| *K. pneumoniae* | 2,207 (9) | 418 (7) |
| *P. mirabilis* | 1,236 (5) | 324 (6) |
| Other Enterobacteriaceae[1] | 3,610 (14) | 893 (16) |
| *P. aeruginosa* | 968 (4) | 341 (6) |
| Other non-fermenters[2] | 502 (2) | 157 (3) |
| Other Gram-negatives[3] | 7 (0) | 4 (0) |
| *E. faecalis* | 2,852 (11) | 773 (14) |
| *E. faecium* | 170 (1) | 121 (2) |
| Other Gram-positives[4] | 3,096 (12) | 677 (12) |

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

**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 2017.

| | *E. coli* | | *K. pneumoniae* | | *P. mirabilis* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|---|---|
| | OPD | IPD | OPD | IPD | OPD | IPD | OPD | IPD |
| **Antibiotic** | | | | | | | | |
| amoxicillin/ampicillin | 45 | 49 | - | - | 23 | 24 | - | - |
| co-amoxiclav[1] - non-uuti | 37 | 40 | 18 | 19 | 7 | 9 | - | - |
| piperacillin-tazobactam | 4 | 5 | 7 | 9 | 0 | 0 | 5 | 8 |
| cefuroxime | 13 | 17 | 17 | 18 | 1 | 1 | - | - |
| cefotaxime/ceftriaxone | 7 | 10 | 9 | 13 | 1 | 1 | - | - |
| ceftazidime | 5 | 8 | 9 | 12 | 0 | 1 | 2 | 2 |
| meropenem/imipenem | 0 | 0 | 0 | 0 | 0 | 0 | - | - |
| meropenem | - | - | - | - | - | - | 1 | 1 |
| imipenem | - | - | - | - | - | - | 5 | 4 |
| ciprofloxacin | 22 | 27 | 18 | 17 | 16 | 18 | 14 | 16 |
| gentamicin | 5 | 7 | 4 | 6 | 7 | 7 | 5 | 3 |
| tobramycin | 6 | 8 | 6 | 10 | 4 | 5 | 1 | 1 |
| fosfomycin | 2 | 2 | 26 | 22 | 12 | 12 | - | - |
| trimethoprim | 31 | 32 | 28 | 27 | 38 | 38 | - | - |
| co-trimoxazole | 28 | 29 | 17 | 21 | 32 | 32 | - | - |
| nitrofurantoin | 4 | 3 | - | - | - | - | - | - |
| **Empiric therapy combinations** | | | | | | | | |
| gentamicin + amoxicillin/ampicillin | 5 | 6 | - | - | 5 | 5 | - | - |
| gentamicin + co-amoxiclav - non-uuti | 4 | 6 | 4 | 5 | 2 | 3 | - | - |
| gentamicin + piperacillin-tazobactam | - | 1 | - | 2 | - | 0 | 1 | 2 |
| gentamicin + cefuroxime | 2 | 4 | 3 | 4 | 0 | 0 | - | - |
| gentamicin + cefotaxime/ceftriaxone | 2 | 3 | 3 | 4 | 0 | 0 | - | - |
| gentamicin + ceftazidime | 1 | 2 | 2 | 3 | 0 | 0 | 0 | 1 |
| tobramycin + ceftazidime | - | - | - | - | - | - | 0 | 0 |
| tobramycin + ciprofloxacin | - | - | - | - | - | - | 0 | 1 |
| **Multidrug resistance** | | | | | | | | |
| HRMO[2] | 10 | 14 | 11 | 16 | 5 | 5 | 2 | 3 |
| multidrug resistance[3] - non-uuti | 9 | 11 | 6 | 8 | 2 | 3 | - | - |

10 Significant and clinically relevant increasing trend since 2013
10 Significant and clinically relevant decreasing trend since 2013
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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, 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 2013 to 2017) 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.

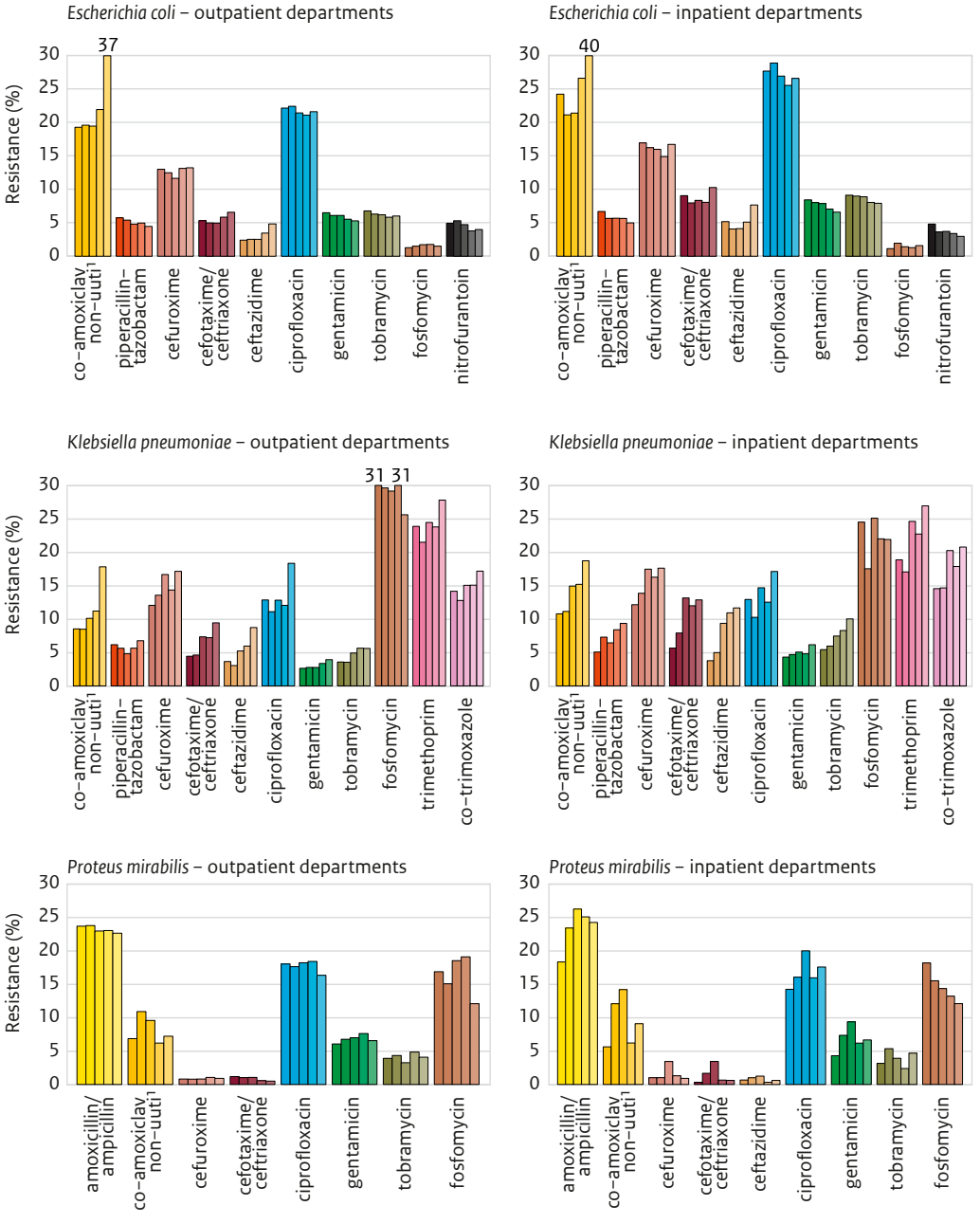
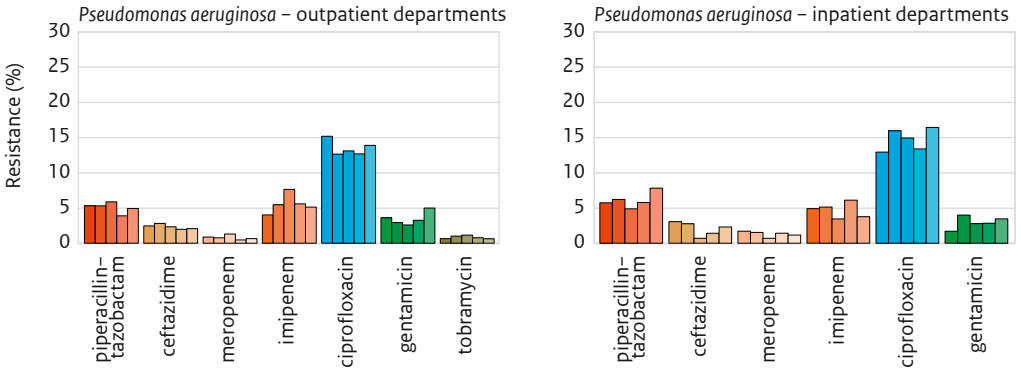


**Figure 4.3.5.1 (continued)** Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments 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 2017.

| | *E. faecalis* | | *E. faecium* | |
|---|---|---|---|---|
| | OPD | IPD | OPD | IPD |
| **Antibiotic** | | | | |
| amoxicillin/ampicillin | - | - | 86 | 96 |
| vancomycin | 0 | 0 | 1 | 1 |
| nitrofurantoin | 1 | 0 | - | - |

*- = Resistance not calculated.*

**Key results**

***Enterobacteriaceae***

- In all Enterobacteriaceae, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤10%, except in *K. pneumoniae* isolates from IPD patients: 13%), ceftazidime (≤9%, except in *K. pneumoniae* isolates from IPD patients: 12%), meropenem/imipenem (0%), gentamicin (≤7%), and tobramycin (≤10%). In addition, levels of 10% or lower were found for fosfomycin (2%) and nitrofurantoin (≤4%) in *E. coli* and for co-amoxiclav (≤9%) and cefuroxime (1%) in *P. mirabilis*.
- In all Enterobacteriaceae, resistance of 20% or higher was found for trimethoprim (≥27%) and co-trimoxazole (≥21%, except in *K. pneumoniae* from OPD patients: 17%). Furthermore, resistance of 20% or higher was found for co-amoxiclav (≥37%) and ciprofloxacin (≥22%) in *E. coli*, for fosfomycin in *K. pneumoniae* (≥22%), and for amoxicillin/ampicillin in *E. coli* and *P. mirabilis* (≥23%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (in OPD from 19% in 2013 to 37% in 2017, and in IPD from 24% to 40%) and *K. pneumoniae* (from 9% to 18% in OPD, and from 11% to 19% 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). Furthermore, in *E. coli*, resistance to amoxicillin/ampicillin (from 54% to 49%) and co-trimoxazole (from 35% to 29%) decreased statistically and to a clinically relevant extent in IPD patients in the last five years. In *K. pneumoniae*, statistically significant and clinically relevant increasing trends in resistance were found for cefotaxime/ceftriaxone and ceftazidime (in OPD patients from 4% in 2013 to 9% in 2017 for both cefotaxime/ceftriaxone and ceftazidime, in IPD patients from 6% to 13% for cefotaxime/ceftriaxone and from 4% to 12% for ceftazidime), and for piperacillin-tazobactam (from 5% to 9%), cefuroxime (from 12% to 18%), tobramycin (from 5% to 10%), trimethoprim (from 19% to 27%), and co-trimoxazole (from 15% to 21%) in IPD patients.
- Resistance to empiric therapy combinations was ≤6%.
- The percentage of HRMO was ≤10% in *P. mirabilis* and *E. coli* (OPD patients only). A statistically significant and clinically relevant increase was observed in *K. pneumoniae* in IPD patients (from 8% in 2013 to 16% in 2017). The percentage of multidrug resistance was ≤9%, except for *E. coli* in IPD patients (11%). In *K. pneumoniae* multidrug resistance increased to a significant and clinically relevant extent in OPD patients (from 3% in 2013 to 6% in 2017) and in IPD patients (from 4% to 8%).

***P. aeruginosa***

- Resistance to each of the selected agents was ≤10%, except for ciprofloxacin (OPD: 14%, IPD: 16%).
- Resistance to empiric therapy combinations and the percentage HRMO were ≤3%.

***E. faecalis and E. faecium***

- Resistance to vancomycin (≤1%) and nitrofurantoin (≤1%, presented for *E. faecalis* only) were both rare.
- In *E. faecium*, resistance to amoxicillin/ampicillin was ≥86%.

## 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' patients and hospital patients (outpatient and inpatient (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 lower respiratory tract infection is suspected. Therefore, the results may be biased towards higher resistance levels by 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 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 2017.

| Pathogen | Lower respiratory tract N (%) | Upper respiratory tract N (%) |
|---|---|---|
| *S. pneumoniae* | 195 (7) | 16 (1) |
| Other Gram-positives[1] | 357 (14) | 1,201 (77) |
| *H. influenzae* | 817 (31) | 55 (4) |
| *M. catarrhalis* | 244 (9) | 11 (1) |
| Other non-fermenters[2] | 404 (15) | 122 (8) |
| Enterobacteriaceae[3] | 587 (22) | 161 (10) |
| Other Gram-negatives[4] | 6 (0) | 0 (0) |

[1] *Staphylococcus spp., S. agalactiae, 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., Proteus spp., Citrobacter spp., Pantoea spp., Morganella spp., Hafnia 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 2017.

| | *S. pneumoniae* | *H. influenzae* | *M. catarrhalis* |
|---|---|---|---|
| **Antibiotic** | | | |
| (benzyl)penicillin (R)[1] | 0 | - | - |
| (benzyl)penicillin (I+R)[1] | 6 | - | - |
| amoxicillin/ampicillin | - | 24 | - |
| co-amoxiclav | - | 10 | 1 |
| erythromycin | 15 | - | 1 |
| doxycycline/tetracycline | 13 | 1 | 1 |
| co-trimoxazole | 10 | 24 | 6 |

*- = 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 2017.

| Pathogen | Blood or cerebrospinal fluid N (%) | Lower respiratory tract N (%) | Upper respiratory tract N (%) |
|---|---|---|---|
| *S. pneumoniae* | 1,479 (5) | 2,742 (9) | 142 (2) |
| Other Gram-positives[1] | 16,718 (56) | 5,628 (18) | 5,028 (61) |
| *H. influenzae* | 191 (1) | 7,675 (24) | 499 (6) |
| *M. catarrhalis* | 18 (0) | 2,340 (7) | 145 (2) |
| Other non-fermenters[2] | 853 (3) | 5,058 (16) | 668 (8) |
| Enterobacteriaceae[3] | 9,863 (33) | 8,232 (26) | 1,690 (21) |
| Other Gram-negatives[4] | 496 (2) | 113 (0) | 7 (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, L. monocytogenes.*

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

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

[4] *B. fragilis, N. meningitidis, C. jejuni, H. pylori.*

**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 2017.

| | *S. pneumoniae* | *H. influenzae* | *M. catarrhalis* |
|---|---|---|---|
| **Antibiotic** | | | |
| (benzyl)penicillin (R)[1] | 0 | - | - |
| (benzyl)penicillin (I+R)[1] | 5 | - | - |
| amoxicillin/ampicillin | - | 22 | - |
| co-amoxiclav | - | 8 | 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 both patient groups) and nonsusceptibility (6% in GP patients and 5% in hospital patients) was ≤10%. Furthermore resistance levels of 10% or lower were found for co-trimoxazole (10% in GP patients and 8% in hospital patients), and erythromycin (10%) and doxycycline/tetracycline (9%) in hospital patients.

***H. influenzae***

- Resistance of 10% or lower was found for doxycycline/tetracycline in both patient groups (1%) and for co-amoxiclav (10% in GP patients and 8% in hospital patients).
- Resistance levels of 20% or higher were found for amoxicillin/ampicillin in GP patients (24%) and hospital patients (22%) and for co-trimoxazole (24% and 23%, respectively).

***M. catarrhalis***

- Resistance to each of the selected agents was ≤10% in both patient groups.

## 4.4 Long-term care facilities

The distribution of pathogens in diagnostic urine and wound or pus samples from long-term care facilities (LTCF) residents is presented in table 4.4.1. The resistance levels in 2017 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 or pus samples in table 4.4.3.

LTCFs usually send urinary, wound, and pus samples for culture and susceptibility testing in case of antimicrobial therapy failure and (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 and pus 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 or pus samples from selected residents of long-term care facilities, ISIS-AR 2017.

| Pathogen | Urine N (%) | Wound or pus N (%) |
|---|---|---|
| *E. coli* | 6,339 (45) | 90 (9) |
| *K. pneumoniae* | 1,267 (9) | 22 (2) |
| *P. mirabilis* | 1,908 (14) | 104 (10) |
| Other Enterobacteriaceae[1] | 1,369 (10) | 91 (9) |
| *P. aeruginosa* | 797 (6) | 121 (11) |
| Other non-fermenters[2] | 124 (1) | 20 (2) |
| Other Gram-negatives[3] | 0 (0) | 16 (2) |
| *S. aureus* | 591 (4) | 466 (44) |
| Other Gram-positives[4] | 1,730 (12) | 124 (12) |

[1] *Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Serratia spp., Hafnia spp., Salmonella spp., Pantoea spp., Escherichia spp. (non-coli).*
[2] *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*
[3] *B. fragilis, H. influenzae.*
[4] *Enterococcus spp., S. agalactiae, S. dysgalactiae equisimilis, S. mitis, S. oralis, S. pyogenes, beta-haemolytic Streptococcus spp. gr C, beta-haemolytic Streptococcus spp. gr G, Staphylococcus spp. (non-aureus), M. tuberculosis.*

**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 2017.

| | *E. coli* | *K. pneumoniae* | *P. mirabilis* | *P. aeruginosa* |
|---|---|---|---|---|
| **Antibiotic** | | | | |
| amoxicillin/ampicillin | 49 | - | 23 | - |
| co-amoxiclav[1] - non-uuti | 41 | 18 | 10 | - |
| piperacillin-tazobactam | 7 | 6 | 1 | 5 |
| cefuroxime | 14 | 15 | 1 | - |
| cefotaxime/ceftriaxone | 6 | 8 | 0 | - |
| ceftazidime | 5 | 7 | 0 | 2 |
| meropenem/imipenem | 0 | 0 | 0 | - |
| meropenem | - | - | - | 2 |
| ciprofloxacin | 22 | 14 | 17 | 11 |
| gentamicin | 6 | 3 | 5 | 4 |
| tobramycin | 7 | 6 | 3 | 0 |
| fosfomycin | 2 | 22 | 14 | - |
| trimethoprim | 27 | 25 | 38 | - |
| co-trimoxazole | 25 | 15 | 29 | - |
| nitrofurantoin | 4 | - | - | - |
| **Multidrug resistance** | | | | |
| HRMO[2] | 11 | 10 | 4 | 1 |
| multidrug resistance[3] - non-uuti | 8 | 5 | 2 | - |

*- = 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 (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone and/or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, 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 and pus isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2017.

| | *S. aureus* |
|---|---|
| **Antibiotic** | |
| flucloxacillin[1] | 2 |
| ciprofloxacin[2] | 25 |
| erythromycin | 12 |
| clindamycin including inducible resistance[3] | 13 |
| doxycycline/tetracycline | 2 |
| fusidic acid | 8 |
| co-trimoxazole | 2 |

[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 for piperacillin-tazobactam (≤7%), cefotaxime/ceftriaxone (≤8%), ceftazidime (≤7%), meropenem/imipenem (0%), gentamicin (≤6%), and tobramycin (≤7%) were ≤10%. In addition, resistance to co-amoxiclav (10%) and cefuroxime (1%) in *P. mirabilis*, and to fosfomycin (2%) and nitrofurantoin (4%) in *E. coli* were ≤10%.
- For all Enterobacteriaceae, resistance levels of 20% or higher were found for amoxicillin/ampicillin (≥23%), and trimethoprim (≥25%). Additionally, resistance levels for co-amoxiclav (41%) and ciprofloxacin (22%) in *E. coli*, fosfomycin (22%) in *K. pneumoniae*, and co-trimoxazole in *E. coli* and *P. mirabilis* (≥25%) were ≥20%.
- The percentage of HRMO was ≤10%, except for *E. coli* (11%).
- The percentage of multidrug resistance was ≤8% in all Enterobacteriaceae.

***P. aeruginosa***

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

***S. aureus***

- Resistance to flucloxacillin (2%), doxycycline/tetracycline (2%), fusidic acid (8%) and co-trimoxazole (2%) were ≤10%.
- Resistance to ciprofloxacin was 25%.

## 4.5 Highly resistant microorganisms

### 4.5.1 Carbapenem-resistant and carbapenemase-producing Enterobacteriaceae

**Introduction**

Carbapenem-resistant Enterobacteriaceae (CRE) and carbapenemase-producing Enterobacteriaceae (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, i.e. a growing public health threat.[4]

Information on CRE is obtained from ISIS-AR data and additionally, information on molecular typing of CPE is obtained from the Type-Ned database.

#### Prevalence and trends of CRE in the Netherlands

**Methods**

The ISIS-AR database (years 2013-2017) was searched for *E. coli* and *K. pneumoniae* isolates that were tested for meropenem and/or imipenem by automated system. Based on the results, they were categorized as either i) non-susceptible to meropenem and/or imipenem based on 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, MICs of screen-positive isolates should be confirmed with gradient tests. Both screening and clinical isolates were included. Only one isolate per patient, i.e. the most resistant and most completely tested isolate, was included.

Based on data on isolates from 35 laboratories, we calculated numbers of non-susceptible and screen-positive isolates in 2017 based on automated testing. Non-susceptible and screen-positive isolates based on automated testing were subsequently categorized by gradient test results. Furthermore, based on data from 26 laboratories that continuously submitted data to ISIS-AR from 2013 to 2017, we calculated 5-years trends in the percentage of isolates with elevated MIC (screen-positive and non-susceptible isolates combined).

**Results**

Results of sequential testing of carbapenem susceptibility in 2017, as prescribed by the NVMM, are presented in Figure 4.5.1.1. Of a total number of 184,159 isolates (159,177 *E. coli* and 24,982 *K. pneumoniae*), an elevated meropenem and/or imipenem MIC on automated testing was found in 0.8% of isolates. Confirmatory testing in eligible isolates using a gradient strip method (performed in 73.5% of eligible isolates) confirmed elevated carbapenem MIC values in 5% of *E. coli* and 25% of *K. pneumoniae*. This means that the overall yield of further testing was low: in the remaining 95% of *E. coli* and 75% of

*K. pneumoniae* isolates, gradient strip testing showed MIC values below the screening breakpoint. Even in isolates non-susceptible using automated testing, 80% of *E. coli* and 47% of *K. pneumoniae* had MIC values below the screening breakpoint using gradient strip testing. In total, 25 carbapenem resistant *E. coli* isolates and 62 carbapenem resistant *K. pneumoniae* isolates were found. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was 0.02% and 0.25% respectively.
Confirmatory testing of elevated carbapenem MIC values has increased over the past years: in 2017 75% of eligible *E. coli* and 73% of *K. pneumoniae* isolates underwent gradient testing, compared to 51% and 62%, respectively, in 2013 (Figure 4.5.1.2). The overall percentage of confirmed *E. coli* and *K. pneumoniae* with elevated MIC was stable over the past five years (0.02% in 2013 and 0.03% in 2017 in *E. coli*, and 0.33% and 0.42% in *K. pneumoniae*, respectively).

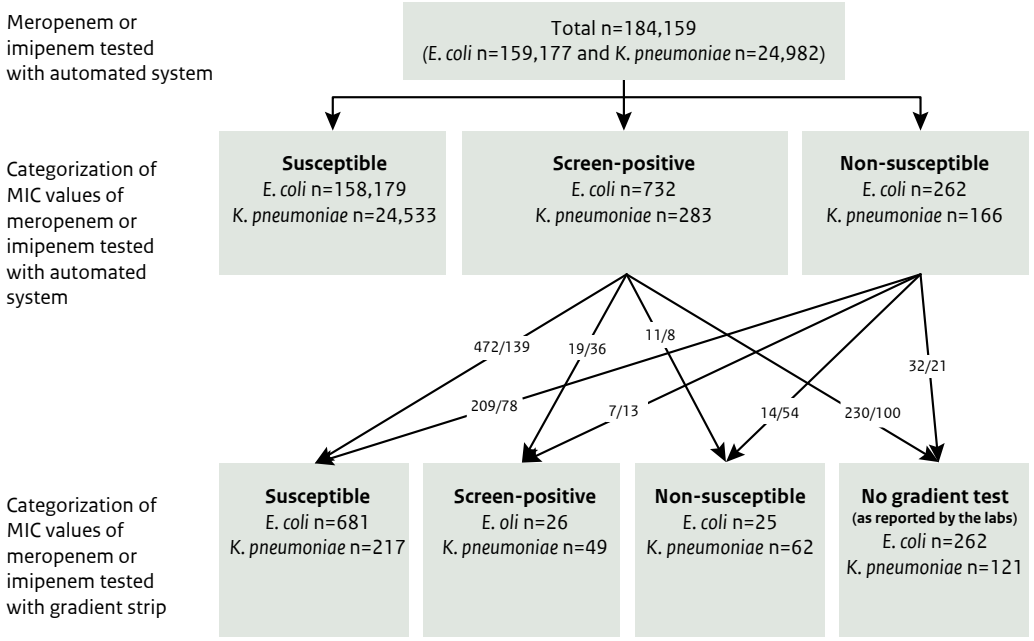
**Discussion**

An elevated carbapenem MIC on automated testing was found in 0.8% of isolates in 2017. This is comparable with previous years. The percentage with a gradient test performed has increased over the past years, to around 75%. This means that the vast majority of the suspected isolates will be investigated further showing the increased effort of laboratories to confirm. The actual percentage of 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.

**Conclusion**

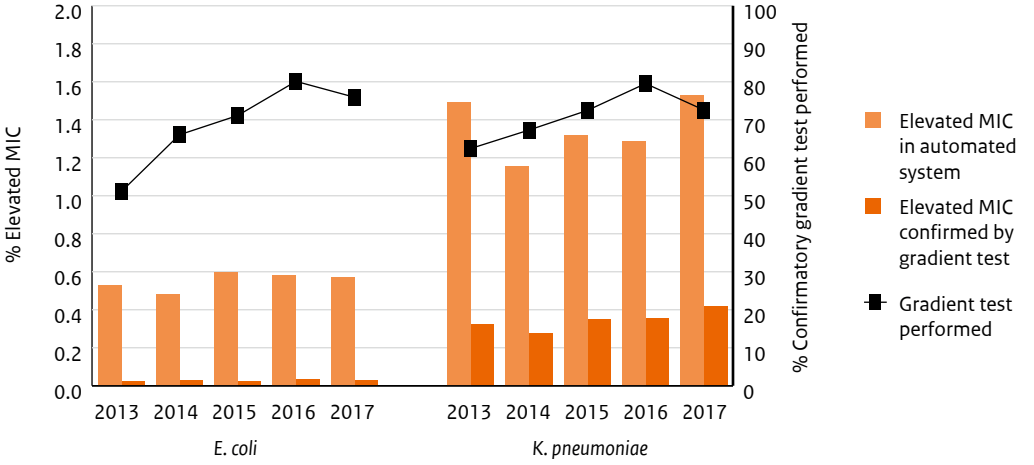
The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing has remained stable (around 0.8%) over the past five years. Confirmatory testing of elevated MIC values with a gradient strip method has increased to around 75% in 2017. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was low (0.03% and 0.42% in 2017, respectively) and stable over the past five years.

**Figure 4.5.1.1** Results of (sequential) testing of carbapenem susceptibility in *E. coli* and *K. pneumoniae* in 2017, according to NVMM guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012) in 35 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 2013 and 2017, in 26 laboratories participating in ISIS-AR.



## Molecular epidemiology

### Methods

For the enhanced surveillance of carbapenemase-producing Enterobacteriaceae (CPE), Dutch MMLs submit isolates with a 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. Nevertheless, the RIVM allows consecutive isolates from the same person when multiple Enterobacteriaceae species and/or multiple different carbapenemase genes were found in an earlier isolate. The RIVM confirms the species and MIC for meropenem, measures the carbapenemase production by the carbapenemase inactivation method (CIM), and assesses the presence of carbapenemase-encoding genes by PCR (carba-PCR). From August 2016 on, next-generation sequencing (NGS) was added to the enhanced CPE surveillance for all isolates that were CIM positive. From 1 September 2016 on, submission of isolates became solely possible via the Type-Ned system.

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 from non-human origin and isolates without a person ID were excluded from further analysis.

### Results

A total of 454 Enterobacteriaceae isolates obtained in 2017 were submitted to the RIVM by 49 Dutch MMLs, of which 406 isolates from 365 persons met the inclusion criteria. The CIM test showed that 235 unique carbapenemase-producing Enterobacteriaceae isolates were obtained from 201 persons submitted by 42 MMLs (mean age 62 years and 56% male). In 2017, one outbreak with carbapenemase-producing Enterobacteriaceae (8 *E. coli* persons with VIM gene) was reported to the Early warning and response meeting of Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR).

The majority 160 (68%) of the 235 isolates, was identified from throat, nose, perineum or rectum swabs, followed by urine (31/235; 13%), wound material or pus (17/235; 7%), sputum/broncho alveolar lavage (11/235; 5%), and blood (10/235; 4%).

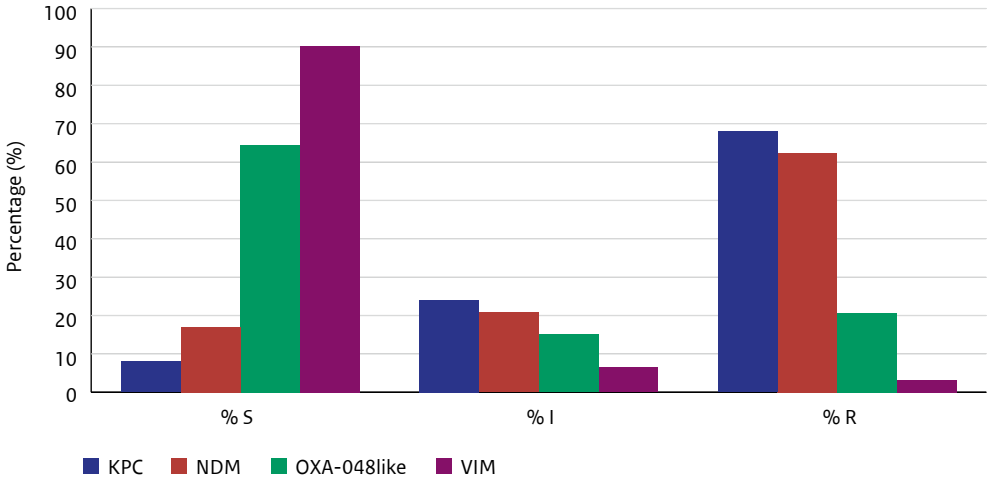
In 173 of the 201 persons, a single carbapenemase-producing species was found, whereas multiple unique carbapenemase-producing species (62 isolates) were isolated from 28 persons. The most frequently identified genes were the genes coding for OXA-048, NDM, VIM and KPC. In six persons both OXA-048 and NDM coding genes were detected in the same isolate. In 17 persons with multiple unique carbapenemase-producing species, this involved the same gene in different species, up to four species in one case. In two persons, a different gene in the same species was found and in eight, a different gene in different species, including one case with a mixture of the same gene in different species and a different gene in different species. Ten unique isolates from 9 patients did not yield a PCR product in the carba-PCR and no carbapenemase gene was identified with NGS. Nine of these isolates were *Enterobacter* species. NGS analysis was performed for 230 isolates originating from 197 persons (Table 4.5.1.1). Meropenem susceptibility for the four major carbapenemases is shown in Figure 4.5.1.3.

**Table 4.5.1.1** Carbapenemases in Enterobacteriaceae isolates submitted in 2017, as detected by PCR and NGS for encoding genes, based on first isolate per patient per year (N=235 unique isolates).

| PCR | NGS, genes coding for | *K. pneumoniae* | *E. coli* | *Enterobacter* species | Other species | Total |
|---|---|---|---|---|---|---|
| KPC | | | | | | 25 |
| | KPC-2 | 8 | 2 | 1 | | 11 |
| | KPC-3 | 13 | | | | 13 |
| | NA | 1 | | | | 1 |
| NDM | | | | | | 53 |
| | NDM-1 | 18 | 7 | 2 | 2 | 29 |
| | NDM-5 | 2 | 16 | | | 18 |
| | NDM-7 | 2 | 3 | | | 5 |
| | NDM-15 | | 1 | | | 1 |
| OXA-023 | | | | | | 1 |
| | OXA-023 | | | | 1 | 1 |
| OXA-048like | | | | | | 107 |
| | OXA-048 | 42 | 24 | 11 | 6 | 83 |
| | OXA-048 + GES-1 | | | 4 | | 4 |
| | OXA-181 | 4 | 6 | | | 10 |
| | OXA-232 | 1 | | | | 1 |
| | OXA-244 | 1 | 5 | | | 6 |
| | NA | 1 | 2 | | | 3 |
| VIM | | | | | | 31 |
| | VIM-1 | 2 | 12 | 6 | 4 | 24 |
| | VIM-4 | | 1 | 1 | 4 | 6 |
| | no gene found | | | 1 | | 1 |
| KPC + NDM | | | | | | 2 |
| | KPC-2 + NDM-1 | | 1 | | 1 | 2 |
| NDM + OXA-048like | | | | | | 6 |
| | NDM + OXA-048 | 3 | | | | 3 |
| | NDM + OXA-162 | | | | 1 | 1 |
| | NDM + OXA-181 | | 1 | | | 1 |
| | NDM + OXA-232 | 1 | | | | 1 |
| no gene found | | | | | | 10 |
| | no gene found | | | 9 | 1 | 10 |
| **Total** | | **99** | **81** | **35** | **20** | **235** |

*NA: NGS not available*

**Figure 4.5.1.3** Meropenem susceptibility for the four major carbapenemases.




*S = meropenem MIC ≤2 mg/L; I = MIC >2 mg/L and ≤8 mg/L; R = MIC >8 mg/L*

Additional epidemiological questionnaire data was available for 150 isolates (150/235; 65%) originating from 125 persons (125/201; 62%) with a confirmed CPE isolate. Most of the questionnaires were complete (136/150; 91%) originating from 112 persons.

Screening was the reason for taking the sample in the majority of the isolates (105/150; 70%), 44 samples were clinical (29%), and one isolate was taken for research purposes (1%). The large majority of the persons were living independently (98/125; 78%).

Risk factors for presence of CPE were analyzed on person level (n=122 with risk factor information available) and not on isolate level. A history of admission to a foreign hospital longer than 24 hours within the previous two months was reported for 61 persons (50%) (country in North Africa (n=17), South Europe (n=16), West Asia (including Turkey) (n=9), South Asia (n=9), West Europe (n=4), Southeast Asia (n=4), Southern Africa (n=1), unknown (n=1)). Ten persons (8%) had been in contact with a hospital abroad in a different way (country in North Africa (n=2), West Asia (n=2), South Europe (n=2), East Europe (n=2), West Europe (n=2), South Asia (n=1), Southeast Asia (n=1)). One person (1%) had been abroad in the previous 6 months without visiting a hospital (country in South Asia). Six persons (5%) were admitted to a health care facility with a known ongoing outbreak of CPE. Work-related contact with livestock animals was not reported for any of the patients, and 4 persons (3%) were already known to be CPE-positive. Overall, no risk factor was identified in 40 patients (33%).

## Discussion

In 2017, more Enterobacteriaceae isolates were submitted (n=454) to the RIVM than in 2015 (n=361) and 2016 (n=403), and as a result more CIM positive isolates were detected. It is unknown to what extent the increase in the number of CPEs submitted to Type-Ned is reflecting the increased awareness among MMLs to 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.

## Conclusion

- The most frequently identified carbapenemase-encoding genes in Enterobacteriaceae were genes encoding for OXA-048, NDM, VIM and KPC.

## References


[1] Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. J Intern Med. 2015 May;277(5):501-12.

[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.

[4] Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect. 2014 Sep;20(9):821-30.

## 4.5.2 Vancomycin-resistant Enterococci

### Introduction

Starting in 2011, a growing number of Dutch hospitals have been confronted with outbreaks of vancomycin-resistant *Enterococcus faecium* (VRE).
VRE outbreaks are reported through the Early warning and response meeting of Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR, see section 4.5.6). In total, since the start of SO-ZI/AMR in April 2012, 72 hospital outbreaks with VRE have been reported in the Netherlands: 9 in 2012, 10 in 2013, 14 in 2014, 16 in 2015, 10 in 2016, and 13 in 2017.

### Methods

From the national routine surveillance system, the ISIS-AR database, the percentage of vancomycin-resistant Enterococci (VRE) was identified among patients in various hospital departments in the Netherlands in 2017. Numbers are based on data from 34 laboratories that continuously reported to the ISIS-AR database during the whole year in 2017. The first *E. faecium* isolate per patient was selected.
As of May 2012, in-depth analysis of the evolutionary relatedness of *E. faecium* genotypes on a population level using Multi Locus Sequence Typing (MLST) data is performed by the UMC Utrecht. Since then, 47 hospitals and laboratories have submitted 958 clinical and surveillance isolates of VRE to the UMC Utrecht (status of March 20th, 2018), of which 919 were identified as *E. faecium*, 2 *E. faecalis* and 1 *E. gallinarum* and of 37 isolates the Enterococcus species was not determined.

### Results

The percentage of vancomycin-resistant Enterococci (VRE) isolates in various hospital departments in the Netherlands based on ISIS-AR is shown in Table 4.5.2.1.
Of the 919 *E. faecium* VRE, submitted to the UMC Utrecht, 529 isolates carried the *vanA* gene cluster, 381 the *vanB* gene cluster, five isolates carried both the *vanA* and the *vanB* gene cluster and four isolates carried the *vanD* gene cluster. Of the 919 *E. faecium* VRE, 801 were typed by Multi Locus Sequence Typing (MLST). This revealed a total of 53 different Sequence Types, suggesting that at least 53 VRE clones circulated in Dutch hospitals. The emergence of VRE in Dutch hospitals can therefore not be attributed to spread of a single clone. On the other hand, 20 STs were found in more than one hospital, suggesting that clonal transmission between hospitals may have contributed to this epidemic rise as well. These highly prevalent STs include ST117 (33 hospitals), ST203 (26 hospitals), ST80 (18 hospitals), and ST18 (15 hospitals).

### Discussion and conclusion

These findings, based on voluntary submission of VRE isolates, give an impression of the molecular epidemiology of VRE in Dutch hospitals. However, it should be noted, that these numbers do not reliably represent the number of VRE isolates/clones circulating in Dutch hospitals, because (i) hospitals send only an unknown fraction of representative VRE to the UMCU for molecular typing, (ii) hospitals send VRE to other laboratories for molecular typing and (iii) hospitals use in-house developed typing schemes for determining genetic relatedness of VRE. This means that there is no completeness and accurate overview of the molecular epidemiology of VRE in Dutch hospitals.

**Table 4.5.2.1** Vancomycin-resistant *E. faecium* (VRE) in the Netherlands in 2017, based on ISIS-AR data.

| Type of department | Tested isolates, N | VRE, N (%) |
|---|---|---|
| GP | 443 | 3 (1) |
| Outpatient departments | 421 | 3 (1) |
| Inpatient departments excluding intensive care units | 2,877 | 23 (1) |
| Intensive care units | 828 | 6 (1) |

*Numbers are based on a selection of 34 laboratories.*
*The first diagnostic E. faecium isolate per patient was selected.*
*Based on laboratory S/I/R interpretation.*
*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.*

## 4.5.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

### Introduction

The Netherlands is a country with a low MRSA prevalence. This may be 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 (MMLs). To monitor the occurrence of MRSA and the molecular characteristics of circulating MRSA types more in-depth at a national level enhanced MRSA surveillance started in 1989 by the National Institute for Public Health and the Environment (RIVM).

### Methods

From the national routine surveillance system, the ISIS-AR database, *S. aureus* isolates including MRSA isolates were identified for unique patients in 2017. Numbers are based on data from 29 laboratories that continuously reported to the ISIS-AR database during the whole year in 2017. The first *S. aureus* isolate per patient was selected.

For the enhanced MRSA surveillance, Dutch MMLs are requested to submit identified MRSA isolates using the Type-Ned system for molecular typing, with the restriction that they only send the first MRSA isolated from a person within a year. With a unique identifier (one-way coded BSN), repeated isolates from the same individual can be recognised. It is assumed that the enhanced MRSA surveillance includes the far majority of all persons found to be MRSA culture-positive by the MMLs. Since 2015, all MRSA isolates submitted through the Type-Ned system are typed using multiple-locus variable number of tandem repeat analysis (MLVA). From November 2016 on, next-generation sequencing (NGS) has been added to the enhanced MRSA surveillance for clinical isolates only.

The data used in this chapter were based on the first MRSA isolate per person in 2017, with the exception that the first clinical isolate is included when both a screening and a clinical sample are submitted from the same person. In addition, samples from non-human origin, *S. aureus* negative for the *mecA* and *mecC* gene, and isolates without a person ID were also excluded from further analysis.

### Results

#### *Prevalence*

The proportion of *S. aureus* that are MRSA in clinical isolates (including blood samples) based on ISIS-AR was 2% (560/28,977) and it was comparable in all types of departments (Table 4.5.3.1). However, because in routine laboratories MRSA's are always registered in their database but most screening isolates that are methicillin susceptible are not, the MRSA prevalence in the population is overestimated if based on all samples. In blood isolates, expected to be most unbiased, the MRSA prevalence was 1.4% (29/2,064).

**Table 4.5.3.1** Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2017, based on ISIS-AR data.

| Type of department | Tested isolates, N | MRSA, N (%)* |
|---|---|---|
| GP | 6,312 | 139 (2) |
| Outpatient departments | 11,126 | 194 (2) |
| Inpatient departments excluding intensive care units | 10,394 | 199 (2) |
| Intensive care units | 1,145 | 28 (2) |
| **Total** | **28,977** | **560 (2)** |

** 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 29 laboratories.*
*The first diagnostic S. aureus isolate per patient was selected.*
*Based on laboratory S/I/R interpretation.*
*Based on re-interpretation according to EUCAST 2017.*

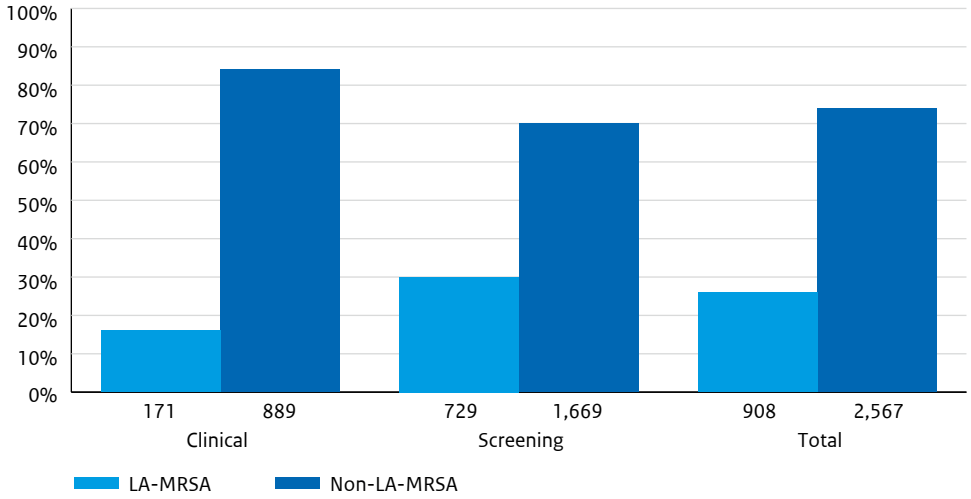
### *Molecular results and epidemiology*

A total of 4,153 isolates obtained in 2017 were genotyped as part of the enhanced MRSA surveillance, of which 3,781 obtained from 3,475 persons (mean age 46 years and 1,886 (54%) male) submitted by 54 MMLs fulfilled the inclusion criteria (*S. aureus mecA* or *mecC* gene positive, from human origin with a known person ID).

The majority of isolates was submitted to the MML by hospitals (2,244/3,475; 65%), followed by GPs (883/3,475; 25%) and nursing or elderly homes (214/3,475; 6%). Based on culture methods and origin of the samples, 69% (2,398/3,475) of the isolates were identified as screening samples (mainly swabs of nose, throat and perineum) (Figure 4.5.3.1). A total of 1,060 samples (31%) were identified as clinical sample (material originating from blood, cerebrospinal fluid, sputum, pus, urine or wound), with the majority being wound material or pus (760/1,060; 72%) and 38 blood samples (4%). For 18 samples (1%), the origin of the sample was unknown.

For 2017, the MRSA population could be divided into 706 MLVA-types, which were grouped into 26 MLVA-complexes (MCs). The most common MLVA-complex in the Dutch enhanced MRSA surveillance in 2017 was MC0398, representing livestock-associated MRSA (LA-MRSA), which was detected in 908/3,475 (26%) of the isolates (Figure 4.5.3.1). Of the LA-MRSA isolates, 19% were clinical isolates, 80% were obtained for screening purposes, and for 1% it was unknown. The most common MLVA types (MTs) among LA-MRSA isolates were MT0398 (484/908; 53%), MT0569 (160/908; 18%), and MT0572 (72/908; 8%). All other types were found in less than 30 isolates in 2017.

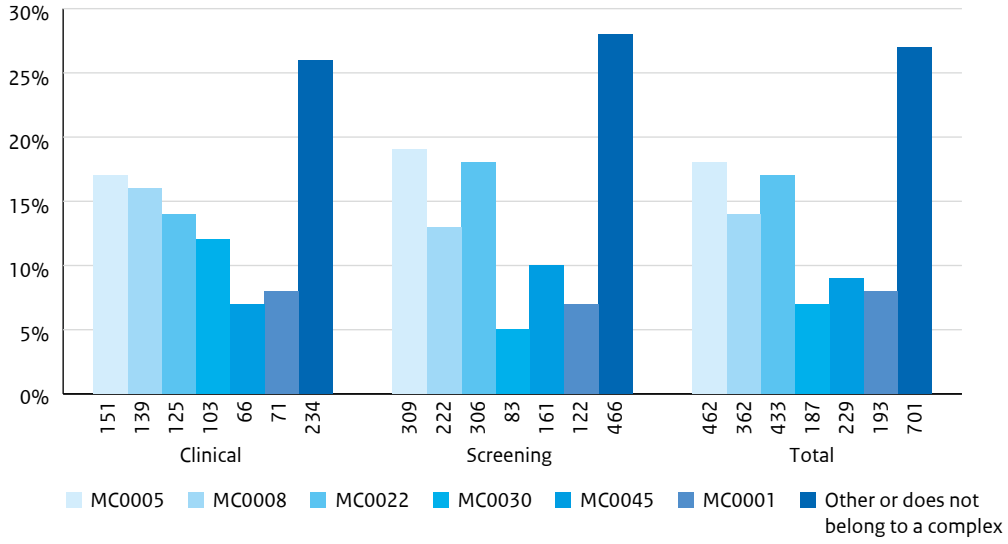
**Figure 4.5.3.1** Distribution of clinical and screening samples for LA-MRSA and non-LA-MRSA among MRSA isolates received in the Dutch enhanced MRSA surveillance in 2017 (N=3,475).




*Only the first MRSA isolate per person was selected.*
*Clinical 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.*
*LA-MRSA represents MC0398.*
*The numbers below the bars represent the absolute numbers of isolates.*
*The total number includes an additional 17 isolates for which it was unknown whether it was a clinical or screening sample (8 LA-MRSA and 9 non-LA-MRSA).*

The number and proportion of clinical isolates was higher among the non-LA-MRSA (889/2,567; 35%) than the LA-MRSA isolates. This distribution is similar to the data observed in 2016. Among the clinical isolates, MC0005, MC0008 and MC0022 were the most prevalent non-LA-MRSA MLVA complexes (Figure 4.5.3.2).

**Figure 4.5.3.2** Distribution of the major non-LA-MRSA MLVA-complexes among MRSA isolates received in the Dutch enhanced MRSA surveillance in 2017 (N=2,567).




*Only the first MRSA isolate per person was selected.*
*Clinical 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.*
*The category Other represents other non-LA-MRSA MLVA complexes than the complexes shown in the graph.*
*The numbers below the bars represent the absolute numbers of isolates.*
*The total number includes an additional 9 isolates for which it was unknown whether it was a clinical or screening sample (2 MC0005, 1 MC0008, 2 MC0022, 1 MC0030, 2 MC0045 and 1 Other).*

Possession of *mecA* was confirmed in 3,449/3,475 (99%) of all isolates, while 26 isolates possessed *mecC* (all non-LA-MRSA, mostly belonging to MC0429 (20/26; 77%)). Panton-Valentine Leukocidin (PVL) positivity was seen in 758/3,475 (22%) of the isolates: 732 (97%) non-LA-MRSA and 26 (3%) LA-MRSA. Most of the PVL-positive LA-MRSA isolates were of MLVA type MT0569 (18/26; 69%) and non-LA-MRSA was often MC0008 (227/732; 31%) with MT0314 (65/227; 29%) and MT0240 (52/227; 23%) as most common MLVA types.

### *Risk groups*

A risk factor questionnaire is requested to be completed as part of the enhanced surveillance. Questionnaire data were available for 3,078 genotyped isolates (3,078/3,781; 81%) originating from 2,827 unique persons (2,827/3,475; 81%): 2,624 (93%) were patients and 202 (7%) were employees. Most of the questionnaires from unique persons were complete (2,745/2,827; 97%). Samples were taken at the outpatient clinic in 910/2,821 (32%) of the persons, during hospital stay in 704/2,821 (25%), at home in 262/2,821 (9%) and at the intensive care unit in 53/2,821 (2%).

Completed data on risk categories based on the WIP guidelines[1] were available for 2,792 (80%) of the persons. Hospitalization abroad during the previous two months was recorded for 164/2,792 persons (6%). Work-related exposure to livestock animals was reported for 426 persons (15%), all except 5 of them (98%) were positive for LA-MRSA (MLVA-complex MC0398). A total of 281 persons (10%) were already known to be MRSA-positive. In 902 persons (32%) screening was performed due to contact with an MRSA-positive person. For 38% (1,066/2,792), no risk factors that meet any of the WIP risk categories for MRSA carriage had been detected at time of screening or afterwards. In 133 persons (5%) it was reported that the sample was taken from an asylum seeker living in an asylum centre. All percentages mentioned were comparable to data from 2016.

Of the clinical isolates, completed data on risk categories were available for 833/1,060 (79%). The large majority was not suspected of MRSA carriage (679/833; 82%), 10% had a high risk for carriage, and 5% was already known to be MRSA positive. Twenty-five (3%) had been hospitalized abroad in the previous two months and eighteen (2%) had work-related exposure to livestock animals. These data were also similar to 2016.

## 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 clinical samples, thereby falsely increasing the proportion of MRSA in clinical isolates.
The distinction between screening and clinical 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 clinical isolates may 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 1.4%. The prevalence in biased samples was 2% in general practices, outpatient departments, hospital departments, and intensive care units.
- LA-MRSA is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance and the distribution of dominant types is similar to 2016.
- Non-LA-MRSA subtypes are more often found in clinical isolates than LA-MRSA.
- A large proportion (38%) of the persons positive for MRSA do not seem to have a risk factor as defined in the WIP risk categories. This is similar to 2016.

## 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. However, the organism may also acquire additional resistance either by chromosomal mutations or by horizontal gene transfer. 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] In *P. aeruginosa*, VIM is the most frequently found carbapenemase and the $bla_{VIM}$ gene is mostly chromosomally located, although plasmids carrying $bla_{VIM}$ have also been described.

### Methods

Data on multidrug resistant (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.

From August 2016 - December 2017 medical microbiology laboratories (MMLs) were asked to submit their carbapenem-resistant *P. aeruginosa* to the RIVM for characterization. Submitted isolates are analyzed to confirm the species by MALDI-ToF, resistance by assessing minimal inhibitory concentrations (MIC) for meropenem by Etest, carbapenemase production by carbapenemase inactivation method (CIM)[2] and presence of carbapenemase-coding genes by multiplex PCR. Isolates shown to produce carbapenemase and/or carry carbapenemase-coding genes were subjected to next-generation sequencing (NGS) to assess the whole genome sequence.

### Results

A search in the ISIS-AR database in 2017 revealed that 1% of the *P. aeruginosa* isolates was MDR. Forty-four percent of the MDR isolates was phenotypically resistant to meropenem and/or imipenem (based on re-interpretation according to EUCAST 2017). This fraction was highest in isolates from ICUs (6/11; 55%) and lowest for isolates obtained from patients attending the GP (10/25; 40%) (table 4.5.4.1).

The RIVM received 694 *P. aeruginosa* isolates sampled between August 2016 and December 2017 ("study period") (one isolate per person per year). Isolates were submitted by 45 MMLs and of these 25 submitted carbapenemase-producing isolates. Of the isolates, 77 (11%) produced carbapenemase. PCR revealed that the majority of the carbapenemase-producing isolates (60/77; 78%) carried a $bla_{VIM}$ gene, five (6%) carried a $bla_{IMP}$ and three (4%) a $bla_{NDM}$ gene. Nine of the carbapenemase-producing isolates (12%) did not yield a PCR product.

NGS of 75 of the 77 carbapenemase-producing isolates revealed that 58 (77%) were VIM-positive, of which all but one (57/77; 76%) carried the $bla_{VIM-2}$ allele and one isolate carried $bla_{VIM-11}$ (table 4.5.4.2). NGS also revealed the presence of $bla_{IMP-1}$, $bla_{IMP-7}$, $bla_{IMP-8}$ and $bla_{IMP-26}$ in five IMP-positive isolates and $bla_{NDM-1}$ in three NDM-positive isolates. Two of the PCR-negative, carbapenemase-producing isolates carried the $bla_{GES-5}$ gene. No known, as so far, carbapenemase-coding gene could be identified in the remaining seven isolates.

During the study period 34% (236/694) of the submitted isolates had MICs above 8 mg/L and 23% (54/236) of those produced carbapenemase. Of the 77 carbapenemase-producing isolates 23 (30%) had MICs ≤8 mg/L (figure 4.5.4.1).

### Discussion

Characterization of meropenem-resistant *P. aeruginosa*, submitted to the RIVM during the time period August 2016 – December 2017, showed that 23% produced carbapenemase. This implies that the majority of the isolates were resistant to carbapenems due to other mechanisms. The majority of the carbapenemase-producing *P. aeruginosa* isolates (76%) carried a $bla_{VIM-2}$ gene. As this gene is usually chromosomally located, transmission and spread of $bla_{VIM-2}$ encoded carbapenem resistance in *P. aeruginosa* may be due to transmission of strains and not by horizontal gene transfer of resistance genes located on mobile elements.

### Conclusions

- In isolates collected during the study period 70% of the carbapenemase producing *P. aeruginosa* had MICs for meropenem above the clinical breakpoint
- Of the *P. aeruginosa* isolates with MICs for meropenem above the clinical breakpoint only 23% produced carbapenemase
- The majority (76%) of carbapenemase producing *P. aeruginosa* carried the chromosomally located $bla_{VIM-2}$ gene

### References


[1] Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Evelina Tacconelli et al. Lancet Infect Dis 2018:18; 318–27 December 21, 2017 http://dx.doi.org/10.1016/S1473-3099(17)30753-3.

[2] The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods. Kim van der Zwaluw,* Angela de Haan, Gerlinde N. Pluister, Hester J. Bootsma, Albert J. de Neeling, and Leo M. Schouls. PLoS One. 2015; 10(3): e0123690.

**Table 4.5.4.1** Multidrug resistant (MDR) *P. aeruginosa* in the Netherlands in 2017, based on ISIS-AR data.

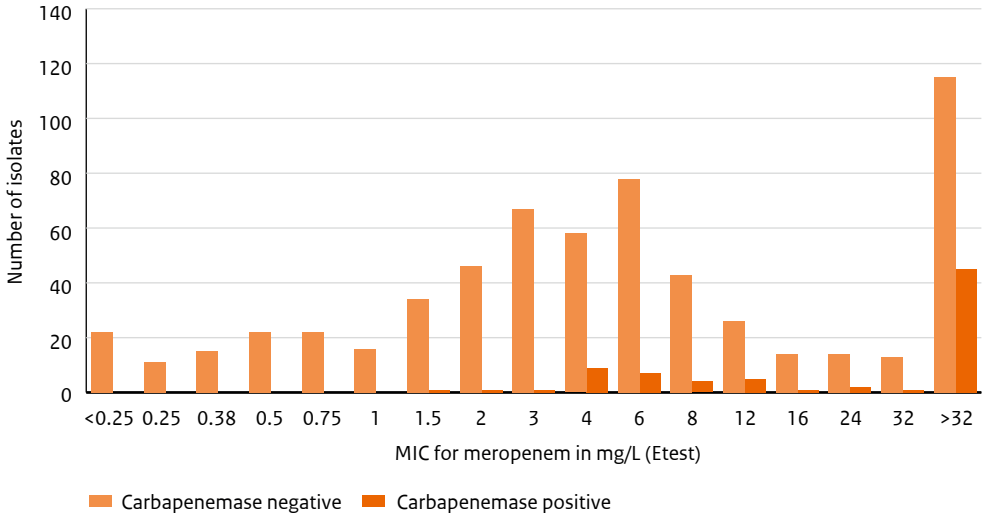
| Type of department | Tested isolates, N | MDR P *aeruginosa* spp., N(%) | Carbapenem resistant MDR P *aeruginosa* spp., N(%) |
|---|---|---|---|
| GP | 3,721 | 25 (1) | 10 (40) |
| Outpatient departments | 3,242 | 49 (2) | 24 (49) |
| Inpatient departments excluding intensive care units | 4,253 | 62 (1) | 25 (40) |
| Intensive care units | 472 | 11 (2) | 6 (55) |
| **Total** | **11,688** | **147 (1)** | **65 (44)** |

*Numbers are based on a selection of 29 laboratories.*
*The first diagnostic P. aeruginosa isolate per patient was selected.*
*Based on re-interpretation according to EUCAST 2017.*
*Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

**Table 4.5.4.2** Allelic distribution of carbapenemase-coding genes in 75 carbapenemase-producing *P. aeruginosa* isolates received during the study period August 2016 – December 2017.

| Carba gene by PCR | Carba allele by NGS | N |
|---|---|---|
| VIM | $bla_{VIM-2}$ | 57 |
| | $bla_{VIM-11}$ | 1 |
| IMP | $bla_{IMP-1}$ | 2 |
| | $bla_{IMP-7}$ | 1 |
| | $bla_{IMP-8}$ | 1 |
| | $bla_{IMP-26}$ | 1 |
| NDM | $bla_{NDM-1}$ | 3 |
| Negative | $bla_{GES-5}$ | 2 |
| | No Carba-gene found | 7 |
| **Total** | | **75** |

**Figure 4.5.4.1** Distribution of MICs among *P. aeruginosa* isolates received during the study period August 2016 – December 2017.



## 4.5.5 Extended spectrum beta-lactamases

### Introduction

Extended spectrum beta-lactamase producing Enterobacteriaceae (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.[1,2] In addition higher carriage rates of ESBL-E is assumed to lead to higher proportion of ESBL-producing isolates in infections.[3] In the Netherlands several recent studies show carriage rates between 5-10% in different populations.[1,4-6] Over the last years, the percentage of ESBLs in clinical isolates of Enterobacteriaceae in the Netherlands was also estimated using the ISIS-AR database. We here present data from ISIS-AR for *E. coli* and *Klebsiella pneumoniae*.

### Methods

Data were extracted from the ISIS-AR database. The percentages of ESBL producing *E. coli* and *Klebsiella 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 2017 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 *Klebsiella pneumoniae* are shown by site, i.e. general practice (GP), outpatient departments, inpatient departments and intensive care units, in 2017. Trends in ESBL percentages (from left to right 2013 to 2017) among clinical isolates of *E. coli* and *Klebsiella pneumoniae* by site are shown in figure 4.5.5.1.

Overall, the percentages of ESBL has increased over the years (more clear in *Klebsiella pneumoniae*) with ESBL percentages between 2 and 6% for *E. coli* and between 4 and 9% for *Klebsiella pneumoniae* depending on type of department in 2017. 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.[2]

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 that can subsequently spread to the environment. One of the factors hypothesized to be an important source of antibiotic resistance in humans is livestock. However, whether ESBL acquisition in humans originate from livestock remains debatable as Dutch studies showed presumably related strains of food-producing animals and humans were not phylogenetically related demonstrated by whole-genome sequencing.[7,8] An important source of ESBL acquisition in humans is international travel, high ESBL acquisition rates up to 88.6% have been found in Dutch travellers to high-endemic countries in 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]

## References


[1] Reuland EA, Al Naiemi N, Kaiser AM, Heck M, Kluytmans JA, Savelkoul PH, Elders PJ, Vandenbroucke-Grauls CM. Prevalence and risk factors for carriage of ESBL-producing *Enterobacteriaceae* in Amsterdam. Journal of Antimicrobial Chemotherapy. 2016;71(4):1076-1082.

[2] European Antimicrobial Resistance Surveillance Network (EARS-Net).

[3] Reddy P, Malczynski M, Obias A, et al. Screening for extended-spectrum β-lactamase-producing *Enterobacteriaceae* among high-risk patients and rates of subsequent bacteremia, Clin Infect Dis , 2007; 45: 846-52.

[4] van den Bijllaardt W, Janssens MM, Buiting AG, Muller AE, Mouton JW, Verweij JJ. Extended-spectrum β-lactamase (ESBL) polymerase chain reaction assay on rectal swabs and enrichment broth for detection of ESBL carriage. J Hosp Infect. 2018 Mar;98(3):264-269.

[5] Wielders CCH, van Hoek AHAM, Hengeveld PD, Veenman C, Dierikx CM, Zomer TP, Smit LAM, van der Hoek W, Heederik DJ, de Greeff SC, Maassen CBM, van Duijkeren E. Extended-spectrum β-lactamase- and pAmpC-producing *Enterobacteriaceae* among the general population in a livestock-dense area. Clin Microbiol Infect. 2017 Feb;23(2):120. e1-120.e8.

[6] Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A, Grobusch MP, Lashof AMO, Molhoek N, Schultsz C, Stobberingh EE, Verbrugh HA, de Jong MD, Melles DC, Penders J. Import and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis. 2017 Jan;17(1):78-85.

[7] de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, et al. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet. 2014;10(12):e1004776.

[8] Dorado-Garcia A, Smid JH, van Pelt W, Bonten MJM, et al. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. J Antimicrob Chemother. 2018;73(2):339-347.

**Table 4.5.5.1** Extended spectrum beta-lactamase (ESBL) producing *E. coli* in the Netherlands in 2017, based on ISIS-AR data.

| Type of department | Tested isolates, N | ESBL |
|---|---|---|
| GP | 79,394 | 2,298 (3) |
| Outpatient departments | 17,886 | 831 (5) |
| Inpatient departments excluding intensive care units | 25,176 | 1,271 (5) |
| Intensive care units | 1,488 | 83 (6) |

*Numbers are based on a selection of 29 laboratories.*
*The first diagnostic isolate per organism per patient was selected.*
*Based on re-interpretation according to EUCAST 2017.*
*The percentage of ESBL producing E. coli was 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).*

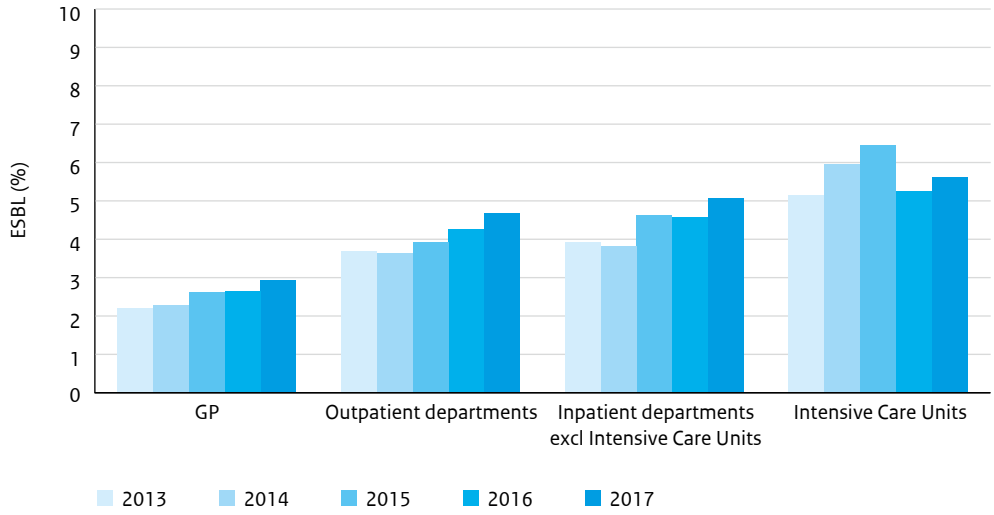
**Table 4.5.5.2** Extended spectrum beta-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2017, based on ISIS-AR data.

| Type of department | Tested isolates, N | ESBL |
|---|---|---|
| GP | 9,813 | 427 (4) |
| Outpatient departments | 3,647 | 238 (7) |
| Inpatient departments excluding intensive care units | 4,901 | 421 (9) |
| Intensive care units | 415 | 37 (9) |

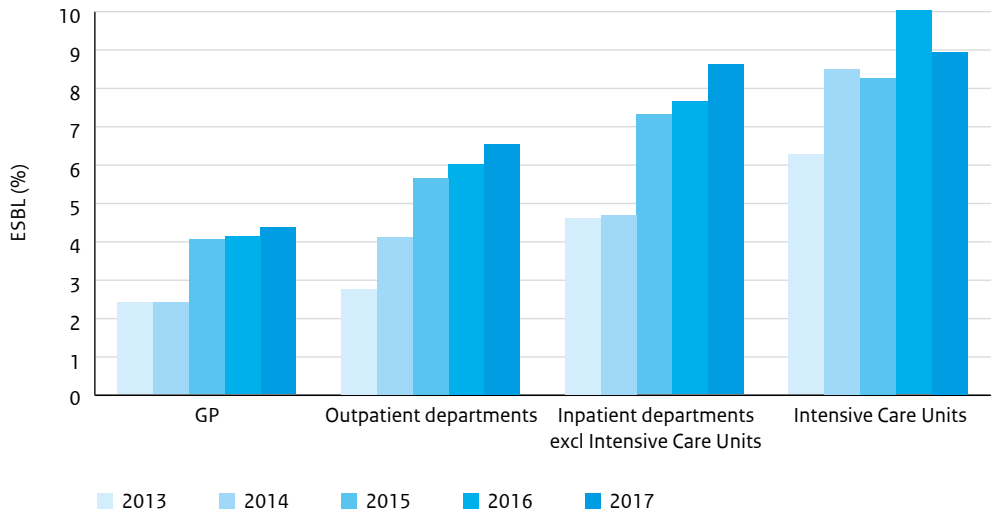
*Numbers are based on a selection of 29 laboratories.*
*The first diagnostic isolate per organism per patient was selected.*
*Based on re-interpretation according to EUCAST 2017.*
*The percentage of ESBL producing K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from 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 and *K. pneumoniae* (from left to right 2013 to 2017) in the Netherlands, based on ISIS-AR data.

*E. coli*




*K. pneumoniae*




*Numbers are based on a selection of 29 laboratories.*
*The first diagnostic isolate per organism per patient per year was selected.*
*Based on re-interpretation according to EUCAST 2017.*
*The percentage of ESBL producing E. coli and Klebsiella 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).*

### 4.5.6 Early warning and response meeting of Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR)

In 2012, the Early warning and response meeting of Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded. The purpose of the SO-ZI/AMR is to prevent further spread and to mitigate large-scale outbreaks in hospitals and nursing homes through early recognition. 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 and course, 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 possible threat to the community exists, it will be classified as phase 3; phase 4 and 5 describe potential management issues.
Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to participate in SO-ZI/AMR. Since 2015 nursing homes and other healthcare facilities are also invited to report HRMO outbreaks.

Table 4.5.6.1 provides an overview of the sixty outbreaks reported in 2017. These were reported by 54 healthcare institutions. These included 31 hospitals, 16 nursing homes, four other institutions (such as long-term care facilities and rehabilitation centers) and three unknown. Most outbreaks (n=50) ended in 2017; seven outbreaks were completed in 2018, three outbreaks, all in phase 2, were still ongoing. As reported in the table, most notifications of outbreaks were motivated by imminent closure of wards; a small proportion was notified because transmission of outbreak strains was ongoing despite infection control measures.

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and norovirus where most often reported, comparable to 2016. Six outbreaks were caused by carbapenemase-producing strains. No *Serratia* outbreaks were reported in contrast to 2016, when three such outbreaks were reported. *Staphylococcus aureus*, enterovirus and rotavirus were also not reported.

Twelve outbreaks included more than 10 patients. The outbreaks classified as phase 2 comprised two MRSA outbreaks, one VRE outbreak, an outbreak with carbapenemase-producing *Enterobacter cloacae* and one outbreak with *E. coli* resistant to aminoglycosides and fluoroquinolones. In 2016 no outbreaks were classified as phase 3 but in 2017 one MRSA outbreak in a nursing home was evaluated as presenting a possible threat to public health.

The time it took to report an outbreak to the SO-ZI/AMR, from the moment that the first patient was identified, was more than three months in five outbreaks. Of these, one was reported after more than two years. In some cases the outbreak was detected not long before reporting, but investigation into the outbreak identified a few patients that appeared to have carried the outbreak strain before the outbreak was detected. Two institutions already requested advice and help to control these outbreaks when notifying this outbreak. Both of these outbreaks were due to a MRSA, one occurred in a nursing home, the other in a home care institution.

**Conclusions**

- On average five outbreaks a month were reported to the SO-ZI/AMR.
- Outbreaks were classified as phase 1 or phase 2, with only one in phase 3.
- Six CPE outbreaks were reported, mainly caused by different organisms.
- Most 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 9.

**Table 4.5.6.1** Characteristics of outbreaks reported to the SO-ZI/AMR in 2017.

| | 2017 n=60<br>n (%) |
|---|---|
| **Microorganism (resistance mechanism)*** | |
| *Staphylococcus aureus* (MRSA) | 30 (50) |
| *Enterococcus faecium* (VRE) | 13 (22) |
| *Acinetobacter* spp. (CPE) | 1 (2) |
| *Enterobacter cloacae* (CPE) | 1 (2) |
| *Escherichia coli* (CPE) | 1 (2) |
| *Escherichia coli* (ESBL) | 2 (3) |
| *Escherichia coli* (AG and FQ) | 1 (2) |
| *Klebsiella pneumoniae* (CPE) | 1 (2) |
| *Klebsiella pneumoniae* (ESBL) | 1 (2) |
| *Pseudomonas aeruginosa* (CPE) | 2 (3) |
| *Clostridium difficile* | 1 (2) |
| Norovirus | 4 (7) |
| Anders | 2 (3) |
| **Reason of reporting** | |
| threatening of ward closure | 35 (58) |
| ongoing transmission | 5 (8) |
| combination of both | 3 (5) |
| HRMO outbreak (not in a hospital) | 13 (22) |
| unknown | 4 (7) |
| **Highest level phase** | |
| phase 1 | 54 (90) |
| phase 2 | 5 (8) |
| phase 3 | 1 (2) |
| phase 4 | 0 (0) |
| phase 5 | 0 (0) |
| **Median number of patients:** (range) | 9 (1-50) |
| **Median duration outbreak in days from reporting date until end of the outbreak:** (range) | 43 (15-270) |
| **Duration in days between detection of the first patient and day of reporting to the SO-ZI/AMR:** (range) | 7 (0-963) |
| **Request for help** | 2 (3) |

** MRSA=methicillin-resistant Staphylococcus aureus; VRE=vancomycin-resistant Enterococcus faecium; ESBL=extended-spectrum beta-lactamase; CPE=carbapenemase-producing Enterobacteriaceae; AG and FQ=resistance to aminoglycosides and fluoroquinolones*

## 4.6 Resistance in specific pathogens

### 4.6.1 *Neisseria meningitidis*

**Introduction**

*Neisseria meningitidis* strains cultured from CSF and/or blood in microbiological laboratories in the Netherlands are submitted to the Netherlands Reference Laboratory for Bacterial Meningitis. 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 gradient test strips (such as Etest), like most assays, are not 100% reproducible, this can give rise to a considerable number of minor and major interpretation errors. Therefore, the PenA amino acid sequence was deduced from the *penA* nucleotide sequence of all isolates.

**Methods**

From 2009- 2017, a total of 369 strains from cerebrospinal fluid (CSF) or CSF and blood and 656 strains from blood were included in the surveillance project of The Netherlands Reference Laboratory for Bacterial Meningitis of the Academic Medical Center, Amsterdam and the National Institute for Public Health and the Environment. The MIC for penicillin was determined by Etest using MHF plates, incubation 18-24 h at 37°C under 5% $CO_2$ . 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 was sequenced, from which the putative amino acid sequence of PenA was deduced.

**Results**

In 2017 one strain from blood was resistant to penicillin (tables 4.6.1.1 and 4.6.1.2), whereas the percentage of strains moderately susceptible to penicillin (MIC 0.06-0.25 mg/L) was around 19% (33/175). In 2017, the 33 moderately susceptible strains from blood and/or CSF belonged to different serogroups: 17 belonged to serogroup B, four to serogroup C, four to serogroup W, seven to serogroup Y and one was non-groupable. In 2017, resistance to ceftriaxone and rifampicin was not found. Alterations in the PenA putative amino acid sequence, associated with non-susceptibility to penicillin according to the Neisseria typing database (https://pubmlst.org/neisseria/), were detected in 22 (13%) of the 175 isolates. Of these isolates, two were phenotypically susceptible and 19 were moderately susceptible by Etest (table 4.6.1.3). One isolate was PenA resistant and also phenotypically resistant. Figure 4.6.1.1 shows the MIC distributions for strains with and without PenA alterations.
PenA genotyping yielded less strains (11%) non-susceptible to penicillin than Etest with EUCAST criteria does (19%) and both methods do not agree completely.

**Conclusions and discussion**

Penicillin resistance is still sporadic (two strains in 2013, zero in the following three years, and one strain in 2017). On the other hand, the percentage of strains moderately susceptible to penicillin (MIC 0.06-0.25 mg/L) increased again for the first time after four years from around 11% to 19% in 2017. Resistance to ceftriaxone was not found in 2017, just as resistance to rifampicin, in contrast to two rifampicin-resistant strains in the previous year after 3 years of absence.

Alterations in PenA associated with non-susceptibility to penicillin are only present in 13% of all isolates compared to 19% with Etest. One or more of the following reasons may be involved: 1) other factors than PenA 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.

**Table 4.6.1.1** Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2009-2017.

| | 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 | **0.0** | **52** |
| 2010 | 43 | **81.1** | 10 | **18.9** | 0 | **0.0** | 0 | **0.0** | **53** |
| 2011 | 29 | **78.4** | 8 | **21.6** | 0 | **0.0** | 0 | **0.0** | **37** |
| 2012 | 24 | **58.5** | 16 | **39.0** | 1 | **2.4** | 0 | **0.0** | **41** |
| 2013 | 35 | **89.7** | 3 | **7.7** | 1 | **2.6** | 0 | **0.0** | **39** |
| 2014 | 26 | **83.9** | 5 | **16.1** | 0 | **0.0** | 0 | **0.0** | **31** |
| 2015 | 31 | **96.9** | 1 | **3.1** | 0 | **0.0** | 0 | **0.0** | **32** |
| 2016 | 34 | **89.5** | 4 | **10.5** | 0 | **0.0** | 0 | **0.0** | **38** |
| 2017 | 37 | **80.4** | 9 | **19.6** | 0 | **0.0** | 0 | **0.0** | **46** |

[a] *MIC values in mg/L*

**Table 4.6.1.2** Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2017.

| | 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 | 77 | **88.5** | 10 | **11.5** | 0 | **0.0** | 0 | **0.0** | **87** |
| 2010 | 67 | **84.8** | 12 | **15.2** | 0 | **0.0** | 0 | **0.0** | **79** |
| 2011 | 34 | **64.2** | 19 | **35.9** | 0 | **0.0** | 0 | **0.0** | **53** |
| 2012 | 27 | **67.5** | 13 | **32.5** | 0 | **0.0** | 0 | **0.0** | **40** |
| 2013 | 53 | **73.6** | 18 | **25.0** | 1 | **1.4** | 0 | **0.0** | **72** |
| 2014 | 37 | **88.1** | 5 | **11.9** | 0 | **0.0** | 0 | **0.0** | **42** |
| 2015 | 46 | **88.5** | 6 | **11.5** | 0 | **0.0** | 0 | **0.0** | **52** |
| 2016 | 89 | **87.3** | 13 | **12.7** | 0 | **0.0** | 0 | **0.0** | **102** |
| 2016 | 104 | **80.6** | 24 | **18.6** | 1 | **0.8** | 0 | **0.0** | **129** |

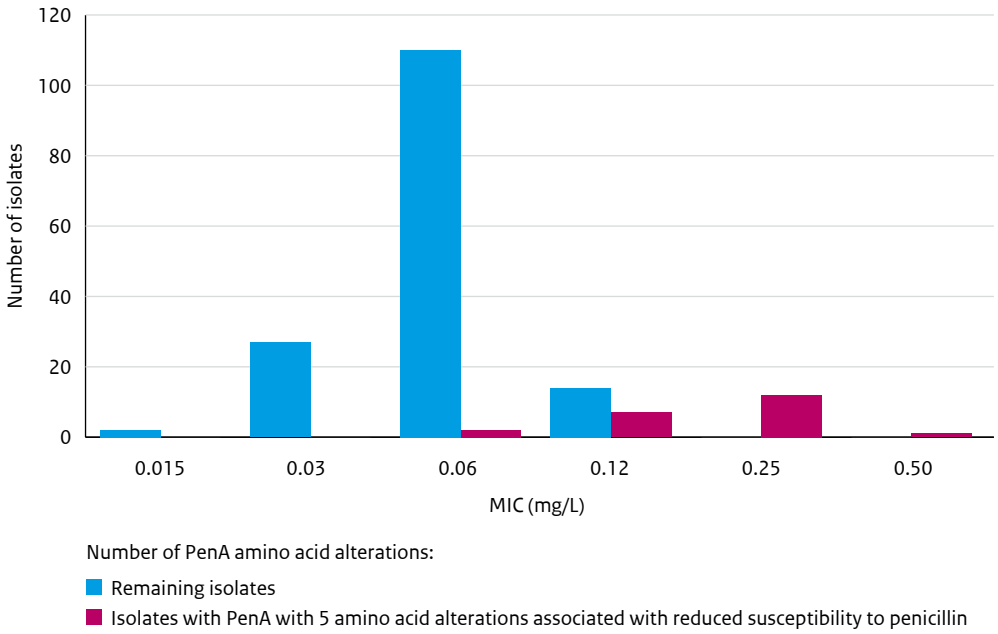
[a] *MIC values in mg/L*

**Table 4.6.1.3** Alterations in PenA and penicillin susceptibility in *N. meningitidis*, 2017.

| Alterations PenA[b] | Number of strains with penicillin MIC[a]: MIC ≤ 0.06 sensitive | 0.064 < MIC ≤ 0.25 | 0.25 < MIC ≤1.0 | MIC >1.0 |
|---|---|---|---|---|
| Yes | 2 | 19 | 1 | 0 |
| No | 139 | 14 | 0 | 0 |
| **Total** | **141** | **33** | **1** | **0** |

[a] *MIC values in mg/L*
[b] *Alterations in PenA associated with non-susceptibility to penicillin*

**Figure 4.6.1.1** MIC distribution of isolates with and without PenA alterations, 2017.



## 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.

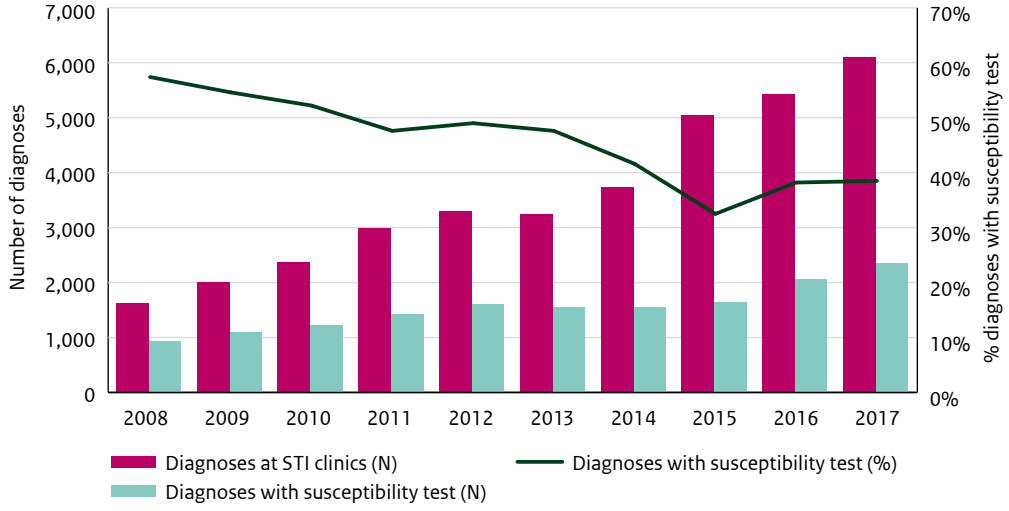
### Methods

The national Gonococcal Resistance to Antimicrobials Surveillance (GRAS) started in 2006, collecting epidemiological data on gonorrhoea and resistance patterns of isolated strains from STI centres across the Netherlands. Eighteen out of 24 STI clinics participated in GRAS in 2017 and they performed 90% of gonorrhoea diagnoses among STI clinic 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].

### Results

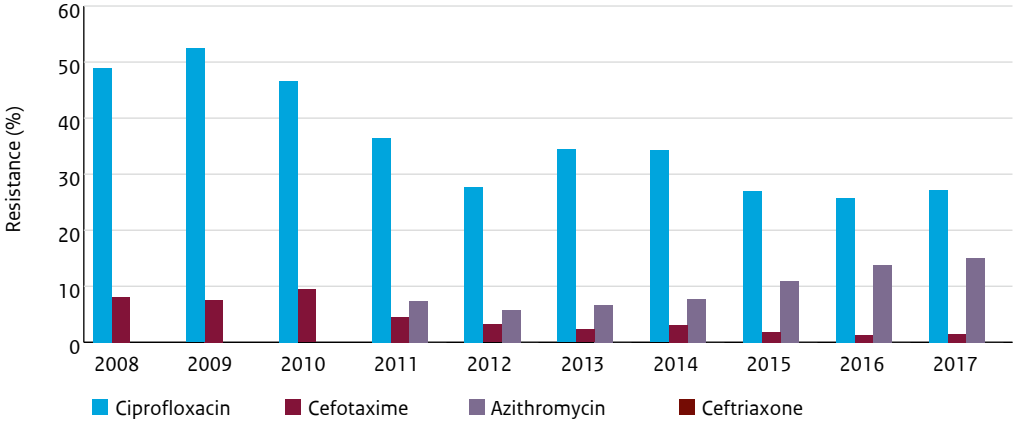
Since 2008, the number of gonorrhoea diagnoses at STI clinics participating in GRAS has increased to 6,100 in 2017, but the percentage of diagnoses including a susceptibility test decreased from 57.4% in 2008 to 38.5% in 2017 (Figure 4.6.2.1). This decline has stabilized since 2014.

**Figure 4.6.2.1** Number of gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test at STI clinics participating in GRAS, 2008-2017.




Gonococcal resistance for ciprofloxacin has decreased over the years from 48.9% in 2008 to 27.2% in 2017. Resistance levels for cefotaxime have also decreased, and were stably around 1.5% in the last three years. For azithromycin, resistance has steadily increased since 2012; from 5.8% to 15.0% in 2017. 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, 2008-2017.




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

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). 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 or MIC of 2 mg/L and higher has been increasing (Figure 4.6.2.3B).

### Discussion

In less than half (38.5%) of all gonorrhoea diagnoses at STI clinics participating in GRAS resistance levels were measured by culture. 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 (3 isolates in 2017). 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.

### Conclusions

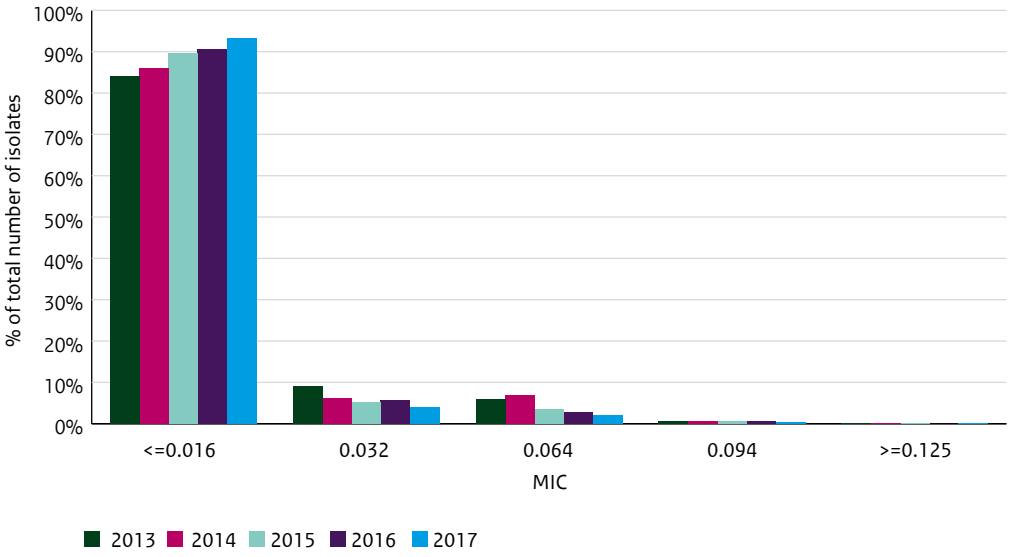
- The number of gonorrhoea diagnoses at STI clinics increases, but the number of diagnoses which include susceptibility tests remains relatively low (38.5% in 2017).
- No resistance to ceftriaxone, the current first-line treatment, has been reported.
- Azithromycin resistance levels continue to increase; from 5.8% in 2012 to 15.0% in 2017.

### References

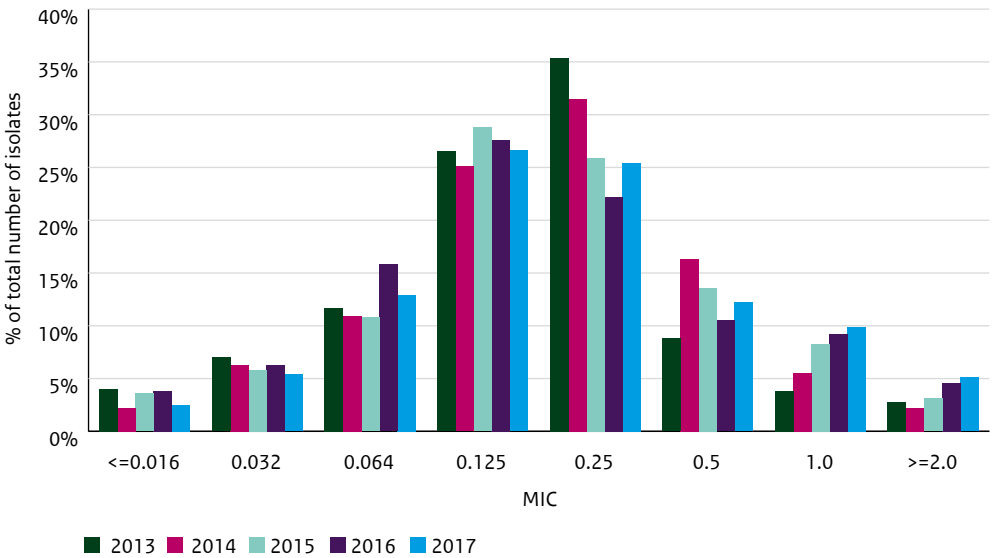
[1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018. Available from http://www.eucast.org/clinical_breakpoints/

**Figure 4.6.2.3** MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2013-2017.

A. MIC distribution for ceftriaxone.




B. MIC distribution for azithromycin.



## 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 causative agent; *Mycobacterium tuberculosis*. In the Netherlands we have reached the elimination phase. Not less than 75% of the TB cases is currently diagnosed in foreign-borns. 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. 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. If resistance is diagnosed in other laboratories, this is verified at the TB reference laboratory at the RIVM. 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 35 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 gold standard in drug susceptibility testing (DST) 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 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. The results on the 25% of cultures for which DST has been performed outside of the RIVM have been collected for the year 2016 and this confirmed this recommendation is followed consistently.

### Results

The presented data on 2017 is preliminary, as not all data is currently available. The *in vitro* generation time of *M. tuberculosis* is long and it takes several weeks before cultures become positive, are sent to the RIVM, and the drug susceptibility testing has been finalized.
In the year 2017, 534 *M. tuberculosis* complex isolates were received at the RIVM for epidemiological typing, of which 329 (62%) were subjected to DST for first line drugs at the RIVM.
In 2017, the number of TB notification cases was 794, of which 534 *M. tuberculosis* complex isolates were received at the RIVM for epidemiological typing.
Until 2010, the rate of INH resistance increased to 9.0%, but since 2011 it decreased over the years to 5.4 % in 2015. In 2016 there was a clear increase in INH resistance to 7.1% (figure 4.6.3.1), but this slightly decreased to 6.7% in 2017. Rifampicin resistance decreased from 3.1% in 2013 to 1.3% in 2014. In 2015 and 2016 the rifampicin resistance increased marginally from to 1.9% to 2.6%. In 2017 rifampicin resistance decreased again to 2.1% of the cases. In 2017, in 0.9% of the cases ethambutol resistance was detected.

**Figure 4.6.3.1** Trends in antibiotic resistance for *M. tuberculosis* 2002-2017.

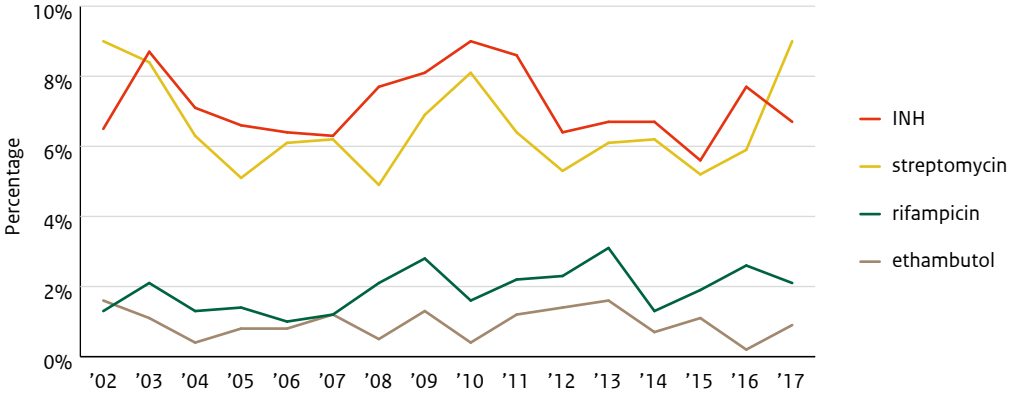
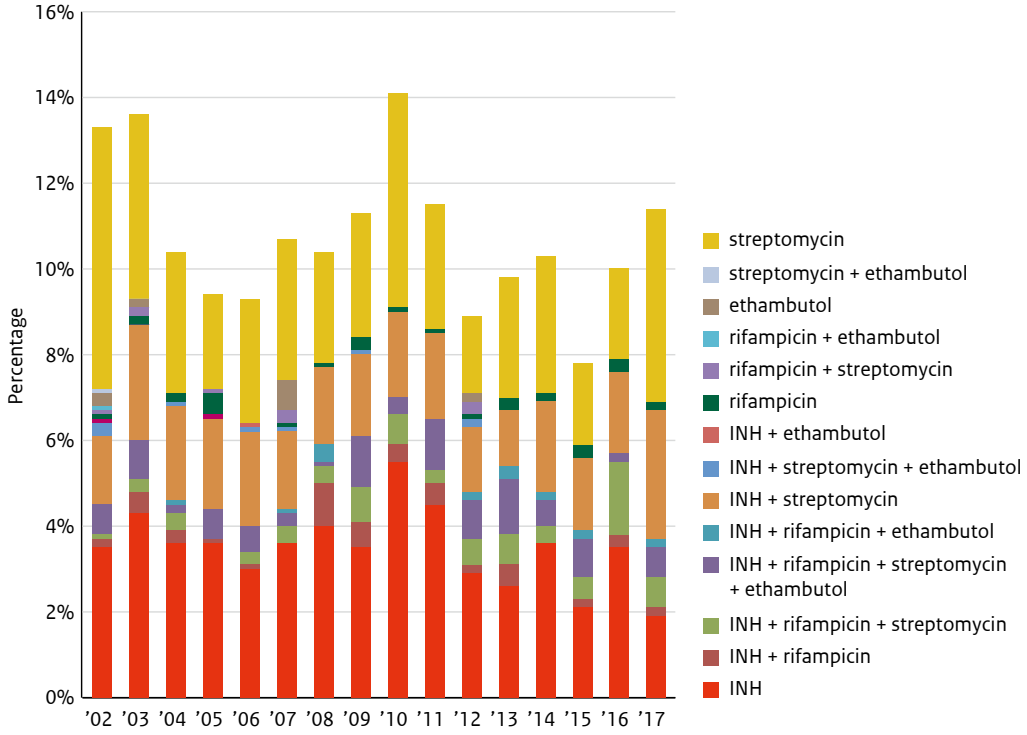



**Figure 4.6.3.2** Trends in combined antibiotic resistance for *M. tuberculosis* 2002-2017.



Multidrug resistant tuberculosis (MDR-TB), defined as resistance to at least INH and rifampicin, was found in 1.1% of the isolates in 2014 and 1.8 % of the isolates in 2015 (figure 4.6.3.2). In 2016, 2.1% of the isolates were reported as MDR-TB. In 2017, 10 MDR-TB cases were diagnosed (1.3%). XDR-TB was not diagnosed in 2017. In recent years mono-resistance to rifampicin was incidentally found; in 2016 in 2 cases and in 2017 one case.

## 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 2017, 10.6% percent of the 534 isolates tested in the Netherlands revealed some form of resistance. Although the number of multidrug resistant isolates remained low and amounted to 10 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. It is being investigated whether the detection of mutations in 23 resistance genes is a reliable predictor of resistance. In the period 2018/2019 WGS will be increasingly used 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 only slightly increased in 2017.
- MDR-TB remained stable in the recent years, and decreased to 1.3% in 2017.
- Tuberculosis notification increased with 6% in 2015 and 3% in 2016, but decreased with 11% in 2017.

## 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.

Drug Susceptibility Testing of *Mycobacterium tuberculosis* Complex by Use of a High-Throughput, Reproducible, AbsoluteConcentration Method. Bert van Klingeren, Mirjam Dessens-Kroon, Tridia van der Laan, Kristin Kremer, and Dick van Soolingena
JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2007, p. 2662-2668.

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 J11, 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 Resistance to 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 ($IC_{50}$). In the absence of known NAI resistance amino acid substitutions detected by genotypic assays, determination of the $IC_{50}$ is the only way to determine the NAI susceptibility of an influenza virus. The major markers for NAI highly reduced inhibition are NA H275Y for N1 subtype viruses and NA E119V and R292K for N2 subtype viruses. For M2B highly reduced inhibition this is M2 S31N. As virtually all influenza A(H1N1)pdm09 and A(H3N2) viruses appear naturally resistant against M2B no active targeted surveillance for M2B resistance is carried out since the 2017/2018 influenza season. As part of the influenza viruses are whole genome sequenced at the end of the season, still some information will be obtained on M2B resistance.

## 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 2017/2018 season, for results obtained so far, no viruses with reduced inhibition for oseltamivir and zanamivir were found.

Oseltamivir and zanamivir prescriptions increased during the 2017/2018 influenza season, similar to levels seen during influenza epidemics since the 2010/2011 influenza season. Amantadine prescriptions during the 2017/2018 season continued to decrease slightly compared to previous seasons, but the vast majority of these prescriptions are for treatment of Parkinson disease.

## Discussion

As in the Netherlands, and globally, virtually all influenza type A viruses carry M2-S31N, 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 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.

## Conclusions

- Overall, over the last 8 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, whilst prescriptions of amantadine showed a continuous slightly decreasing trend.

**Table 4.6.4.1** (Highly) reduced inhibition of influenza viruses by NAIs and M2Bs in the Netherlands, 2010/2011 - 2017/2018[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[10] | 0/258 | 1/1 (100%) | 0/145 | 1/1 (100%) | 0/112 |

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;*

2 *Two viruses with highly reduced inhibition by oseltamivir due to the H275Y 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 H275Y 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.*

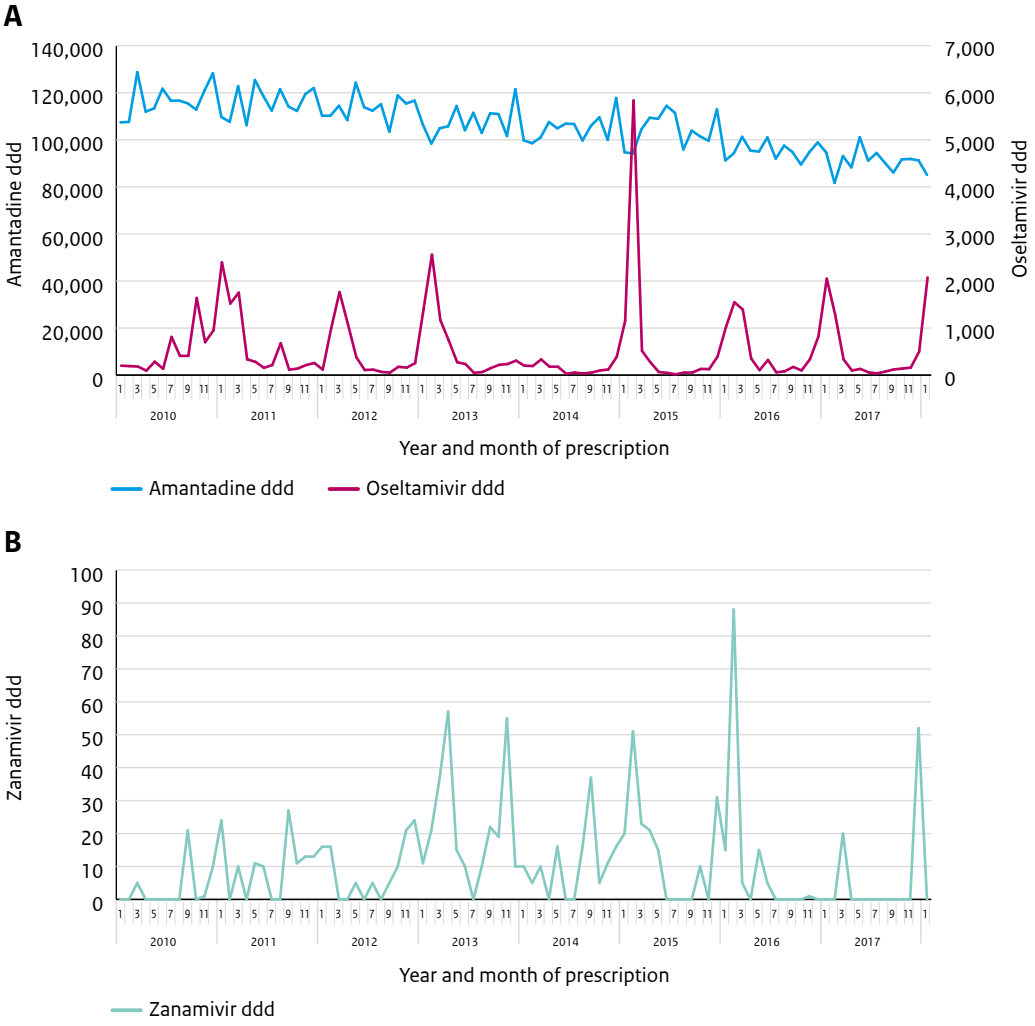
7 *One virus with highly reduced inhibition by oseltamivir due to mixture 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

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 an H275Y amino acid substitution. The patient was treated with oseltamivir prior to specimen collection.*

10 *Preliminary data, status by 13 March 2018.*

**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] Gubareva LV, Besselaar TG, Daniels RS, Fry A, Gregory V, Huang W, Hurt AC, Jorquera PA, Lackenby A, Leang SK, Lo J, Pereyaslov D, Rebelo-de-Andrade H, Siqueira MM, Takashita E, Odagiri T, Wang D, Zhang W, Meijer A. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2015-2016. Antiviral Res. 2017 Oct;146:12-20.

## 4.6.5 The antibiotic susceptibility profile of anaerobic bacteria

### Introduction

Following the reports in earlier years, we report the antibiotic susceptibility profiles of anaerobic bacteria isolated at the University Medical Center Groningen in 2017. The profiles are assumed to be representative for susceptibility of anaerobes in The Netherlands.

### Material and methods

About 67% of clinical samples from which anaerobic bacteria were isolated, derived from primary sterile sites. Anaerobic clinical isolates were identified using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The antibiotic profiles for amoxicillin, amoxicillin-clavulanic acid (only gram-negative anaerobic bacteria), clindamycin, metronidazole and meropenem (only species belonging to the genera *Bacteroides* and *Prevotella*) were assessed. The MIC for the different antibiotics were determined using Etest (bioMerieux, France) according to the manufacturer's recommendations. To determine resistance the breakpoints according to the EUCAST guidelines were used.

### Results and discussion

The antibiotic susceptibility profiles of the different anaerobic genera, are shown in Table 4.6.5.1.

*Gram-negative anaerobic bacteria*

For amoxicillin the resistance rates remained similar as for the previous years. However, the percentage resistant strains among the genus *Fusobacterium* seems to be increasing, from 0 till 22% in the previous years, to 27% in this year. The resistance rates for amoxicillin-clavulanic acid remained stable. Expect for some strains within the *Bilophila* genus, which for the first time since three years showed resistance to amoxicillin-clavulanic acid. All strains belonging to the genera *Fusobacterium*, *Bilophila* and *Veillonella* were susceptible for clindamycin. An increase in resistance was observed for *Porphyromonas* strains, from 11 till 17% in the previous years, to 38% in this year. The resistance rate among the other gram-negative anaerobic genera remained similar. Again we observed metronidazole resistance among the genera *Bacteroides* and *Prevotella*. All strains belonging to the genera *Bacteroides* and *Prevotella* were susceptible for meropenem.

*Gram-positive anaerobic bacteria*

Resistance for amoxicillin was only observed within the genus *Clostridium*, which was low compared to other years. Resistance rates for clindamycin remained similar to the previous years and no resistance for metronidazole was observed. In contrast to previous years we looked separately at gram-positive anaerobic cocci (GPAC) based on the different genera (Table 4.6.5.2). *Finegoldia magna* and *Peptostreptococcus* spp. showed the highest MIC for amoxicillin. The genera *Finegoldia* and *Peptoniphilus* had the highest resistance rates for clindamycin.

**Table 4.6.5.1** The $MIC_{50}$, $MIC_{90}$ and percentage resistance for different genera of anaerobic bacteria, isolated from human clinical specimens in 2017.

| | 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. (173-179)[a] | 32 | >256 | 96.5 | 0.38 | 1.5 | 0.6 | 1.5 | >256 | 23.7 | 0.25 | 0.75 | 0.6 | 0.125 | 0.5 | 0 |
| *Parabacteroides* spp. (n=18) | >256 | >256 | 66.7 | 2 | 6 | 0 | 4 | 12 | 27.8 | 0.38 | 0.5 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Fusobacterium* spp. (n=25) | 0.064 | >256 | 24 | 0.047 | 1.5 | 8 | 0.032 | 0.19 | 0 | 0.016 | 0.125 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Prevotella spp.* (n=87-88)[a] | 0.5 | 64 | 41.3 | 0.064 | 0.75 | 0 | 0.016 | 0.094 | 9.1 | 0.19 | 0.75 | 1.1 | 0.032 | 0.094 | 0 |
| *Porphyromonas* spp. (n=13) | 0.016 | 3 | 15.4 | <0.016 | 0.25 | 0 | 0.016 | >256 | 38.4 | 0.016 | 0.5 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Bilophila* spp. (n=14) | 48 | 192 | 85.7 | 1 | 1.5 | 7.1 | 0.5 | 1 | 0 | 0.047 | 0.38 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Veillonella* spp. (n=19-21)[a] | 0.5 | 2 | 4.7 | 0.38 | 1.5 | 0 | 0.094 | 0.19 | 0 | 1 | 1.5 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| | | | | | | | | | | | | | | | |
| Gram-positive anaerobes | | | | | | | | | | | | | | | |
| *Actinomyces* spp. (n=172) | 0.125 | 0.38 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.19 | 1 | 5.2 | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] |
| *Atopobium* spp. (n=9-13)[a] | 0.064 | 0.75 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.38 | 6 | 15.4 | 0.125 | 0.19 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Bifidobacterium* spp. (n=8) | 0.19 | 0.25 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.032 | 0.064 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] |
| *Clostridium* spp. (n=37) | 0.25 | 0.75 | 2.7 | n.a.[b] | n.a.[b] | n.a.[b] | 1.5 | 16 | 29.7 | 0.5 | 0.75 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Eggerthella lenta* (n=6-7)[a] | 0.75 | 1 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.25 | 1 | 0 | 0.19 | 0.25 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |
| *Cutibacterium* spp. (n=163) | 0.047 | 0.125 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.032 | 0.125 | 3.7 | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] | n.a.[b] |
| GPAC (n=120-122)[a] | 0.064 | 0.25 | 0 | n.a.[b] | n.a.[b] | n.a.[b] | 0.19 | >256 | 12.5 | 0.19 | 0.75 | 0 | n.a.[b] | n.a.[b] | n.a.[b] |

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

**Conclusions**

- Amoxicillin resistance among fusobacteria seems to be increasing, which makes testing for beta-lactamase production a necessity.
- An increase in clindamycin resistance among *Porphyromonas* species was observed.
- As in previous years metronidazole resistance among the genera *Bacteroides* and *Prevotella* was present. However, all strains were susceptible for meropenem.
- Among the gram-positive anaerobic bacteria resistance for amoxicillin was only encountered for *Clostridium* species.
- Antibiotic susceptibility profiles differ between the different GPAC genera.

**Table 4.6.5.2** The $MIC_{50}$ $MIC_{90}$ and percentage resistance for the genera categorized under the term gram-positive anaerobic cocci.

| | amoxicillin | | | clindamycin | | | metronidazole | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$MIC_{50}$** | **$MIC_{90}$** | **%R** | **$MIC_{50}$** | **$MIC_{90}$** | **%R** | **$MIC_{50}$** | **$MIC_{90}$** | **%R** |
| GPAC (n=120-122) | 0.064 | 0.25 | 0 | 0.19 | >256 | 12.5 | 0.19 | 0.75 | 0 |
| *Anaerococcus* spp. (n=25-26)[a] | 0.047 | 0.094 | 0 | 0.094 | >256 | 12 | 0.25 | 1 | 0 |
| *Finegoldia magna* (n=33) | 0.19 | 0.38 | 0 | 1 | >256 | 21.2 | 0.19 | 0.38 | 0 |
| *Parvimonas micra* (n=25-26)[a] | 0.032 | 0.125 | 0 | 0.125 | 0.75 | 4 | 0.064 | 0.25 | 0 |
| *Peptoniphilus* spp. (n=24) | 0.016 | 0.094 | 0 | 0.25 | >256 | 16.7 | 0.25 | 0.75 | 0 |
| *Peptostreptococcus* spp. (n=12) | 0.19 | 0.5 | 0 | 0.094 | 0.25 | 0 | 0.125 | 0.38 | 0 |

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

## 4.6.6 *Clostridium 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 *C. difficile* at the Leiden University Medical Centre (LUMC) soon after recognition of fluoroquinolone resistant C.*difficile* 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 CDI surveillance programme has been initiated in 2009 in order to monitor CDI incidence rates and circulating ribotypes in an endemic situation.

### Methods

Currently, 24 acute care hospitals are participating in the sentinel surveillance programme.[1] In these hospitals, all hospitalized patients >2 years with clinical signs and symptoms of CDI in combination with a positive test for *C. difficile* toxins or toxigenic *C. difficile* are included. Clinical data and outcomes after 30 days are registered. Isolates of all included CDI cases are sent to the LUMC for PCR ribotyping. Antibiotic resistance was determined for a selection of *C. difficile* sentinel surveillance isolates. MIC testing was performed using agar dilution test.[2]

### Results

From May 2016 to May 2017, a mean CDI incidence rate of 3.08 cases per 10,000 patient-days was found through sentinel surveillance.[1] The most frequently encountered PCR ribotypes were 014/020 (20%) and the closely related ribotypes 078 and 126 (12%). One outbreak due to PCR ribotype 001 was reported. Ribotype 027 was found in 0.6% of sentinel surveillance samples (May 2015-May 2016: 1.2%). Among samples submitted for ad hoc typing, PCR ribotype 027 was the predominant type (17%). One outbreak due to PCR ribotype 027 was reported in a healthcare facility not participating in the sentinel surveillance.[1]

Antibiotic resistance was determined for 50 randomly selected *C. difficile* sentinel surveillance isolates, collected between January 2017 and December 2017 (Table 4.6.6.1). No resistance was detected, using CLSI/EUCAST cut-off levels.[2,3]

**Table 4.6.6.1** $MIC_{50}$, $MIC_{90}$ and range (mg/L) of 52 C. difficile sentinel surveillance isolates.

| | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|
| Fidaxomicin | <0.06 | 0.125 | <0.06-0.125 |
| Metronidazole | 0.5 | 0.5 | <0.06-0.5 |
| Vancomycin | 0.25 | 0.5 | 0.06-0.5 |

## Discussion

The CDI incidence and most frequently encountered PCR ribotypes were similar to previous years. The proportion of PCR ribotype 027 among sentinel surveillance samples was slightly lower than last year, and significantly lower than in some of the previous years. However, among samples submitted for ad hoc typing, PCR ribotype 027 was the most frequently encountered PCR ribotype (17%), similar to last year. None of the tested isolates were found to be resistant to the therapeutic drugs metronidazole, vancomycin and fidaxomicin.

## Conclusion

- A low proportion of PCR ribotype 027 was found in sentinel surveillance samples, but this ribotype is still the most frequently encountered PCR ribotype among samples submitted for ad hoc typing.
- No resistance of C. *difficile* to metronidazole, vancomycin or fidaxomicin was found

**Note.** In December 2017, a clinical isolate of C. *difficile* PCR ribotype 014 was found with MIC=8 mg/L to metronidazole. Preliminary data suggest a novel form of metronidazole resistance and further research is currently performed.

## References


[1] Crobach MJT, Dorp van Sofie, Terveer EM, Harmanus C, Sanders IMJG, Kuijper EJ, Notermans DW, Greeff de SC, Albas J, Dissel v JT. 2017. Eleventh Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance.

[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*. This fungus causes invasive and non-invasive diseases in humans depending on the immune status of the host. The triazoles represent the most important class of agents used for the management of aspergillus diseases, with only the polyene amphotericin B and echinocandines as alternative treatment options. Here we report the triazole resistance frequency in *A. fumigatus* as detected in the Netherlands in 2017.

### Methods

In five University Medical Centers all clinical *A. fumigatus* isolates were screened for triazole resistance using a four-well agar plate (VIPcheck™, Nijmegen, 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.[1] Growth on the azole 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 is performed using the EUCAST microbroth dilution method.[2] 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 2017 *A. fumigatus* isolates from 774 (range 75 to 240 per center) culture-positive patients were screened for the presence of triazole resistance. Overall 114 patients (14.7%) harbored a triazole-resistant isolate. In all centers the resistance frequency exceeded 10%, ranging from 10.6% in Radboudumc, Nijmegen to 23.7% in LUMC, Leiden. Compared with previous years the detected resistance frequency has increased (Table 4.6.7.1). The underlying diseases of patients with triazole-resistant *A. fumigatus* were diverse, but most frequently involved patients with chronic lung disease and CF (58 of 97 (60%) patients with known underlying conditions) (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 68% (range 46% to 84%) and 16% (range 7% to 24%) of resistance mutations, respectively. One $TR_{46}$isolate harbored an additional M172I mutation. Overall the proportion of $TR_{46}$ had increased compared with 2016 from 9% to 16%. Similar to previous years, 15% of triazole-resistant *A. fumigatus* isolates harbored no resistance mutations in the *Cyp51A*-gene, indicating the presence of other uncharacterized resistance mechanisms.

## Discussion

In 2017 the detected triazole resistance frequency further increased compared to previous years with now 14.7% of screened isolates showing a triazole-resistant phenotype. In one center the resistance frequency had remained stable compared with 2016, while in all others an increase was noted ranging from 0.7% to 3.2%. The underlying resistance mutations remained dominated by those associated with the environment accounting for 83% of resistance mutations found. A recent retrospective cohort study involving patients from three surveillance centers showed an excess mortality of 19% to 32% in patients with culture-positive triazole-resistant invasive aspergillosis compared with triazole-susceptible cases. In these patients an escalation strategy had been followed, i.e. treatment was started with voriconazole and switched to liposomal amphotericin B or combination therapy once resistance was detected. However, this approach was associated with 27% higher mortality in patients with triazole-resistant invasive aspergillosis. The SWAB-guideline for the management of invasive mycoses was revised in December 2017 now recommending routine triazole resistance testing and coverage of triazole resistance in initial antifungal therapy of patients suspected of invasive aspergillosis.[3]

To increase the geographical coverage of resistance surveillance six new centers will screen all clinical *A. fumigatus* isolates including centers in Nijmegen, Veldhoven, Nieuwegein, Enschede, Alkmaar and Leeuwarden. Furthermore, an intensified surveillance by the Centre of Infectious Diseases of the National Institute for Public Health and the Environment, will commence in 2018 initially in the University Medical Centers to collect more structural data, including epidemiological, clinical and microbiological information on patients suspected of having invasive aspergillus infection (both culture-positive and -negative cases).

## Conclusions

- The triazole resistance frequency in *A. fumigatus* continues to increase to 14.7% of unselected culture positive patients harboring a resistant isolate.
- The triazole resistance mutations remain dominated by those associated with environmental resistance selection, as they were found in 83% of triazole-resistant isolates.
- The national guideline for the management of invasive aspergillosis has been revised now recommending triazole resistance testing and initial coverage of resistance in patients suspected of invasive aspergillosis.

## References


[1] Arendrup MC, Verweij PE, Mouton JW, Lagrou K, Meletiadis J. Multicentre validation of 4-well azole agar plates as a screening method for detection of clinically relevant azole-resistant *Aspergillus fumigatus*. J Antimicrob Chemother. 2017;72:3325-3.

[2] EUCAST definitive document E.DEF 9.3.1. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds. (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9_3_1_Mould_testing__definitive.pdf).

[3] Dutch Working Party on Antibiotic policy. SWAB Guidelines for the management of invasive fungal infections. (http://www.swab.nl/swab/cms3.nsf/uploads/3AA7A56CE879587BC12581 F80061297F/$FILE/SWAB%20Richtlijn%20Mycosen%202017%20(final).pdf)

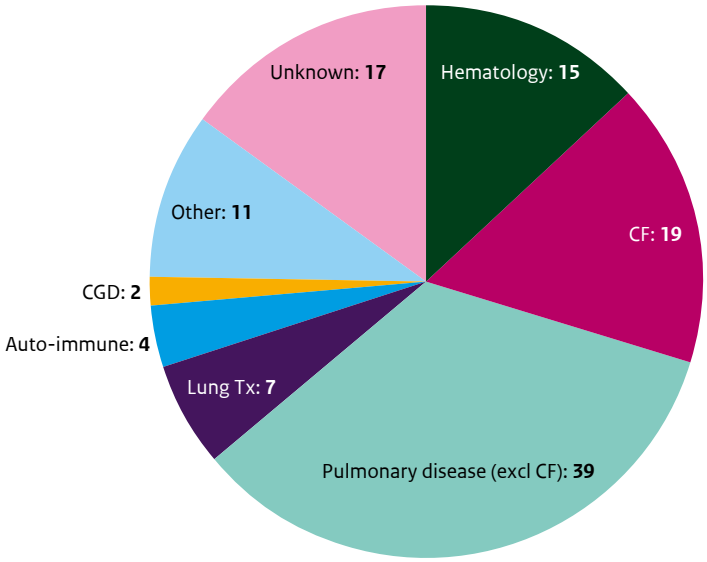
**Table 4.6.7.1** Triazole resistance frequency in unselected clinical *A. fumigatus* isolates in five University Medical Centers, 2013 to 2017.

| | 2013 | | 2014 | | 2015 | | 2016 | | 2017 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | screened | azoleR (%) | screened | azoleR (%) | screened | azoleR (%) | screened | azoleR (%) | screened | azoleR (%) |
| ErasmusMC | 231 | 10 (4.3) | 265 | 10 (3.8) | 22 | 7 (31.8)* | 186 | 24 (12.9) | 147 | 19 (12.9) |
| LUMC | 99 | 19 (19.2) | 113 | 15 (13.3) | 141 | 23 (16.3) | 88 | 18 (20.5) | 114 | 27 (23.7) |
| Radboudumc | 123 | 6 (4.9) | 143 | 7 (4.9) | 145 | 12 (8.3) | 210 | 20 (9.5) | 198 | 21 (10.6) |
| UMCG | 194 | 16 (8.2) | 191 | 18 (9.4) | 225 | 15 (6.7) | 215 | 26 (12.1) | 240 | 35 (14.6) |
| VUmc | 113 | 8 (7.1) | 104 | 9 (8.7) | 89 | 14 (15.7) | 85 | 13 (15.3) | 75 | 12 (16) |
| **Total** | **760** | **58 (7.6)** | **814** | **59 (7.2)** | **600** | **64 (10.7)**** | **784** | **101 (12.9)** | **774** | **114 (14.7)** |

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

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

**Figure 4.6.7.1** Distribution of underlying diseases of 114 patients with recovery of triazole-resistant *A. fumigatus*.




*CF: cystic fibrosis; Lung Tx: lung transplantation; CGD: chronic granulomatous disease*

# 5 Antimicrobial stewardship monitor in hospitals

**Introduction**

The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by antimicrobial stewardship teams (A-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 2017, an electronic survey was sent to all 80 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 46 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.

**Results**

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

Sixty-four of 80 hospitals returned the survey, resulting in a response rate of 80%. The mean number of hospital beds was 483 (range 50-1048). Thirteen percent of the hospitals were university hospitals (100% response rate), 53% non-university teaching hospitals (76% response rate) and 34% non-teaching hospitals (81% response rate). Of the responding hospitals, 60 (94%) had an A-team and 4 (6%) were preparing one. All 60 operational A-teams consisted of at least one hospital pharmacist, one medical microbiologist and 68% had at least one infectious disease specialist. Ten percent of the

A-teams employed a nurse. Authorization by the hospital boards of directors had been granted to 84% of the A-teams; 41% of the hospital boards of directors provided a budget for the A-teams, with a median financial support of 0.5 FTE (range 0.05-1.5). IT support was available for 66% of the A-teams and was mainly used for the following antimicrobial stewardship-related activities: data reporting (72% of the A-teams with IT support available), selection of specified patients (67%), point prevalence survey (51%), and decision support (44%). The time dedicated by the A-team to antimicrobial stewardship-related activities was a mean of 19.8 hours per week (range 3-58).

*Stewardship activities*

Fifty-seven A-teams provided data on stewardship activities. A-teams received reports at least annually on cumulative antimicrobial susceptibility in 83% and reports on quantitative use of antimicrobials in 72%. Incidental monitoring by means of point prevalence surveys (PPS) was performed by 72% of the A-teams. Appropriate antimicrobial use was monitored in several ways, as shown in Table 5.1.1. Fifty-two (91%) of the hospitals had interventions in place to monitor and/or improve the use of restricted antimicrobials, i.e. drugs that only should be prescribed for microorganisms that are resistant to the usual drugs (Table 5.1.2). Table 5.1.3 summarizes the performance and monitoring of bedside consultation.

**Table 5.1.1** Percentage of hospitals that continuously, occasionally, and/or incidentally monitored stewardship activities (n=57).

| Stewardship objective | Total | Continuous | Intermittent | Incidental | Combination |
|---|---|---|---|---|---|
| Restricted antimicrobials (correct indication) | 52 (91%) | n.a. | n.a. | n.a. | n.a. |
| Guideline adherence (correct indication)* | 19 (33%) | 6 (11%) | 10 (18%) | 6 (11%) | 3 (5%) |
| Bedside consultation | 35 (61%) | 35 (61%) | n.a. | n.a. | n.a. |
| IV-oral switch | 31 (54%) | 21 (37%) | 9 (16%) | 1 (2%) | 0 (0%) |
| Therapeutic drug monitoring | 37 (65%) | 34 (60%) | 3 (5%) | 0 (0%) | 0 (0%) |
| De-escalation | 21 (37%) | 14 (25%) | 5 (9%) | 2 (4%) | 0 (0%) |
| Discontinuation | 21 (37%) | 13 (23%) | 7 (12%) | 1 (2%) | 0 (0%) |
| Correct diagnostics | 19 (33%) | 9 (16%) | 8 (14%) | 3 (5%) | 1 (2%) |

*Continuous: 4-7 days a week; Intermittent (in regular basis): 1-3 days a week; Incidental: audit or PPS; N.a., not available*
** for non-restricted antimicrobials*

**Table 5.1.2** Interventions in hospitals performed to monitor and improve the use of restricted antimicrobials (n=57).

| | |
|---|---|
| Post prescription review | 25 (44%) |
| Post-authorization | 22 (41%) |
| Formulary restriction | 15 (26%) |
| Computerized alert | 14 (25%) |
| Check of performed diagnostics | 10 (18%) |
| Pre-authorization | 9 (16%) |
| Order form | 3 (5%) |
| Mandatory bedside consultation | 1 (2%) |
| Stop order | 1 (2%) |

**Table 5.1.3** Patient categories for which the hospital (n=57) agreed to perform a compulsory bedside consultation by an infectious disease specialist and for which A-teams monitor the performance.

| Category | Compulsory bedside consultation, % of 57 hospitals | Monitoring of bedside consultation, % of hospitals with indication for consultation |
|---|---|---|
| No recommended bedside consultation | 17 (30%) | Not applicable |
| Staphylococcus aureus bacteremia | 41 (72%) | 23 (56%) |
| Infective endocarditis | 19 (33%) | 2 (10%) |
| Prosthetic joint infection | 13 (23%) | 2 (10%) |
| Vascular prosthesis infection | 13 (23%) | 1 (8%) |
| Invasive fungal infection | 16 (28%) | 2 (13%) |

Ninety-two percent (range: 81% – 100%) of the 57 hospitals provided individual recommendations on stewardship objectives by telephone, 62% face-to-face (range: 51% – 73%), and 27% (range: 17% – 42%) of hospitals by computerized alerts.

Forty-eight of the 57 (84%) A-teams provided feedback of the PPS or audit(s) results to the frontline health care workers. Education on antimicrobial stewardship was provided to residents in 33 of 57 hospitals (58%) and to medical specialists in 16 of 57 hospitals (28%). Attendance to these sessions was mandatory in 43% and 14%, respectively. Subjects discussed were antimicrobial resistance (residents/specialists: 94%/94%), specific syndromes (94%/94%), restricted antimicrobials (52%/63%), IV-oral switch (61%/56%), streamlining (61%/75%), therapeutic drug monitoring (39%/63%), and/or PPS/audit (15%/28%).

## 5.2 Quality of antimicrobial use in hospitals

**Methods**

Based on A-teams' preferences and feasibility, three stewardship objectives were selected for inclusion in the antimicrobial stewardship monitor: 1) prescribe restricted antimicrobials according to the local guideline; 2) switch from intravenous to oral therapy (IV-oral switch); 3) perform bedside consultation in patients with a *Staphylococcus aureus* bacteremia (SAB).

From September 2017 until December 2017, a pilot was performed in ten hospitals to evaluate the feasibility to extract data on the performance of these stewardship objectives from the hospital electronic medical records (EMR) and associated lab systems. In addition, participating hospitals were offered the opportunity to provide data manually in a web-based portal. The data were used to compute process indicator performance scores.

**Results**

Eight of the ten hospitals that participated in the pilot could provide data. One hospital had insufficient IT support and in one hospital the board of directors did not grant approval to participate. Three hospitals provided data only electronically, three only manually, and two hospitals both electronically and manually. The pilot ended 31st of December 2017.

*Restricted antimicrobials*

It proved to be technically feasible to extract antimicrobial prescriptions from the EMR. Also, it was feasible to collate the individual prescriptions to one antibiotic course in case of dose adjustments or changes in route of administration. An example from one hospital is shown in Figure 5.2.1. Assessment of the appropriateness of these prescriptions by the A-team was performed for a part of these courses. Data on these assessments could not be extracted from the EMR, for technical reasons. Therefore, this information was added manually to the stewardship monitor, leading to the quality indicator performance as shown in Figure 5.2.1.

*IV-oral switch*

As a proxy for timely IV-oral switch, two types of indicators could be calculated from data provided electronically: first, the mean duration of intravenous administration of an antibiotic course and second, the percentage of patients that were switched to oral administration within 72 hours after initiation or in whom the antibiotic treatment was stopped. An example from one hospital is shown in Figure 5.2.2.

*Bedside consultation*

Positive blood cultures with *S. aureus* could be automatically extracted from the lab systems. The challenge to count a patient with several positive blood cultures as one episode of SAB was overcome. However, whether or not bedside consultation was performed was not documented as discrete variable in this hospital, and had to be manually completed in order to calculate quality indicator performance (80% [16/20]).

**Figure 5.2.1** Number of antibiotic courses (total column), number of antibiotic courses assessed (green + violet column), of antibiotic courses assessed as appropriate (green column), of antibiotic courses assessed as inappropriate (violet column) for carbapenems, piperacillin-tazobactam, and fluoroquinolones. The percentages show the performance of the quality indicator "prescribe restricted antimicrobials according to the local guideline".

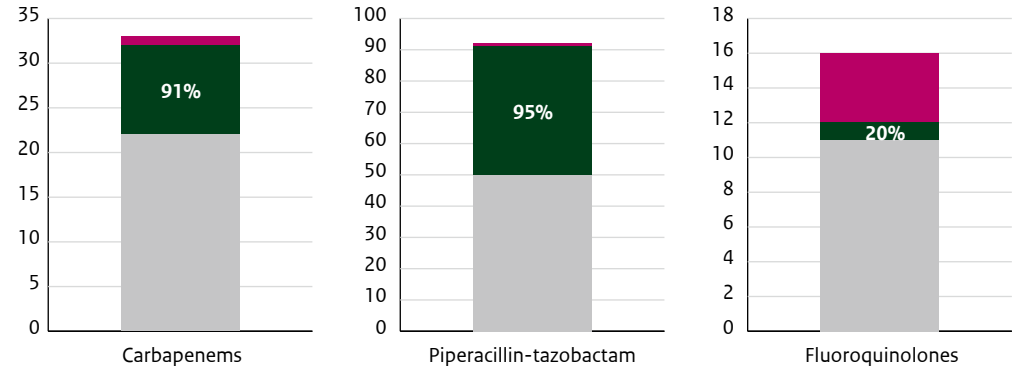
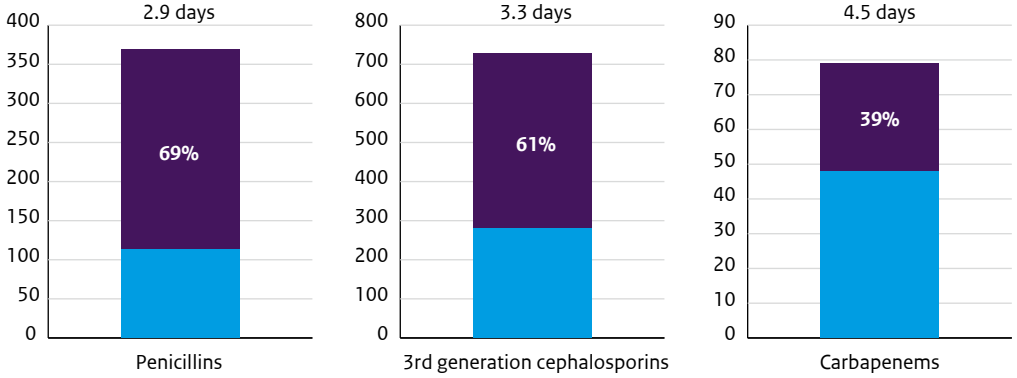



**Figure 5.2.2** Number of antibiotic courses that were administered intravenously for >72 hours (blue column) or ≤72 hours (purple column; percentage). The mean duration of the intravenous administration for the different antibiotics is shown above the columns.



## Discussion

This pilot has yielded valuable information on 1) the possibilities of data-extraction for the purpose of surveillance and 2) barriers (and solutions) for its implementation.

Dutch hospitals engage actively in antimicrobial stewardship. This inquiry shows that 60 of the 64 responding hospitals (94%) have established an A-team and that almost all monitor the quality of antimicrobial use. A-teams focus on the use of restricted antimicrobials (91%), followed by IV-oral switch, and bedside consultation (~60%). These activities come on top of baseline functions of the three core specialties, and complement those with proactive and persistent efforts to measure the quality of antimicrobial use, facilitating improvement strategies and feedback to prescribers. There is, however, room for improvement. Not all stewardship objectives are covered, and structural documentation facilitating analysis of the quality of antimicrobial use needs to be improved. A likely explanation is the lack of human resources, since only 41% of the A-teams were financially supported by the hospital boards of directors. If budget was provided (median of 0.5 FTE), this was significantly less than the recommended national staffing standards.

The quality improvement cycle is the core of antimicrobial stewardship. For this purpose, data on the quality of antibiotic use and its determinants are crucial. To facilitate this process, the SWAB performed a pilot to test the feasibility of automated data-extraction from the EMR and lab systems. This pilot shows that relevant data indeed can be extracted for the calculation of quality indicators. Half of the hospitals could provide data electronically, although only for part of the data. Data requiring an assessment by the A-team, such as the appropriateness of antimicrobial use, could only be provided manually, since these are not available as discrete data in the EMR. Thus, standardized documentation in the EMR is crucial. Another prerequisite for data-extraction is IT support. This was insufficient for the hospitals that could not provide data electronically. This is in line with the results of the survey. Only nine percent of the A-teams had structural IT support.

This pilot illustrates that data extraction from EMR and lab systems is feasible, but faces several challenges. Efforts are made to include missing variables in EMR and A-teams should claim structural IT support in their hospitals.

We pursue a steady increase in hospitals participating in the antimicrobial stewardship monitor, with ultimately an interactive dashboard showing the quality of antimicrobial use. Collected data from multiple hospitals will enable benchmarking, but challenges remain, for example to correct for the selections made for the prescriptions monitored by the A-teams. For the performance of bedside consultations in SAB, currently only the performance of bedside consultation is reported. As of 2018, the stewardship monitor will be integrated with the national initiative of a SAB registry, and more aspects of care for patients with SAB will be included.

# MARAN 2018

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2017

June 2018

## Colophon

This report is published under the acronym MARAN-2018 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-2018 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-2018 is published in a combined back-to-back report with NETHMAP-2018. 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-2018 can be ordered from the secretariat of WBVR, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

**Editors**
Dr. K.T. Veldman[1], Prof. Dr. D.J. Mevius [1,2]
[1] Wageningen Bioveterinary Research (WBVR), Lelystad
[2] Dept. I&I, Faculty of Veterinary Medicine, Utrecht University
Ing. B. Wit,
Food and Consumer Product Safety Authority (NVWA), Utrecht
Dr. W. van Pelt,
National Institute for Public Health and the Environment (RIVM), Bilthoven
Prof. Dr. D. Heederik,
Netherlands Veterinary Medicines Institute (SDa), Utrecht

**The following persons contributed to the writing of MARAN 2018**
*Part I Total sales of antibiotics and usage in livestock*
Dr. I.M. van Geijlswijk, Prof. Dr. D. J.J. Heederik, Prof. Dr. J. Wagenaar, Prof. Dr. J. W. Mouton, Dr. J. H. Jacobs, P. Sanders Msc, SDa, Utrecht

*Part II Resistance data*
Dr. K.T. Veldman, Dr. M. Swanenburg, Dr. D. Ceccarelli, Prof. Dr. D.J. Mevius
WBVR, Lelystad
Ing. B. Wit,
NVWA, Utrecht
Dr. W. van Pelt,
RIVM, Bilthoven
Dr. J. Hordijk, Prof. Dr. J. Wagenaar
FD Utrecht

**People involved in providing data for the surveillance of antimicrobial resistance**

WBVR, Lelystad:
Joop Testerink, Marga Japing, Arie Kant, Yvon Geurts

RIVM, Bilthoven:
Max Heck, Henny Maas, Wilfrid van Pelt, Lapo Mughini Gras, Anjo Verbruggen
NVWA, Utrecht
Ben Wit, Petra Dop, Rianne Hagen-Lenselink, Michel Rapallini

Ministry of Economic Affairs, The Hague
Bart van den Assum, Gertine van Ingen-ter Brinke

## Acknowledgements

This study was primarily financed by the Ministry of Economic Affairs, through the project 'Antimicrobial Resistance Research in Animals', grant number WOT-01-002-03.02, project leader in 2017 Dr. K.T. Veldman.

The Food and Consumer Product Safety Authority within the domain Microbiology financed by the Ministry of Health, Welfare and Sport provided additional financing for the work of Ing. B. Wit in animal products and the contribution to several chapters by Dr. W. van Pelt.

The authors thank Mr. Drs. J.F. Schutte and Drs. B.G.M. Eussen from FIDIN for providing detailed insight into the national sales data.

The authors thank Xerox/OBT, Den Haag for the layout.

# Contents

# 1 Summary

**Antibiotic Usage**

Sales of antimicrobial veterinary medicinal products (AVMP's) in 2017 (181 tonnes) showed an increase of 3% compared to 2016 (176 tonnes). In 2016, sales barely covered monitored and extrapolated use; reasons for the increase of sales in 2017 could be an increase in stock (catching up) and increased use in growing unmonitored sectors.

In most sectors, veal valves, pigs, broilers and turkeys, a reduction in consumption has been realized. In dairy cows and other cattle a small increase in consumption is noted. The calculation of consumption is based on national conversion factors (DDDA's) of authorized drugs. Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3$^{rd}$ and 4$^{th}$ generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these AVMP's 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 VMP's.

**Antimicrobial resistance**

In 2017 *S.* Enteritidis (25.6%) followed by *S.* Typhimurium (15.9%) together with the monophasic variant of Typhimurium: *S.* enterica subspecies *enterica* 1,4,[5],12:i:- (15.7%), were most frequently isolated from humans suffering from salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated. In poultry (including poultry products and broilers), the number of *S.* Paratyphi B var. Java was equal to 2016. The most isolated serovar in poultry meat in 2017 was *S.* Heidelberg. The highest proportions of resistance were observed in the *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky, and to a lesser extent in *S.* Typhimurium. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Kentucky (81.3% resistant), *S.* Infantis (26.2%) and Enteritidis (21.5%).

In 2017, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.8% concerning seven different serovars, isolated from human samples. Cefotaxime resistance was

detected in 67.6% of the *Salmonella* isolates obtained from (outside EU) imported poultry products. No cefotaxime resistant isolates were found in fresh meat from Dutch retail (produced within EU). No carbapenemase producing *Salmonella* were found in 2017.

Proportions of resistance in *C. jejuni* from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2017, compared to 2016. Resistance to macrolides was rarely detected in isolates from livestock and humans and almost exclusively found in *C. coli* isolates from broilers and pigs. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with an increase in 2017), 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.

Proportions of resistance to ampicillin, sulfamethoxazole and trimethoprim in human STEC O157 isolates were somewhat higher in 2017, compared to 2016 (10.7% to 16.1% for ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). There is an increasing tendency for resistance against these antimicrobials since 2009. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 3.2% of human STEC O157 isolates. For the first time since seven years one cefotaxime resistant, ESBL-producing isolate was detected.

In 2017, resistance proportions of indicator *E. coli* in caecal samples showed a tendency to decrease in broilers, to stabilize in pigs, and showed a slight increase in veal calves. In dairy cattle the resistance proportions remained at a constant low level. As in former years, resistance proportions in *E. coli* from chicken and turkey meat, were substantially higher than in pork and beef. The proportion of *E. coli* isolates resistant to third-generation cephalosporins was low in faecal samples from broilers and pigs and they were not detected in dairy cattle and veal calves. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still high in broilers, pigs, veal calves and chicken and turkey meat. Levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Within the randomly isolated indicator *E. coli* in caecal samples from broilers a continuous low proportion of ESBL/AmpC-producing *E. coli* was observed in the last five years (<3%) and this was confirmed in 2017 (1.7%). No ESBL/AmpC-producing indicator *E. coli* were detected by random isolation in faecal samples from pigs, veal calves and dairy cattle. Selective culturing in livestock faeces showed a further decrease in the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* in broilers. For the second year in a row, an increase was observed in white and rosé veal calves carrying ESBL/AmpC-producing *E. coli*, using selective culturing. 2017 was the first year a higher prevalence was recorded in veal calves than in broilers (36.7% vs 32.6%).
The most prevalent ESBL/AmpC gene was $bla_{\text{CTX-M-1}}$ in all animal species. $bla_{\text{CTX-M-15}}$ was found frequently in veal calves and dairy cows (30%). $bla_{\text{CMY-2}}$ in broilers (25%), followed by $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52c}}$ and $bla_{\text{CTX-M-14}}$.

A comparable gene distribution was observed in corresponding meat samples. The overall prevalence of ESBL/AmpC-producing *E. coli* in meat in 2017 was 9.6%. After three years of decreasing prevalence (67% to 24% in 2014-2016), in 2017 31.6% of fresh chicken meat samples were found positive, resulting in a similar prevalence as in broilers (32.6%). Imported chicken meat was more frequently positive (56.1%). Also lamb and veal meat were more frequently found positive than in previous years.
The proportion of human ESBL/AmpC-producing *Salmonella* in 2017 was 1.8%, confirming a continuous low level (≤2%) since 2014. Most represented ESBL/AmpC genes were $bla_{\text{CTX-M-14b}}$, generally associated with S. Kentucky, $bla_{\text{CTX-M-9}}$ in S. Typhimurium, and $bla_{\text{CMY-2}}$ in S. Typhimurium and S. Agona. The majority (84%) of ESBL/AmpC-producing *Salmonella* from humans were highly multidrug resistant (5-8 antibiotics).

No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance in livestock. Only $bla_{\text{OXA-48-like}}$ genes were detected in six samples (three broilers, two slaughter pigs and one dairy cow) and all associated with *Shewanella* spp..

In an ongoing prospective study of faecal samples of companion animals one dog was found to be carrier of *E. coli* carrying $bla_{\text{OXA-48}}$. This was the first time such a carbapenemase producing isolate was detected in a dog in the Netherlands. Molecular analysis of the isolate is ongoing but preliminary analysis suggests that the $bla_{\text{OXA-48}}$ gene is transferable because it is located on a mobile element.

Colistin resistance gene *mcr-1* was identified at a low-level in *E. coli* from livestock (1.2%) and at higher levels in retail meat from chicken (7.7%), but not in *Salmonella*.

It can be concluded that the sales of antibiotics for animals remained stable compared to 2016. In 2017 a clear reduction in antibiotic use was only observed in broilers and turkeys, while in use pigs and veal calves showed a small reduction and use in dairy cattle showed a small increase. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3$^{rd}$ and 4$^{th}$ generation) remains to be very minimal.
The usage data are to a large extend reflected in the resistance data of 2017 where proportions of resistant *E. coli* stabilized in pigs compared to constant decreasing tendencies since 2009. In veal calves the resistant proportions have been stable since 2012 and showed a slight increase in 2017. In broilers the continuous reduction in use resulted in an ongoing decrease in proportions of resistant *E. coli* for most antibiotic classes tested. Also the concentration of ESBL/AmpC-producing *E. coli* in broiler faeces and on poultry meat was again lower than in previous years. In contrast to broilers, in 2017 the prevalence of ESBL-carriers again increased in both white and rosé veal calves. This shows that the measures implemented in Dutch livestock production to reduce the overall antibiotic use and to stop the use of 3$^{rd}$-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 antimicrobial resistance remained stable and ESBL occurrence increased. As in previous years carbapenemase producing *Enterobacteriaceae* or the colistin 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 2017

### 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 (AVMP's) on package level sold in 2017 in the Netherlands, as extracted from the Vetindex and supplemented with AVMP's 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, veal calves, dairy- and other cattle) covered 90.6% of sales in 2017.
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 AVMP's, 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 involves for 70% dairy goats, and has grown since 2010, and is now half of the sheep sector.

Dairy cattle experienced a major decrease in number of animals because of the phosphate production limitations after the increase of the preceding two years which occurred as a result of the abandoning

of milk quota. With the exception of piglets (and as a result the mass of the pig sector as a whole) and goats, all major production sectors showed a decrease in numbers of animals, while the mass of sold antimicrobial substances increased with 3% in 2017 compared to 2016.

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

| Number of animals x1000 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|---|---|---|---|---|
| Piglets (less than 20 kg) | 4,809 | 4,649 | 4,797 | 4,993 | 4,920 | 5,115 | 5,408 | 4,986 | 5,522 |
| Sows | 1,100 | 1,098 | 1,106 | 1,081 | 1,095 | 1,106 | 1,053 | 1,022 | 1,066 |
| Fattening pigs | 6,199 | 6,459 | 6,200 | 4,189 | 4,209 | 4,087 | 4,223 | 4,140 | 3,967 |
| Other pigs | 2,100 | 2,040 | 2,021 | 1,841 | 1,789 | 1,765 | 1,769 | 1,733 | 1,741 |
| Turkeys | 1,060 | 1,036 | 990 | 827 | 841 | 794 | 863 | 762 | 671 |
| Broilers | 52,323 | 54,367 | 57,811 | 43,912 | 44,242 | 47,020 | 49,107 | 48,378 | 48,237 |
| Other poultry | 46,383 | 48,218 | 40,442 | 52,356 | 54,345 | 56,924 | 58,636 | 57,172 | 56,947 |
| Veal calves | 886 | 921 | 906 | 908 | 925 | 921 | 909 | 956 | 953 |
| Other cattle | 3,112 | 3,039 | 2,993 | 3,045 | 3,064 | 3,230 | 3,360 | 3,353 | 3,082 |
| Dairy cattle | 1,562 | 1,518 | 1,504 | 1,541 | 1,597 | 1,610 | 1,717 | 1,794 | 1,665 |
| Sheep | 1,091 | 1,211 | 1,113 | 1,093 | 1,074 | 1,070 | 1,032 | 1,032 | 1,015 |
| Goats | 374 | 353 | 380 | 397 | 413 | 431 | 470 | 500 | 533 |
| Fattening rabbits | 271 | 260 | 262 | 284 | 270 | 278 | 333 | 318 | 300 |
| Dows | 41 | 39 | 39 | 43 | 41 | 43 | 48 | 45 | 43 |

### 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 63.38 % over the years 2009-2017, the Governmental 70% reduction goal has not been reached yet.

Sales of AVMP's in 2017 (181 tonnes) showed an increase of 3% compared to 2016 (176 tonnes). In 2016, sales barely covered monitored and extrapolated use; reasons for the increase of sales could be an increase in stock (catching up) and increased use in growing unmonitored 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, aminoglycosides and cephalosporins of 1$^{st}$ and 2$^{nd}$ generation). A steady decrease over the years is noted for fixed combinations (mainly mastitis injectors), and the critically important antimicrobials fluoroquinolones, polymyxins, cephalosporins of 3$^{rd}$ and 4$^{th}$ generation, and for trimethoprim/sulfonamides (-13% in 2017 compared to 2016). Sales of amphenicols dropped with 4% in 2017 after increases in earlier years. Also sales of 1$^{st}$ and 2$^{nd}$ generation cephalosporins (-15%) decreased. The sales of quinolones increased (+3%), other antimicrobials (mainly metronidazole and

bacitracin) and macrolides increased each with 5%. Pleuromutilins sales increased with 20%.

**Figure ABuse01** Antimicrobial veterinary medicinal product sales 1999-2017 in kg (thousands).

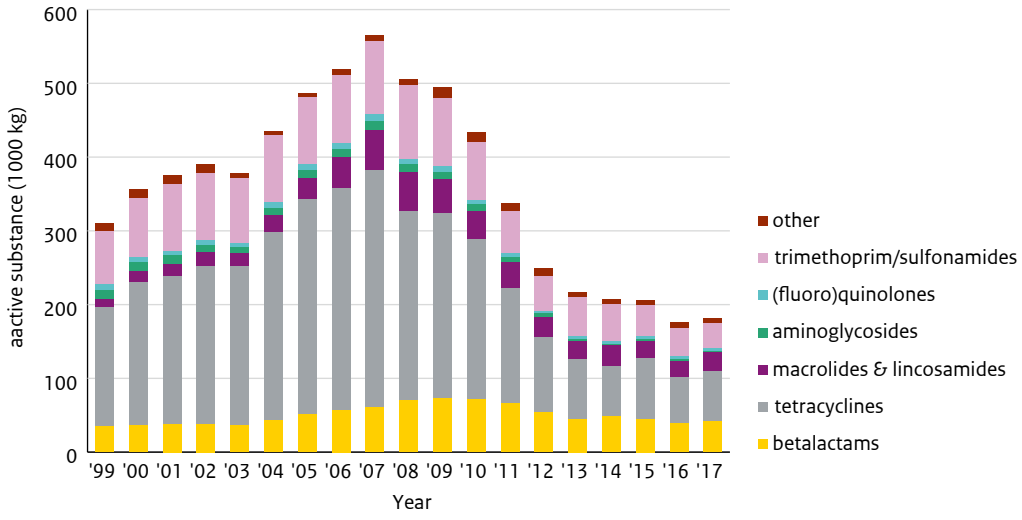
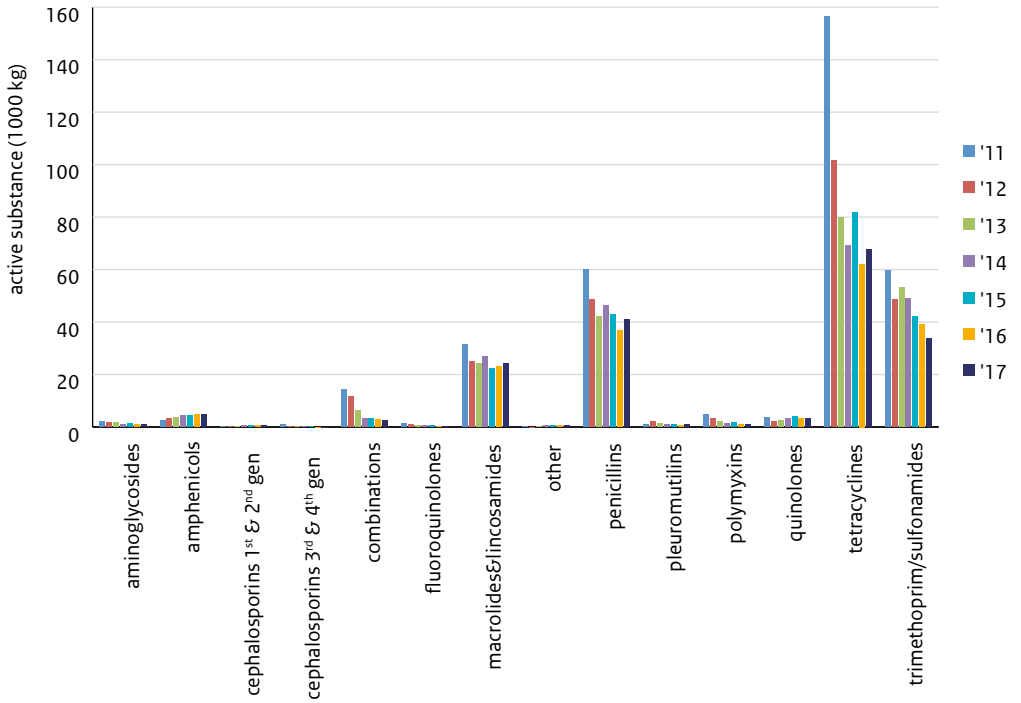



**Table ABuse02** Antimicrobial veterinary medicinal product sales from 1999-2017 in kg (thousands) (FIDIN, 2018).

| year | '99 | '00 | '01 | '02 | '03 | '04 | '05 | '06 | '07 | '08 | '09 | '10 | '11 | '12 | '13 | '14 | '15 | '16 | '17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| betalactam antibiotics | 35 | 36 | 38 | 38 | 36 | 43 | 51 | 57 | 61 | 70 | 73 | 71 | 66 | 54 | 45 | 48 | 45 | 39 | 42 |
| tetracyclines | 162 | 194 | 200 | 214 | 216 | 256 | 292 | 301 | 321 | 257 | 251 | 217 | 157 | 102 | 80 | 69 | 82 | 62 | 68 |
| macrolids & lincosamides | 10 | 15 | 17 | 19 | 17 | 23 | 28 | 42 | 55 | 52 | 46 | 39 | 34 | 26 | 25 | 28 | 23 | 23 | 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 |
| (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 |
| trimethoprim/ sulfonamides | 72 | 80 | 92 | 92 | 88 | 91 | 91 | 93 | 99 | 100 | 92 | 78 | 58 | 48 | 53 | 49 | 42 | 39 | 34 |
| other antibiotics | 11 | 12 | 11 | 11 | 7 | 6 | 6 | 8 | 8 | 7 | 15 | 13 | 10 | 10 | 8.1 | 7.8 | 7.5 | 7.4 | 7.2 |
| total sales | 310 | 356 | 376 | 390 | 378 | 434 | 487 | 519 | 565 | 506 | 495 | 433 | 338 | 249 | 217 | 207 | 206 | 176 | 181 |

**Figure ABuse02** Antimicrobial veterinary medicinal product sales by pharmaco-therapeutic class 2011-2017 in kg (thousands)




### Tetracyclines

In contrast to 2016, total mass of tetracyclines sold increased with 9% in 2017, while the use decreased in all monitored sectors. This pattern also occured in 2015. The fraction of doxycycline increased to 49% of the total sales of tetracyclines (47% in 2016, 42% in 2015, 41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

### Penicillins

Second place in mass again, penicillin sales increased 11% compared to 2016; the increase was limited to the broad spectrum aminopenicillins, sales of narrow spectrum penicillines decreased. As a result, the overall figures amount to 75% broad and 25% narrow spectrum penicillines of the mass sold respectively.

### Trimethoprim/sulfonamides

The use of trimethoprim/sulfonamides decreased further in 2017, and due to the increase of penicillins, it ranks third in mass sold.

### (Fluoro)quinolones

The sales of fluoroquinolones decreased with 82 kg (25%) in 2017. An overall reduction of 83% was realized in comparison with 2011. 78% of the sales are applied in the monitored sectors. The sales of quinolones increased in 2017, compared with 2011 an overall decrease of 14% was noticed, 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 AVMP's for companion animals were reported for the first time. Sales of these VMPs were relatively stable over the period 2015 to 2017. The sales of 3rd and 4th generation cephalosporins halved in 2017 with 1 kg (from 2 kg). 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 AVMP's have to be imported.

### Polymyxins

Colistin sales and use decreased in 2017. Compared to 2011 a reduction of 80% has been accomplished. 95% are oral VMP's, 5% are injectables combined with aminopenicillins.

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

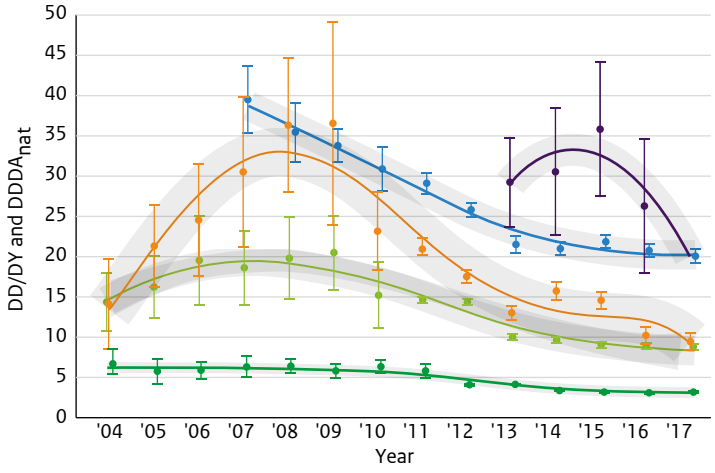
Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands by Wageningen University for sentinel farms. These data were, in cooperation with Utrecht University, converted to the number of defined doses per animal year (DD/AY). The calculation method is similar to the method applied in human drug use. Applied antimicrobial veterinary medicinal products (AVMP's) 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 consumption reports are prepared by the Netherlands Veterinary Medicines Institute (SDa) using consumption data from *all* farms in the largest sectors of food production animals: pigs, veal calves, broilers, cattle (since 2012) and turkeys (since 2013). In 2016 rabbits are also monitored but are not included in this report because of transition problems with data transfer. Since 2017 also antimicrobials use in poultry sectors additionally to broilers is made available. 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 AVMP's consumption data are reported for in 2013 – 2017 (pigs, veal calves, cattle, broilers and turkeys).
Table ABuse04 gives animal weights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In most sectors (veal valves, pigs, broilers and turkeys) a reduction in consumption has been realized. In dairy cows and other cattle a small increase in consumption is noted. 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 2011-2016 as $DDDA_{NAT}$) are depicted in Figure Abuse03, and specification of applied antimicrobial groups in the different sectors for 2013-2017 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 VMP's 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 2017, CBS (Centraal Bureau voor de Statistiek, National Institute of Statistics) 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 | 2013 | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|---|---|
| pigs | 710,688 | 710,802 | 704,937 | 706,025 | 686,638 | 690,093 |
| veal calves | 156,602 | 159,547 | 158,828 | 156,751 | 164,890 | 163,935 |
| diary cows | 924,600 | 958,200 | 966,000 | 1,030,200 | 1,076,400 | 999,000 |
| other cattle | 597,900 | 573,800 | 649,000 | 649,800 | 600,100 | 542,000 |
| broilers | 43,846 | 44,242 | 47,020 | 49,107 | 48,378 | 48,237 |
| turkeys | 4,961 | 5,046 | 4,763 | 5,178 | 4,572 | 4,023 |
| rabbits | 872 | 830 | 860 | 1,004 | 948 | 901 |

**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 2011-2017 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 by the sector quality systems.
This consumption is calculated with a detailed denominator, to facilitate refined benchmarking.
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 2013-2017.

* *categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2015*

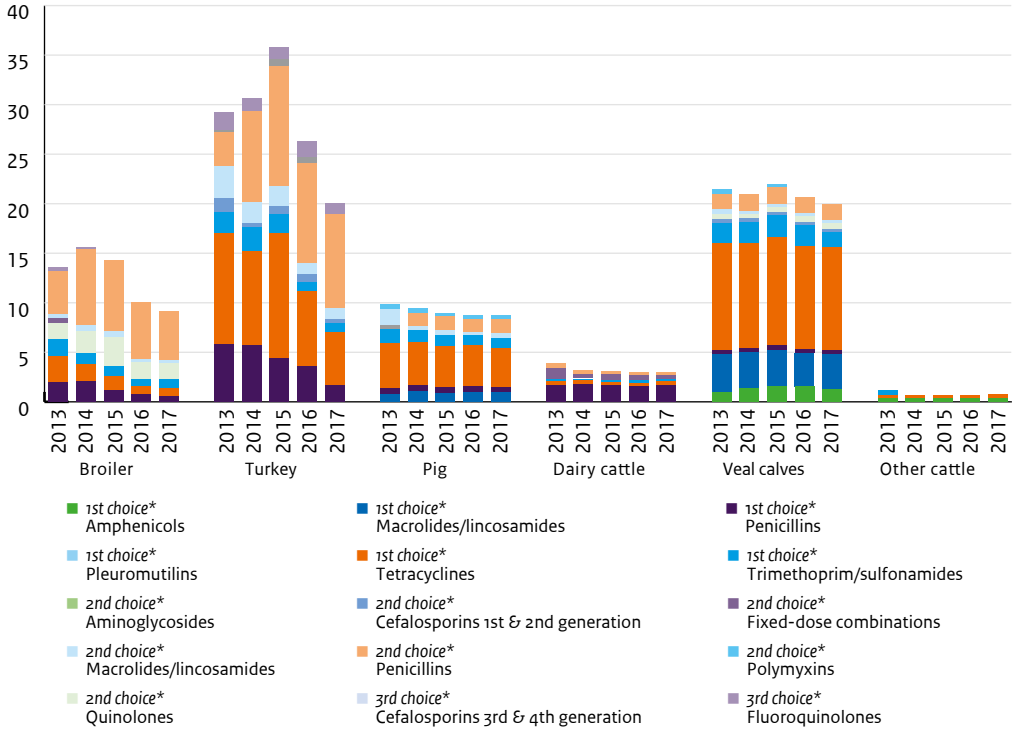


**Table ABuse05** Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2013 - 2017.

| | Animalsector | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Veal calfs* | | | | | Dairy cattle | | | | | Cattle | | | | |
| Year | 2013 | 2014 | 2015 | 2016 | 2017 | 2013 | 2014 | 2015 | 2016 | 2017 | 2013 | 2014 | 2015 | 2016 | 2017 |
| Number of farms with prescriptions | 2125 | 2061 | 1978 | 1928 | 1868 | 18005 | 17747 | 17737 | 17529 | 17121 | 13644 | 13359 | 12971 | 12548 | 12790 |
| Pharmacotherapeutic group | | | | | | | | | | | | | | | |
| Aminoglycosides | 0.53 | 0.34 | 0.19 | 0.23 | 0.23 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |
| Amphenicols | 1.23 | 1.52 | 1.63 | 1.59 | 1.44 | 0.05 | 0.06 | 0.06 | 0.06 | 0.05 | 0.11 | 0.10 | 0.10 | 0.11 | 0.11 |
| Cefalosporins 1st & 2nd generation | - | - | - | - | - | 0.03 | 0.02 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 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 | 0.00 |
| Combinations | 0.09 | 0.01 | 0.00 | 0.00 | 0.01 | 1.01 | 0.48 | 0.42 | 0.38 | 0.34 | 0.08 | 0.04 | 0.03 | 0.03 | 0.04 |
| Fluoroquinolones | 0.03 | 0.02 | 0.02 | 0.03 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | | | | 0.00 |
| Macrolides/lincosamides | 3.84 | 3.72 | 3.88 | 3.54 | 3.65 | 0.06 | 0.10 | 0.10 | 0.07 | 0.06 | 0.22 | 0.20 | 0.16 | 0.17 | 0.19 |
| Other | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Penicillins | 2.11 | 2.15 | 2.33 | 2.25 | 2.21 | 2.20 | 2.00 | 1.87 | 1.86 | 2.00 | 0.19 | 0.18 | 0.16 | 0.16 | 0.18 |
| Pleuromutilins | - | - | - | | - | - | - | - | - | - | - | - | - | - | - |
| Polymyxins | 0.36 | 0.15 | 0.19 | 0.07 | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 |
| Quinolones | 0.30 | 0.49 | 0.58 | 0.66 | 0.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.03 | 0.02 | 0.03 | 0.02 |
| Tetracyclines | 10.87 | 10.66 | 11.01 | 10.47 | 10.35 | 0.42 | 0.39 | 0.37 | 0.35 | 0.32 | 0.59 | 0.47 | 0.42 | 0.44 | 0.45 |
| Trimethoprim/sulfonamides | 2.14 | 2.08 | 2.22 | 2.05 | 1.61 | 0.22 | 0.24 | 0.25 | 0.24 | 0.24 | 0.16 | 0.11 | 0.10 | 0.10 | 0.09 |
| Total | 21.50 | 21.15 | 22.05 | 20.88 | 20.13 | 4.03 | 3.30 | 3.11 | 3.01 | 3.06 | 1.40 | 1.15 | 1.00 | 1.07 | 1.10 |

* *Population data derived from CBS (formerly from Eurostat)*

**Table ABuse05 (continued)** Trends in $DDDA_{nat}$ in the Netherlands in livestock.

| | Animalsector | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pigs | | | | | Broilers | | | | | Turkeys | | | | |
| Year | 2013 | 2014 | 2015 | 2016 | 2017 | 2013 | 2014 | 2015 | 2016 | 2017 | 2014 | 2014 | 2015 | 2016 | 2017 |
| Number of farms with prescriptions | 6588 | 6072 | 5824 | 5462 | 5297 | 770 | 797 | 816 | 849 | 852 | 40 | 40 | 47 | 43 | 45 |
| Pharmacotherapeutic group | | | | | | | | | | | | | | | |
| Aminoglycosides | - | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.03 | 0.02 | 0.01 | 0.03 | 1.24 | 0.40 | 0.71 | 0.69 | 0.05 |
| Amphenicols | 0.09 | 0.17 | 0.18 | 0.24 | 0.25 | - | - | - | - | - | 0.02 | - | - | - | - |
| Cefalosporins 1st & 2nd generation | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Cefalosporins 3rd & 4th generation | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Combinations | 0.10 | 0.05 | 0.04 | 0.03 | 0.02 | 0.37 | 0.08 | 0.11 | 0.05 | 0.01 | - | - | - | - | - |
| Fluoroquinolones | 0.00 | 0.00 | - | 0.00 | | 0.24 | 0.18 | 0.07 | 0.07 | 0.05 | 1.76 | 1.29 | 1.20 | 1.60 | 1.06 |
| Macrolides/lincosamides | 1.02 | 1.09 | 1.04 | 1.08 | 1.13 | 0.31 | 0.35 | 0.48 | 0.25 | 0.20 | 3.55 | 2.12 | 1.98 | 1.18 | 1.30 |
| Other | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Penicillins | 2.17 | 2.05 | 1.93 | 1.97 | 1.96 | 6.34 | 9.96 | 8.44 | 6.48 | 5.58 | 9.34 | 14.89 | 16.61 | 13.75 | 11.01 |
| Pleuromutilins | 0.12 | 0.09 | 0.08 | 0.07 | 0.09 | - | - | - | - | - | - | - | 0.12 | - | 0.10 |
| Polymyxins | 0.44 | 0.34 | 0.38 | 0.28 | 0.26 | 0.08 | 0.05 | 0.06 | 0.04 | 0.03 | 0.18 | 0.08 | 0.63 | 0.61 | - |
| Quinolones | 0.03 | 0.05 | 0.03 | 0.02 | 0.03 | 1.65 | 2.22 | 2.86 | 1.51 | 1.72 | 0.23 | 0.02 | 0.10 | 0.01 | 0.26 |
| Tetracyclines | 4.58 | 4.34 | 4.15 | 4.07 | 4.05 | 2.52 | 1.77 | 1.49 | 1.01 | 0.95 | 11.19 | 9.58 | 12.57 | 7.63 | 5.51 |
| Trimethoprim/sulfonamides | 1.40 | 1.33 | 1.20 | 1.10 | 0.90 | 1.46 | 1.45 | 1.07 | 0.78 | 0.82 | 1.80 | 2.37 | 2.01 | 0.95 | 0.86 |
| Total | 9.97 | 9.52 | 9.05 | 8.87 | 8.70 | 13.01 | 15.76 | 14.59 | 10.19 | 9.40 | 29.31 | 30.74 | 35.94 | 26.42 | 20.16 |

* *Population data derived from CBS (formerly from Eurostat)*

**Table ABuse06** Applied bodyweights for $DDDA_F$ 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 expressed in the number of international units DDDVET of the European Surveillance of Veterinary Antimicrobial Consumption in pigs, veal calves, cattle, broilers and turkeys in the Netherlands per animal-year

A comparison of the number of DDDA with the internationally established ESVAC $DDD_{VET}$ was conducted for the 2016 and 2017 data, 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 AVMP's 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, AVMP's 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.

In contrast to the SDa $DDDA_{NAT}$ calculations, $DDD_{VET}$ results decreased for all sectors, even in dairy cows and other cattle. In dairy cows this could imply that the increase was caused by mastitis and dry cow treatments, being excluded from calculation in de ESVAC method. In veal calves this explanation is less obvious.

**Conclusion**

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The unexpected increase in sales of antimicrobial VMP's in the Netherlands in 2017 may be the result of an adjustment or compensation for the relatively low 2016 sales, which is not supported by 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 3$^{rd}$ and 4$^{th}$ generation) is reduced to an absolute minimum, even in the unmonitored sectors. Import of these VMP's 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 VMP's.

**Table ABuse07** number of DDDAVET/animal year in monitored sectors 2016-2017.

| | Broilers | | Turkeys | | Pigs | | Dairy cattle (excluding intramammary and intrauterine administrations) | | Veal calves | | Other cattle | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDDA | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDD$_{VET}$ | #DDDA | #DDD$_{VET}$ | #DDD$_{VET}$ |
| First choice* | 4.02 | 3.71 | 16.12 | 11.37 | 6.91 | 6.62 | 0.95 | 0.92 | 19.51 | 18.52 | 0.95 | 0.95 |
| % 1st choice of total | 34.84% | 34.36% | 57.72% | 49.48% | 79.13% | 77.72% | 90.33% | 89.76% | 78.93% | 87.61% | 81.28% | 86.12% |
| Amphenicols | - | - | - | - | 0.18 | 0.19 | 0.04 | 0.04 | 1.22 | 1.11 | 0.09 | 0.08 |
| Macrolides/lincosamides | - | - | - | - | 0.81 | 0.85 | 0.03 | 0.03 | 3.81 | 3.94 | 0.17 | 0.19 |
| Penicillins | 0.68 | 0.58 | 3.64 | 1.61 | 0.57 | 0.54 | 0.15 | 0.15 | 0.26 | 0.26 | 0.05 | 0.05 |
| Pleuromutilins | - | - | - | 0.14 | 0.07 | 0.10 | - | - | - | - | - | - |
| Tetracyclines | 1.32 | 1.27 | 10.71 | 9.20 | 3.46 | 3.42 | 0.24 | 0.22 | 10.88 | 10.61 | 0.47 | 0.48 |
| Trimethoprim/sulfonamides | 1.78 | 1.86 | 0.49 | 0.42 | 1.81 | 1.51 | 0.47 | 0.48 | 3.34 | 2.61 | 0.17 | 0.15 |
| Second choice* | 7.47 | 7.03 | 10.21 | 10.54 | 1.82 | 1.90 | 0.10 | 0.10 | 5.18 | 2.59 | 0.22 | 0.15 |
| % 2nd choice of total | 64.55% | 65.15% | 36.55% | 45.89% | 20.87% | 22.28% | 9.34% | 9.97% | 20.97% | 12.23% | 18.68% | 13.81% |
| Aminoglycosides | 0.00 | 0.03 | 0.20 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.09 | 0.09 | 0.01 | 0.01 |
| Cefalosporins 1st & 2nd generation | - | - | - | - | - | - | - | - | - | - | - | - |
| Combinations | 1.08 | 0.02 | 0.01 | - | 0.02 | 0.03 | 0.00 | 0.04 | 0.85 | 0.01 | 0.04 | 0.03 |
| Macrolides/lincosamides | 0.33 | 0.19 | 1.28 | 1.40 | 0.08 | 0.53 | 0.04 | 0.01 | 0.00 | 0.14 | 0.03 | 0.01 |
| Penicillins | - | 5.53 | - | 8.95 | 0.41 | 1.01 | 0.01 | 0.05 | 0.12 | 1.59 | 0.01 | 0.07 |
| Polymyxins | 6.28 | 0.02 | 9.56 | 0.00 | 0.97 | 0.31 | 0.04 | 0.00 | 4.05 | 0.02 | 0.13 | 0.00 |
| Quinolones | 0.03 | 1.23 | 0.44 | 0.19 | 0.34 | 0.02 | 0.01 | 0.00 | 0.07 | 0.74 | 0.01 | 0.03 |
| Third choice* | 0.07 | 0.05 | 1.60 | 1.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.03 | 0.00 | 0.00 |
| % 3rd choice of total | 0.61% | 0.49% | 5.73% | 4.63% | 0.00% | 0.00% | 0.33% | 0.27% | 0.10% | 0.16% | 0.03% | 0.07% |
| Cefalosporins 3rd & 4th generation | - | - | - | - | - | - | 0.00 | 0.00 | - | - | 0.00 | - |
| Fluoroquinolones | 0.07 | 0.05 | 1.60 | 1.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.03 | 0.00 | 0.00 |
| Total | 11.57 | 10.78 | 27.93 | 22.98 | 8.73 | 8.52 | 1.05 | 1.03 | 24.72 | 21.15 | 1.17 | 1.10 |

* *Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2015.*

# 3 Resistance data

This chapter describes susceptibility test results as determined in 2017 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

In this chapter, resistance percentages of *Salmonella* isolates are presented. These isolates were sampled from humans suffering from clinical enteral infections/acute gastroenteritis, 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 2017 *S.* Enteritidis (25.6%) followed by *S.* Typhimurium (15.9%) together with the monophasic variant of Typhimurium: *S.* enterica subspecies enterica 1,4,[5],12:i:- (15.7%), were most frequently isolated from humans suffering from salmonellosis.
2. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, *S.* Typhimurium and *S.* Dublin were most commonly isolated.
3. In poultry (including poultry products and broilers), the number of *S.* Paratyphi B var. Java was equal to 2016. The most isolated serovar in poultry meat in 2017 was *S.* Heidelberg.
4. The highest proportions of resistance were observed in the *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky, and to a lesser extent in *S.* Typhimurium.
5. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Kentucky (81.3% resistant), *S.* Infantis (26.2%) and Enteritidis (21.5%).
6. In 2017, the proportions cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.8%, among seven different serovars, isolated from human samples. Cefotaxime resistance was detected in 67.6% of the *Salmonella* isolates (predominantly S. Heidelberg) obtained from imported poultry products. No cefotaxime resistant isolates were found in fresh retail meat.
7. In 2017 no carbapenemase producing *Salmonella* were found.

## *Salmonella* serovar prevalence

In the Netherlands, an extensive 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). A summary of the serotyping results of *Salmonella* isolated from humans and farm animals (pigs, cattle and poultry) is presented in Table S01.

From all human *Salmonella* isolates sent to the RIVM by regional public health and other clinical laboratories a selection of 1222 isolates was sent to WBVR for susceptibility testing. These strains were the first isolates recovered from patients with salmonellosis. Also, 475 isolates from other sources were tested consisting of: isolates from pigs (N = 50) and cattle (N = 40) sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections in animals. The isolates from broilers (N = 58) and layers and reproduction poultry (N = 8) were mainly nonclinical *Salmonella* isolates derived from a diversity of monitoring programs on farms, slaughterhouses and at retail. Isolates from a diversity of other sources (N = 319 from animal feed and food products; other animals from animal husbandry (e.g. sheep, goats) have also been serotyped and tested. In addition, NVWA tested 143 *Salmonella* isolates obtained from raw meats (mainly poultry), spices, herbs and seafood. The results of these isolates were not included in Tables S02, S03, S04 and S05, but are shown in Table S06.

In 2017, Enteritidis 02-10(11)-07-03-02 outbreak in humans was a continuation of the Polish egg outbreak in 2016; Monophasic Typhimurium 03-12-09-00-211 outbreak was at the German border and supposedly related to "junkfood" involving predominantly adolescents; Bovismorbificans outbreak related to the consumption of uncooked ham products; Kentucky outbreak took place in a nursery home; Newport , Agbeni and Infantis elevations could not be traced to a source.

As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most frequently isolated serovars from human clinical infections. In 2017, the most frequently isolated from humans suffering from

salmonellosis were S. Enteritidis (25.6%), followed by S. Typhimurium (15.9%) together with the monophasic variant of Typhimurium (*S. enterica subspecies enterica* 1,4,[5],12:i:-) (15.7%).
S. Typhimurium and its monophasic variant were mainly associated with pigs and cattle, but were also found in poultry. S. Enteritidis was mainly isolated from poultry, broilers and layers (Table S01).
In pigs, the most isolated serovar was S. Typhimurium and especially its monophasic variant. In cattle, S. Typhimurium and S. Dublin were most commonly isolated. In poultry many different serovars were found. In 2017, the most isolated serovar was S. Heidelberg (27.2%) all from imported poultry meat or meat preparations, followed by S. Enteritidis (12.9%), which was the predominant serovar in 2016.
The presence of S. Paratyphi B var. Java (S. Java) and S. Infantis was approximately the same as in 2016 (9.6% and 6.6% respectively).
Reported travel, on average 10%, contributed up to 34% of the cases of human salmonellosis over the years 2014-2017, but differed per serovar. Relative high contributions of travel (≥30%) were noted for the serovars Kentucky, Typhi/Paratyphi A,B,C, Schwarzengrund, Stanley, Virchow and Corvallis.
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* three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice, have been added to the panel and three antimicrobials of less importance for treatment of human infections (florfenicol, kanamycin and streptomycin) have been deleted since the implementation (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 former years, colistin resistance was not reported in *Salmonella* in 2017. 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 (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*.
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 2016 and 2017 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 related | Humans | | Pigs | | Cattle | |
|---|---|---|---|---|---|---|---|---|
| | | 2014-2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| N Total | | | 1529 | 1242 | 63 | 163 | 58 | 80 |
| N tested | Tested | | 1473 | 1222 | 52 | 66 | 49 | 55 |
| Enteritidis | 841 | 12% | 438 | 318 | | | 3 | 1 |
| Typhimurium | 601 | 4% | 260 | 197 | 14 | 56 | 16 | 28 |
| Typhimurium (monofasisch) | 530 | 4% | 229 | 195 | 33 | 86 | 14 | 10 |
| Infantis | 182 | 10% | 37 | 44 | 1 | 1 | | |
| Paratyphi B. var. Java | 86 | 24% | 34 | 20 | | | 1 | 1 |
| Kentucky | 85 | 30% | 36 | 40 | | | | |
| Dublin | 82 | 3% | 28 | 7 | 1 | 1 | 17 | 27 |
| Heidelberg | 82 | 10% | 5 | 1 | | | | |
| Bovismorbificans | 69 | 5% | 42 | 28 | 1 | 2 | | 1 |
| Typhi/Paratyphi A,B,C | 62 | 34% | 31 | 24 | | | | |
| Derby | 60 | 7% | 20 | 12 | 5 | 5 | | |
| Brandenburg | 58 | 4% | 11 | 7 | 4 | 2 | 3 | 1 |
| Newport | 57 | 21% | 23 | 24 | | | | 1 |
| Montevideo | 53 | 21% | 4 | 9 | | | | 3 |
| Livingstone | 49 | 4% | 5 | 4 | 1 | 2 | | |
| Agona | 45 | 24% | 13 | 10 | | | | 1 |
| Schwarzengrund | 44 | 30% | 9 | 6 | | | | |
| Napoli | 42 | 9% | 31 | 10 | | | | |
| Senftenberg | 42 | 16% | 5 | 1 | | | | |
| Kedougou | 41 | n.a. | | | | | | |
| Chester | 35 | 16% | 16 | 16 | | | | |
| Mbandaka | 33 | 28% | 6 | 2 | | | | |
| Anatum | 32 | 28% | 1 | 2 | | | | |
| Stanley | 29 | 31% | 14 | 13 | | | | |
| Give | 28 | 18% | 4 | 6 | | | | |
| Thompson | 27 | 3% | 9 | 7 | | | | |
| Virchow | 24 | 30% | 9 | 11 | | | | |
| Saintpaul | 23 | 25% | 13 | 9 | | | | |
| Corvallis | 22 | 34% | 9 | 9 | | | | |
| Goldcoast | 21 | 3% | 8 | 6 | 1 | 1 | 2 | 1 |
| Braenderup | 19 | 24% | 12 | 7 | | | | |
| Tennessee | 18 | 3% | 1 | | | | | |
| Weltevreden | 18 | 31% | 10 | 6 | | | | |
| Javiana | 17 | 19% | 6 | 12 | | | | |
| Rissen | 17 | 19% | 5 | 3 | 2 | | | |
| Panama | 17 | 8% | 4 | 6 | | 2 | | |
| Agbeni | 16 | 0% | 1 | 16 | | | | |
| Bredeney | 15 | 22% | 4 | 3 | | | | |
| Ohio | 15 | 13% | 1 | 3 | | | | |
| Poona | 15 | 26% | 6 | 7 | | | | |
| Hadar | 12 | 27% | 5 | 5 | | | | |

**Table S01 (continued)** Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 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 | |
|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| N Total | 318 | 272 | 98 | 160 | 112 | 24 | 1028 | 926 |
| N tested | 199 | 197 | 76 | 139 | 49 | 8 | 354 | 337 |
| Enteritidis | 183 | 35 | 39 | 6 | 85 | 5 | 33 | 47 |
| Typhimurium | 9 | 19 | 1 | 7 | 2 | 4 | 38 | 107 |
| Typhimurium (monofasisch) | 24 | 21 | 13 | 5 | 3 | 7 | 41 | 78 |
| Infantis | 32 | 18 | 20 | 10 | 2 | | 74 | 33 |
| Paratyphi B. var. Java | 23 | 26 | 9 | 22 | 2 | | 16 | 7 |
| Kentucky | | | | | | | 6 | 8 |
| Dublin | | 1 | | | | | 7 | 1 |
| Heidelberg | 2 | 74 | 2 | 73 | | | 2 | 1 |
| Bovismorbificans | | 2 | | | | | 1 | |
| Typhi/Paratyphi A,B,C | | | | | | | | |
| Derby | | 1 | | | | | 35 | 15 |
| Brandenburg | 1 | 5 | 1 | 5 | | | 42 | 22 |
| Newport | 1 | 1 | 1 | 1 | | | 3 | 2 |
| Montevideo | 2 | 2 | | | 1 | 1 | 31 | 24 |
| Livingstone | | | | | | | 146 | 109 |
| Agona | | 2 | | 1 | | | 18 | 14 |
| Schwarzengrund | 2 | 8 | 2 | 8 | | | 11 | 17 |
| Napoli | | | | | | | 3 | 3 |
| Senftenberg | | 2 | | | | | 29 | 22 |
| Kedougou | | 6 | | 2 | | | 19 | 65 |
| Chester | | | | | | | 7 | 2 |
| Mbandaka | 2 | 1 | | 1 | 2 | | 16 | 16 |
| Anatum | 2 | 3 | | 3 | 1 | | 68 | 15 |
| Stanley | | | | | | | 3 | 4 |
| Give | 3 | | 1 | | | | 4 | 21 |
| Thompson | | 11 | | | | 4 | 4 | 8 |
| Virchow | 2 | 2 | 2 | 1 | | | 3 | 3 |
| Saintpaul | | | | | | | 4 | 5 |
| Corvallis | 2 | | 1 | | | | 2 | 2 |
| Goldcoast | | | | | | | 1 | 4 |
| Braenderup | | | | | | | 2 | 2 |
| Tennessee | 1 | | 1 | | | | 43 | 7 |
| Weltevreden | | | | | | | 6 | 1 |
| Javiana | | | | | | | 1 | 1 |
| Rissen | | | | | | | 8 | 3 |
| Panama | 2 | | | | 2 | | 4 | 2 |
| Agbeni | | | | | | | | |
| Bredeney | | 1 | | | | 1 | 18 | 2 |
| Ohio | | | | | | | 5 | 13 |
| Poona | | | | | | | 1 | 3 |
| Hadar | 1 | | | | | | 6 | 5 |

**Table S01 (continued)** Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 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 related | | Humans | | Pigs | | Cattle | |
|---|---|---|---|---|---|---|---|---|
| | 2014-2017 | | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| N Total | | | 1529 | 1242 | 63 | 163 | 58 | 80 |
| N tested | Tested | | 1473 | 1222 | 52 | 66 | 49 | 55 |
| Oranienburg | 12 | 21% | 5 | 8 | | | | |
| Bareilly | 11 | 21% | 6 | 4 | | | | |
| Kottbus | 10 | 24% | 5 | 4 | | | | 1 |
| Muenchen | 10 | 17% | 2 | 6 | | 1 | 1 | |
| Cerro | 8 | 25% | 1 | | | | | |
| Goettingen | 8 | 0% | 3 | 1 | | | | 1 |
| Jerusalem | 8 | n.a. | | | | | | |
| London | 8 | 6% | 1 | 3 | | 1 | | |
| Indiana | 7 | 10% | 3 | | | | | |
| Mikawasima | 6 | 0% | 4 | 2 | | | | |
| OVERIGE | 352 | 18% | 94 | 108 | | 3 | 1 | 3 |

MIC-distributions and resistance percentages of 1697 *Salmonella's* from different sources tested for susceptibility in 2017 are presented in Table S02. The resistance rates were approximately at the same level as in 2016. Highest proportions of resistance were again observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, chloramphenicol and trimethoprim. The proportions of resistance to ciprofloxacin and cefotaxime/ceftazidime seem to fluctuate a little since 2013. Resistance to the carbapenem antibiotic meropenem was not detected, indicating that carbapenemase producers were not present in the tested isolates (see also chapter 4.2). Like in 2015 and 2016, low proportions of resistance were found for tigecycline (1.3%) and azithromycin (1.0%), almost exclusively in human isolates.

Table S03 shows resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2017. Resistance profiles varied considerably among serovars. High resistance proportions were observed in *S.* Heidelberg, monophasic *S.* Typhimurium and in *S.* Kentucky (64.6-81.3%), and to a lesser extent in *S.* Typhimurium.
Most serovars have acquired resistance against more than one antimicrobial. Again, the most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

### Quinolone resistance

The class of fluoroquinolones is widely 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, 13.8% of *Salmonella* isolates (N =234/1697) demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02).
The dominant serovars of ciprofloxacin resistant isolates were *S.* Heidelberg (100%), *S.* Kentucky (81%) from humans, *S.* Infantis (26%) from broilers, and *S.* Enteritidis (22%) from both humans and broilers.

**Table S01 (continued)** Most prevalent *Salmonella* serotypes isolated in 2016 and 2017 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 | |
|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| N Total | 318 | 272 | 98 | 160 | 112 | 24 | 1028 | 926 |
| N tested | 199 | 197 | 76 | 139 | 49 | 8 | 354 | 337 |
| Oranienburg | | | | | | | 7 | 3 |
| Bareilly | | | | | | | | |
| Kottbus | | | | | | | | |
| Muenchen | | | | | | | 2 | 2 |
| Cerro | | | | | | | 5 | 5 |
| Goettingen | | | | | | | | |
| Jerusalem | 4 | 8 | | 5 | 4 | | 2 | 1 |
| London | | | | | | | 5 | 2 |
| Indiana | 2 | | | | 2 | | 3 | 1 |
| Mikawasima | | | | | | | 1 | |
| OVERIGE | 18 | 23 | 5 | 10 | 6 | 2 | 242 | 218 |

In meat (Table S06) the proportion of isolates resistant to ciprofloxacin was very high (89%). The majority of these isolates were obtained from chicken meat (both from imported meat and fresh retail meat). In chicken meat *S.* Heidelberg (N=72) (all from imported meat) was the most predominant isolate followed by *S.* Schwarzengrund (N = 8) and *S.* Infantis (N = 7). The high proportion of resistance to fluoroquinolones in poultry meat reflects the frequent usage of fluoroquinolones in the international poultry production chain.

### ESBL's in *Salmonella*

The emergence of multidrug resistant *Salmonella* strains with resistance to fluoroquinolones and third-generation cephalosporins is a serious development, which results in severe limitations for effective treatment of human infections (WHO, factsheet 139, 2005). The total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates in 2017 was 31/1697 (1.8%), among seven different serovars, all isolated from human samples: predominantly *S.* Kentucky (N = 18) and, *S.* Typhimurium (N = 8). The other serovars were *S.* Infantis (N = 2), monophasic *S.* Typhimurium (N = 1), S. Agona (N=1) and *S.* Virchow (N=1).
In isolates from imported meat samples (from outside EU) the proportion of cefotaxime resistance in imported chicken meat was high with 67.6% (Table S06). The serovars were *S.* Heidelberg (N = 65), *S.* Minnesota (N = 2) and *S.* Schwarzengrund (N = 1). No cefotaxime resistance was detected in samples from fresh retail chicken meat, or other fresh meat products.

**Table S02** MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N=1697) tested for antibiotic susceptibility during 2017.

| *Salmonella* | MIC (%) distribution mg/L | | | | | | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N = 1697 | 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 | | | | | | | 37.9 | 32.2 | 1.8 | 0.1 | | | 0.1 | 27.9 | | | | | 27.9 | 25.8 - 30.1 |
| Cefotaxime | | | | | 97.1 | 1.0 | 0.1 | | | 1.8 | | | | | | | | | 1.8 | 1.2 - 2.6 |
| Ceftazidime | | | | | | 95.1 | 3.2 | 0.2 | 0.5 | 0.5 | 0.5 | | | | | | | | 1.4 | 0.9 - 2.1 |
| Gentamicin | | | | | | 85.9 | 9.8 | 0.9 | 0.4 | 0.1 | 1.2 | 0.9 | 0.8 | | | | | | 3.4 | 2.6 - 4.4 |
| Tetracycline | | | | | | | | 71.9 | 2.5 | 0.4 | 0.1 | 0.9 | 2.2 | 22.0 | | | | | 25.2 | 23.1 - 27.3 |
| Sulfamethoxazole | | | | | | | | | | 45.1 | 24.6 | 3.5 | 0.5 | 0.1 | | | 0.1 | 26.2 | 26.2 | 24.1 - 28.4 |
| Trimethoprim | | | | | 68.4 | 23.0 | 1.0 | 0.3 | 0.1 | | | | 7.3 | | | | | | 7.4 | 6.2 - 8.7 |
| Ciprofloxacin | 15.1 | 69.0 | 2.1 | 0.8 | 4.7 | 4.0 | 1.4 | 0.3 | | 0.6 | 1.9 | | | | | | | | 13.8 | 12.2 - 15.6 |
| Nalidixic acid | | | | | | | | | 79.8 | 6.4 | 2.1 | 1.7 | 0.1 | 0.2 | 9.7 | | | | 11.7 | 10.2 - 13.3 |
| Chloramphenicol | | | | | | | | | | 87.5 | 6.2 | 0.4 | 0.2 | 0.6 | 5.2 | | | | 6.3 | 5.2 - 7.6 |
| Azitromycin* | | | | | | | | 0.1 | 24.3 | 69.5 | 5.2 | 0.3 | 0.2 | 0.5 | | | | | 1.0 | 0.6 - 1.6 |
| Colistin** | | | | | | | 79.4 | 13.1 | 7.1 | 0.4 | | | | | | | | | - | - |
| Meropenem | | 86.3 | 13.6 | 0.1 | | | | | | | | | | | | | | | 0.0 | 0 - 0.2 |
| Tigecyclin | | | | | 55.2 | 37.5 | 6.1 | 1.2 | 0.1 | | | | | | | | | | 1.3 | 0.8 - 2.0 |

*The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (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*

**Table S03** Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2017 (N tested).

| | Enteritidis (326) | Typhimurium (272) | 1,4,[5],12:i:- (235) | Infantis (65) | Kentucky (48) | Bovismorbificans (34) | Kedougou (32) | Montevideo (31) | Dublin (26) | Livingstone (26) | Newport (25) | Paratyphi B var Java (25) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ampicillin | 8.6 | 60.3 | 90.6 | 4.6 | 79.2 | 0.0 | 0.0 | 0.0 | 11.5 | 0.0 | 0.0 | 0.0 |
| Cefotaxime | 0.0 | 2.9 | 0.4 | 4.6 | 37.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ceftazidime | 0.0 | 0.7 | 0.4 | 1.5 | 37.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gentamicin | 0.3 | 5.1 | 2.1 | 1.5 | 64.6 | 0.0 | 0.0 | 0.0 | 7.7 | 0.0 | 0.0 | 0.0 |
| Tetracycline | 2.1 | 43.0 | 88.9 | 20.0 | 70.8 | 2.9 | 0.0 | 0.0 | 15.4 | 0.0 | 12.0 | 0.0 |
| Sulfamethoxazole | 2.1 | 46.7 | 88.9 | 26.2 | 68.8 | 2.9 | 0.0 | 3.2 | 19.2 | 7.7 | 12.0 | 8.0 |
| Trimethoprim | 0.0 | 20.2 | 6.4 | 15.4 | 6.3 | 0.0 | 0.0 | 3.2 | 11.5 | 7.7 | 12.0 | 16.0 |
| Ciprofloxacin | 21.5 | 6.6 | 4.3 | 26.2 | 81.3 | 0.0 | 0.0 | 6.5 | 3.8 | 0.0 | 12.0 | 8.0 |
| Nalidixic acid | 21.2 | 2.2 | 2.6 | 26.2 | 81.3 | 0.0 | 0.0 | 3.2 | 3.8 | 0.0 | 4.0 | 8.0 |
| Chloramphenicol | 0.0 | 22.8 | 8.9 | 4.6 | 6.3 | 0.0 | 0.0 | 0.0 | 15.4 | 0.0 | 8.0 | 0.0 |
| Azithromycin | 0.6 | 0.7 | 3.0 | 0.0 | 2.1 | 0.0 | 0.0 | 0.0 | 7.7 | 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 |
| Tigecycline | 0.0 | 3.3 | 0.4 | 6.2 | 12.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

### *S.* Typhimurium

S. Typhimurium represented 15.9% (197/1242) of all human *Salmonella* isolates as characterized by RIVM in 2017 (Table S01). This is less than in 2015 and 2016 (19.4% and 17.0% respectively), and approximately the same as in 2014 (16.2%). *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 isolates and also for trimethoprim in pig isolates and isolates from other sources (including broilers, sheep, goats, food and feed). Resistance to chloramphenicol was also found in isolates from humans, pigs and other sources, at a somewhat lower level. About 20% 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 similar to the proportion in previous years (except for 2015). Resistance to the clinically important drug cefotaxime was only detected in human isolates at a low level (3.9%). Resistance to fluoroquinolones was present in isolates from humans (7.8%), but was much less frequently found than in 2016 (19.2%). In cattle and pig isolates no resistance to fluoroquinolones was measured in 2017. After an increased finding of fluoroquinolone resistance in

S. Typhimurium isolates in 2016, proportions of resistance in 2017 were at the same level as in 2015. Borderline resistance to tigecycline was rarely observed in human isolates (N = 3), goats (N=3), sheep (N = 1) and pigs (N = 1). These isolates all exhibit slightly elevated MIC-values caused by an unknown resistance mechanism (if any).

**Table S04** Resistance percentages of S. Typhimurium (N tested) isolated from humans, cattle, pigs and other sources in 2017.

| | *S.* Typhimurium (272)[a] | | | |
|---|---|---|---|---|
| | Humans (206) | Cattle (16) | Pigs (18) | Other sources (33)[b] |
| Ampicillin | 54.6 | 62.5 | 83.3 | 81.8 |
| Cefotaxime | 3.9 | 0.0 | 0.0 | 0.0 |
| Ceftazidime | 1.0 | 0.0 | 0.0 | 0.0 |
| Gentamicin | 3.9 | 37.5 | 0.0 | 0.0 |
| Tetracycline | 33.7 | 81.3 | 61.1 | 72.7 |
| Sulfamethoxazole | 38.0 | 81.3 | 61.1 | 75.8 |
| Trimethoprim | 13.7 | 25.0 | 38.9 | 48.5 |
| Ciprofloxacin | 7.8 | 0.0 | 0.0 | 6.1 |
| Nalidixic acid | 2.4 | 0.0 | 0.0 | 3.0 |
| Chloramphenicol | 21.0 | 56.3 | 16.7 | 21.2 |
| Azithromycin | 1.0 | 0.0 | 0.0 | 0.0 |
| Meropenem | 0.0 | 0.0 | 0.0 | 0.0 |
| Tigecycline | 1.5 | 0.0 | 5.6 | 15.2 |

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

*b. including broilers, sheep, goats, food and feed products.*

Resistance proportions in S. Typhimurium isolates from human samples showed an increasing tendency until 2010, after which resistance showed a tendency to decrease until 2015, with a slight increase for some antimicrobials in 2014, and an increase for most antimicrobials in 2016. Resistance proportions for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated from 2014 to 2016 (Figure S01). In 2017, resistance proportions for most antimicrobials were a bit lower, compared to 2016, except for ampicillin, trimethoprim, gentamicin and cefotaxime.

Resistance proportions in S. Typhimurium isolates from animal samples (cattle and pigs shown in figure S01) vary considerably over the years. This seemed to decrease from 2013, but an increase was seen in 2016. In 2017, resistance for almost all antimicrobials decreased in the isolates from pigs and increased in the isolates from cattle. However, these figures should be interpreted with care, because of the relatively small number of isolates per year.

## 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. The four dominant MLVA-types (03-10-05-04-01, 03-11-05-04-01, 03-09-05-04-01 and 02-10-07-03-02) were found in isolates from humans and broilers and were similar to the most predominant MLVA types in 2013 to 2016. In 2017, the most predominant (N = 77) S. Enteritidis again was MLVA type (02-09-07-03-02) part of the outbreak associated with the consumption of Polish eggs in 2016.

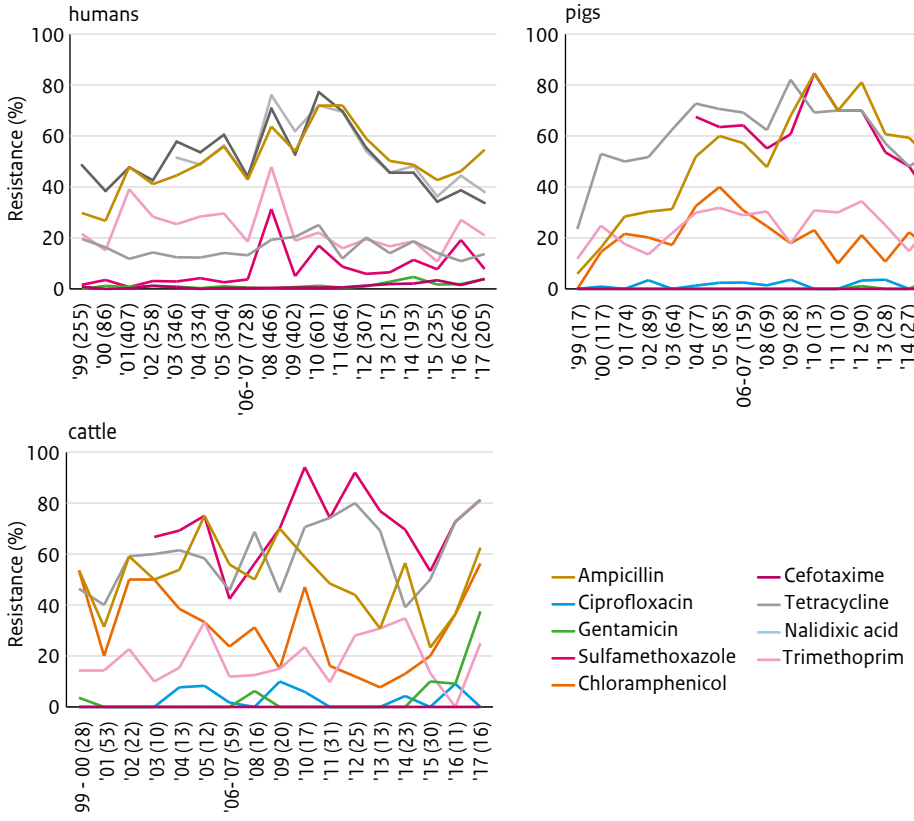
Compared to many other *Salmonella* serovars, resistance in S. Enteritidis is relatively low (Table S03). Table S05 presents resistance proportions in S. Enteritidis isolates from human samples and broilers. In 2017 no isolates from laying hens were tested, so we cannot compare with previous years.

The resistance percentage for fluoroquinolones in human isolates was 22.8%. For ampicillin a resistance rate of 9.1% was found. For all other antimicrobials resistance proportions of human S. Enteritidis isolates was very low or not detected. All isolates (N = 18) from broilers were fully susceptible. The trends in resistance of S. Enteritidis over the years in human isolates are summarized in Figure S02. Resistance in human isolates for chloramphenicol and ciprofloxacin increased, compared to 2016. In general, resistance proportions in human isolates seem to be very stable over years, with an increasing trend for ciprofloxacin resistance since 2010.

**Table S05** Resistance percentages of S. Enteritidis (N tested) isolated from humans and broilers in 2017.

| | S. Enteritidis (325) | |
|---|---|---|
| | Humans (307) | Broilers (18) |
| Ampicillin | 9.1 | 0.0 |
| Cefotaxime | 0.0 | 0.0 |
| Ceftazidime | 0.0 | 0.0 |
| Gentamicin | 0.3 | 0.0 |
| Tetracycline | 2.3 | 0.0 |
| Sulfamethoxazole | 2.0 | 0.0 |
| Trimethoprim | 0.0 | 0.0 |
| Ciprofloxacin | 22.8 | 0.0 |
| Nalidixic acid | 22.5 | 0.0 |
| Chloramphenicol | 0.0 | 0.0 |
| Azithromycin | 0.7 | 0.0 |
| Meropenem | 0.0 | 0.0 |
| Tigecycline | 0.0 | 0.0 |

**Figure S01** Trends in resistance (%) of S. Typhimurium isolated from humans and food-animals in 1999 - 2017.




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

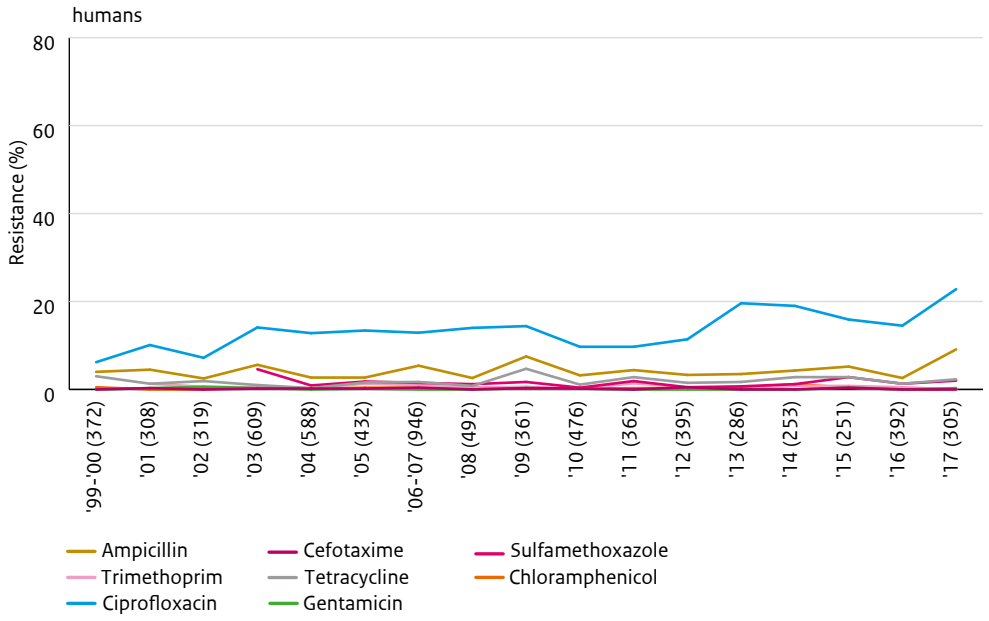
Since 2016, S. Java was not the most predominant serovar isolated in broiler production anymore, as it was in the period before 2015. Figure S03 shows resistance proportions of human and poultry isolates of S. Java. Since 2012, the resistance proportions seem to fluctuate, and a real increasing or decreasing trend cannot be seen. Resistance to trimethoprim was 100%, like in former years. Like in 2016, resistance to chloramphenicol was not detected. The resistance level for ciprofloxacin further increased to 58.8% in 2017.

All 20 tested S. Java strains, isolated from human infections, were trimethoprim susceptible and therefore not considered to be related to the clone spreading in Dutch poultry.

### *Salmonella* from chicken meat, other meat sources and herbs and spices

Table S06 and Figure S04 show resistance data of *Salmonella* isolates from raw meat (chicken and other), herbs and spices (due to oversampling S. Heidelberg was not included in the analysis in Figure S04). S. Heidelberg (54%) was the most abundant serovar found in imported chicken meat in 2017, followed by S. Paratyphi B variation Java (11%) and S. Infantis (8%). Isolates from other meat samples were resistant against a fewer number of antimicrobials and at lower levels than isolates from chicken meat. Resistance proportions for the quinolones (ciprofloxacin and nalidixic acid) were very high in isolates from chicken meat, especially in imported meat (89.2% and 86.3% respectively); resistance proportions in isolates from other meat samples were a bit lower than in 2016, but the number of isolates was low. Borderline resistance to tigecycline was observed in one S. Infantis isolate (6,3%) from retail meat and in eighteen S. Heidelberg (17,6%) and one S. Schwarzengrund isolate (1,0%) all from imported chicken meat samples. Like in S. Typhimurium, MIC-values were slightly elevated. Tigecycline resistance was not detected in samples from other meat. Resistance to cephalosporins (cefotaxime and ceftazidime) was not detected in chicken meat samples from retail and other meat samples, but was very frequently found in isolates from imported chicken meat samples (67.6% for both drugs).
Only 8 strains were isolated from "other products" (herbs, spices, sea food). Resistance proportions in Table S06 are therefore not representative for those products in general. Resistance to quinolones (ciprofloxacin and nalidixic acid), ampicillin and tetracycline were 12.5% (1 of 8 isolates). For the other antimicrobials no resistance was detected in the isolates from other products.

**Figure S02** Trends in resistance (%) of S. Enteritidis isolated from humans from 1999 - 2017.



The overall resistance proportions of *Salmonella* from poultry products over the years are shown in Figure S04. Resistances fluctuate since 2001, with an increasing trend for ciprofloxacin; the resistance proportion for tetracycline also increased since 2001, but is decreasing since 2015. 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. The increase in 2014/2015 could reflect the relatively high proportion of strains from imported poultry products included. It should be noticed that the fluctuating resistance proportions during the years, could be influenced by the varying proportions of imported products sampled per year.

**Figure S03** Trends in resistance (%) of S. Paratyphi variant Java isolated in humans and broilers from 1999 - 2017.

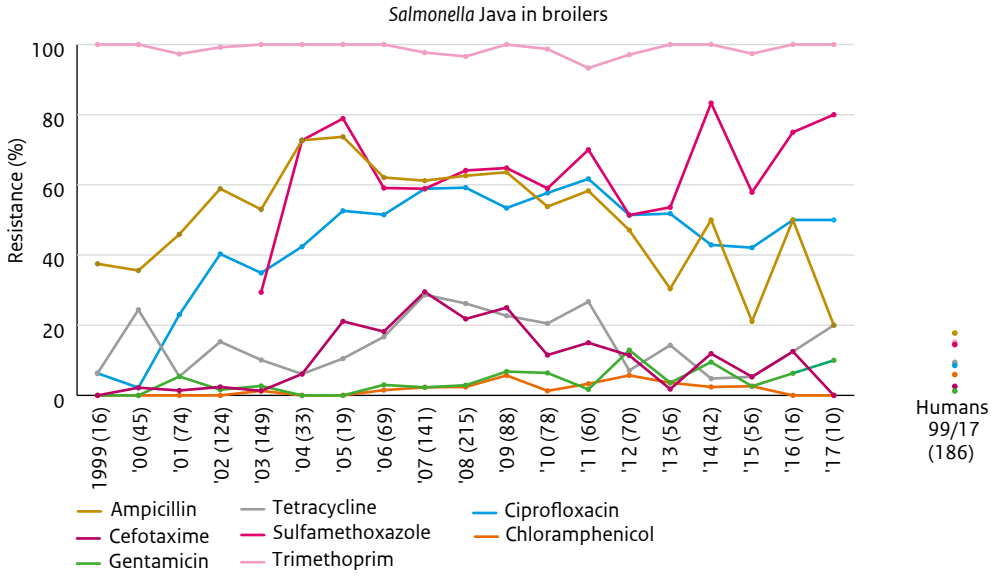


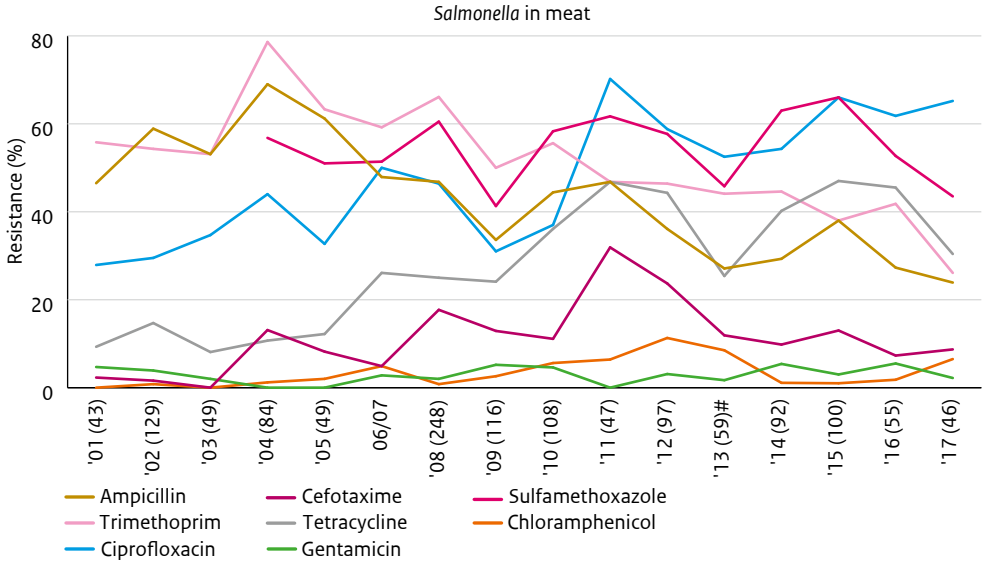
**Table S06** Resistance (%) of *Salmonella* enterica isolated from different types of raw meat, herbs, spices and seafood in the Netherlands in 2017.

| | Chicken Retail N = 16 | Chicken Imported N = 102 | Other meat[a] N = 17 | Other products[b] N = 8 |
|---|---|---|---|---|
| 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 = 4) , beef (n = 3), lamb (n = 3), turkey (n = 2), frog (n = 4) and cangaroo (n = 1).*

*b. Other products includes spices and herbs (n = 6) and seafood (n = 2).*

**Figure S04** Trends in resistance (%) of *Salmonella* enterica isolated from poultry meats in the Netherlands from 2001-2017.




*# Due to an oversampling, S. Heidelberg was excluded from the analysis in 2013 (see Nethmap/MARAN2014).*

## 3.1.2 Campylobacter

This chapter describes the occurrence and trends in antimicrobial resistance in *Campylobacter jejuni* and *C. coli*. Isolates were sampled 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 products). In addition to broilers, in 2017 also *C. jejuni* isolates from faecal samples collected at beef cattle farms, and *C. coli* isolates from pig caecal samples were tested for resistance.

The MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated at WBVR from caecal samples of broilers and pigs in 2017 are presented in table C01. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers, cattle, pigs and poultry meat are shown in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products over the last 12 to 16 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 2007 onwards 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 did not substantially change in 2017, compared to 2016.
2. In *C. jejuni* from faecal samples of beef cattle and *C. coli* from caecal samples of pigs, proportions of resistance were lower.
3. Resistance to macrolides was rarely detected in isolates from livestock and humans and almost exclusively found in *C. coli* isolates from broilers and pigs.
4. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
5. In *C. jejuni* from cattle, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry.
6. In. *C. coli* from pigs, high resistance levels were found for streptomycin and tetracycline.
7. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with an increase in 2017), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.
8. 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.

In 2017, the highest proportions of resistant *C. jejuni* and *C. coli* from broilers and pigs were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid, and in *C. coli* isolates from pigs also for streptomycin (Table C01). Table C02 shows that resistance percentages were high in isolates from broilers and poultry meat (both *C. jejuni* and *C. coli*), and lower for *C. jejuni* isolates from beef cattle and *C. coli* isolates from pigs.

In recent years, in broilers and poultry meat *C. jejuni* resistant to erythromycin, streptomycin and gentamicin were only incidentally found. In 2017, resistance to these antimicrobials could not be detected in isolates from broilers, as was the case for gentamicin in poultry meat. Resistance to tetracycline was more frequently detected in both poultry meat and broilers in 2017 (59.2% in broilers and 55.8% in poultry meat). Resistance to ciprofloxacin showed fluctuation over the years and was over 60% since 2014 for broilers and poultry meat (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 affected by the lower number of isolates in the survey (Figure C02). Resistance in *C. coli* from broilers and poultry meat could not be detected for gentamicin. However, resistance in *C. coli* was low for erythromycin and streptomycin in 2016, but was more frequently found in 2017 for both broilers and poultry meat. Resistance percentages for ciprofloxacin in broilers have been fluctuating since 2001, but increased to a very high level (94.4%) in 2017. However, because of the low number of *C. coli* isolates tested in 2016 (N = 23) and 2017 (N = 36) these results might not be very representative. Resistance to tetracycline in broilers seem to follow the same trend as ciprofloxacin resistance, at approximately equal percentages (Figure C02); in poultry meat the resistance level for tetracycline increased to 69.4% in 2017.

Overall, 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. Resistance against streptomycin and erythromycin was at low levels for the *C. jejuni* isolates from broilers, poultry meat and beef cattle. A high resistance rate against streptomycin was detected in *C. coli* isolates from pigs (73.5%). Lower levels of resistance against streptomycin were detected in *C. coli* isolates from broilers (8.0%) and poultry meat (11.1%).

**Table C01** MIC distribution (in %) for *Campylobacter jejuni* (N = 157) and *C. coli* (N = 50) isolated from caecal samples of broilers and *C. coli* (N= 83) from pigs in 2017.

| *C. jejuni*, *broilers* | MIC (%) distribution mg/L | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (N = 157 ) | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | | |
| Ciprofloxacin | 29.9 | 2.5 | 0.0 | 0.0 | 0.0 | 1.9 | 31.8 | 26.8 | 7.0 | | | | 67.5 | 59.6 - 74.7 |
| Nalidixic acid | | | | 0.0 | 4.5 | 29.3 | 0.0 | 0.0 | 0.0 | 0.0 | 66.2 | | 66.2 | 58.3 - 73.6 |
| Erythromycin | | | | 68.8 | 29.3 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 - 2.3 |
| Gentamicin | 68.2 | 31.2 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | 0.0 | 0 - 2.3 |
| Streptomycin | | 3.8 | 54.8 | 40.8 | 0.6 | 0.0 | 0.0 | 0.0 | | | | | 0.0 | 0 - 2.3 |
| Tetracycline | | | 37.6 | 3.2 | 0.0 | 0.0 | 0.0 | 5.1 | 2.5 | 7.6 | 43.9 | | 59.2 | 51.1 - 67.0 |

| *C. coli*, *broilers* | MIC (%) distribution mg/L | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (N = 50 ) | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | | |
| Ciprofloxacin | 16,0 | 6,0 | 0,0 | 0,0 | 0,0 | 22,0 | 36,0 | 20,0 | 0,0 | | | | 78,0 | 64,0 - 88,5 |
| Nalidixic acid | | | | 0,0 | 0,0 | 14,0 | 8,0 | 0,0 | 0,0 | 0,0 | 78,0 | | 78,0 | 64,0 - 88,5 |
| Erythromycin | | | | 66,0 | 16,0 | 2,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 16,0 | 16,0 | 7,2 - 29,1 |
| Gentamicin | 4,0 | 76,0 | 20,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | | | | | 0,0 | 0 - 7,1 |
| Streptomycin | | 0,0 | 2,0 | 78,0 | 12,0 | 0,0 | 0,0 | 2,0 | 6,0 | | | | 8,0 | 2,2 - 19,2 |
| Tetracycline | | | 16,0 | 4,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 80,0 | | 80,0 | 66,3 - 90,0 |

| *C. coli*, *pigs* | MIC (%) distribution mg/L | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (N = 83 ) | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | | |
| Ciprofloxacin | 74,7 | 9,6 | 0,0 | 0,0 | 0,0 | 1,2 | 6,0 | 4,8 | 3,6 | | | | 15,7 | 8,6 - 25,3 |
| Nalidixic acid | | | | 0,0 | 0,0 | 47,0 | 30,1 | 7,2 | 0,0 | 1,2 | 14,5 | | 15,7 | 8,6 - 25,3 |
| Erythromycin | | | | 51,8 | 36,1 | 6,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 6,0 | 6,0 | 2,0 - 13,5 |
| Gentamicin | 6,0 | 62,7 | 31,3 | | | | | | | | | | 0,0 | 0 - 4,4 |
| Streptomycin | | 0,0 | | 13,3 | 12,0 | 1,2 | 1,2 | 24,1 | 48,2 | | | | 73,5 | 62,7 - 82,6 |
| Tetracycline | | | 7,2 | 2,4 | 2,4 | 1,2 | 2,4 | 1,2 | 1,2 | 7,2 | 74,7 | | 88,0 | 79,0 - 94,1 |

**Table C02** Resistance percentages of C. *jejuni* and C. *coli* isolated from faecal samples of broilers, cattle and pigs and from poultry meat in 2017.

| | *C. jejuni* | | | *C. coli* | | |
|---|---|---|---|---|---|---|
| | Broilers | Poultry meat | Cattle | Broilers | Poultry meat | Pigs |
| N | 157 | 77 | 90 | 50 | 36 | 83 |
| Ciprofloxacin | 67,5 | 64,9 | 23,3 | 78,0 | 94,4 | 15,7 |
| Nalidixic acid | 66,2 | 62,3 | 25,6 | 78,0 | 94,4 | 15,7 |
| Erythromycin | 0,0 | 0,0 | 1,1 | 16,0 | 16,7 | 6,0 |
| Gentamicin | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Streptomycin | 0,0 | 2,6 | 1,1 | 8,0 | 11,1 | 73,5 |
| Tetracycline | 59,2 | 55,8 | 17,8 | 80,0 | 69,4 | 88,0 |

### Quinolones

The high proportion of *Campylobacter* spp. isolates from animal origin resistant to the quinolones (Figures C01 and C02) and especially from human patients (Figure C03) is a public health concern.
The proportion of *C. jejuni* isolates from broilers resistant to quinolones remains at a continuous high level in the last decade with 67.5% in 2017. The proportion of quinolone resistance in *C. jejuni* from poultry meat is comparably high with 64.9% in 2017. Ciprofloxacin resistance proportions in *C. jejuni* isolates from beef cattle and from *C. coli* isolates from pigs were at medium levels in 2017 (23.3% and 15.7% respectively). For *C. coli* from pigs resistance was higher than in 2013 (6.1%).
A continuation of high levels of ciprofloxacin resistance were also observed in the *C. coli* isolates from broilers with 78% in 2017. 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. Nevertheless, the proportion of resistance to quinolones was remarkably high in 2017: 94.4% for both ciprofloxacin and nalidixic acid. The resistance levels for fluoroquinolone in human campylobacter isolates were also high (61.6%), which was approximately at the same level as in the years 2014-2016 (58.3 - 61.4%).

### 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 was low: it could not be detected in *C. jejuni* from caecal samples of broilers in 2017, and 2.0% of human isolates from 2013-2017 were classified as resistant. 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*).
Like the years before, also in 2017, erythromycin resistance was very rare in *C. jejuni* isolates, with no resistance in broilers and poultry meat, and in one erythromycin resistant *C. jejuni* isolate (1.1%) isolated in a faecal sample from a beef cattle farm (Table C02). Erythromycin resistance was detected in *C. coli* from caecal samples of broilers (16.0%) and in poultry meat (16.7%) and in slaughter pigs (6%).

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

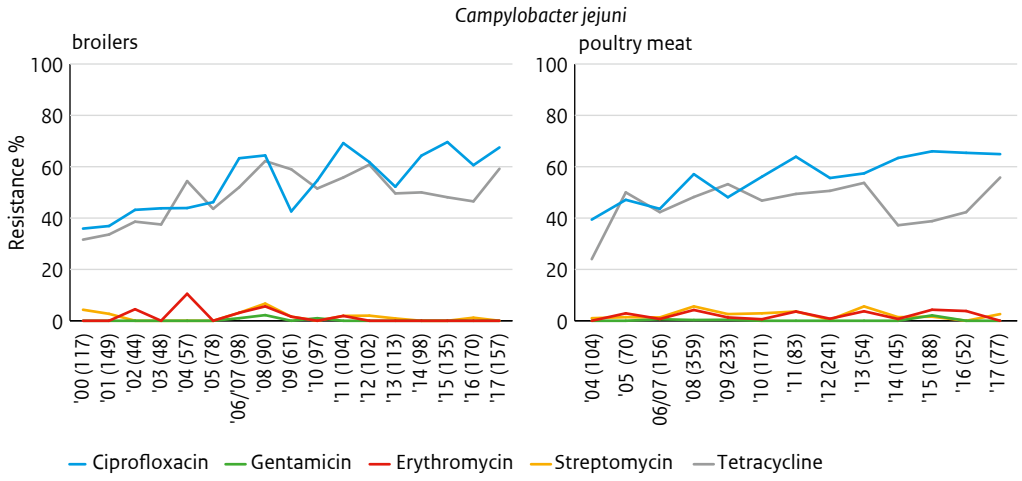



**Figure C02** Trends in resistance (%) of *Campylobacter* coli isolated from broilers and poultry meat in the Netherlands.

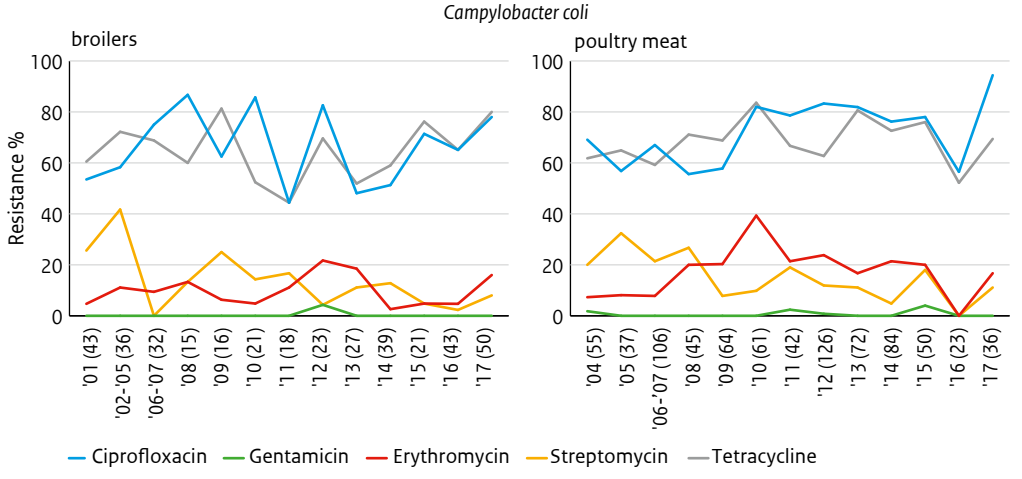


**Table C03** Domestically acquired and travel related resistance in C. *jejuni* and *C. coli* isolated from humans from 2007 - 2017 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

| | 2007-2012 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Domestically acquired | | | | Travel related | | | |
| | *C. jejuni* | | *C. coli* | | *C. jejuni* | | *C. coli* | |
| | N | R% | N | R% | N | R% | N | R% |
| Fluoroquinolone | 18374 | 51.3 | 1340 | 51.5 | 958 | 65.4 | 101 | 60.4 |
| Tetracycline | 10875 | 21.9 | 897 | 31.3 | 290 | 31.7 | 49 | 28.6 |
| Erythromycin | 15261 | 2.2 | 1131 | 7.5 | 726 | 3.9 | 80 | 12.5 |

| | 2013-2017 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Domestically acquired | | | | Travel related | | | |
| | *C. jejuni* | | *C. coli* | | *C. jejuni* | | *C. coli* | |
| | N | R% | N | R% | N | R% | N | R% |
| Fluoroquinolone | 13673 | 58.7 | 970 | 63.1 | 952 | 76.7 | 111 | 75.7 |
| Tetracycline | 9259 | 41.0 | 625 | 62.2 | 584 | 58.4 | 64 | 65.6 |
| Erythromycin | 11956 | 2.0 | 802 | 13.7 | 858 | 3.7 | 102 | 32.4 |

| | *Campylobacter* spp. (R%) | | | | | |
|---|---|---|---|---|---|---|
| | 2017 | 2016 | 2015 | 2014 | 2013 | 2007/12 |
| Fluoroquinolone | 61.6 | 58.3 | 61.4 | 60.6 | 57.6 | 51.9 |
| Tetracycline | 47.6 | 42.0 | 42.3 | 43.9 | 38.5 | 30.0 |
| Erythromycin | 3.5 | 2.6 | 2.9 | 3.2 | 3.2 | 2.7 |

### 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 2017 no samples were collected from laying hens, ducks and turkey meat.

Table Co2 shows that 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 were also high for tetracycline and quinolones. Resistance to gentamicin was not observed in *C. jejuni* and *C. coli* isolates. Resistance to erythromycin and streptomycin was rarely observed in *C. jejuni* and more frequently found in *C. coli*. Moreover, streptomycin resistance was commonly detected in *C. coli* isolates from pigs (73.5%).

In general, higher resistance rates were observed for most 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.

### Cattle and pigs

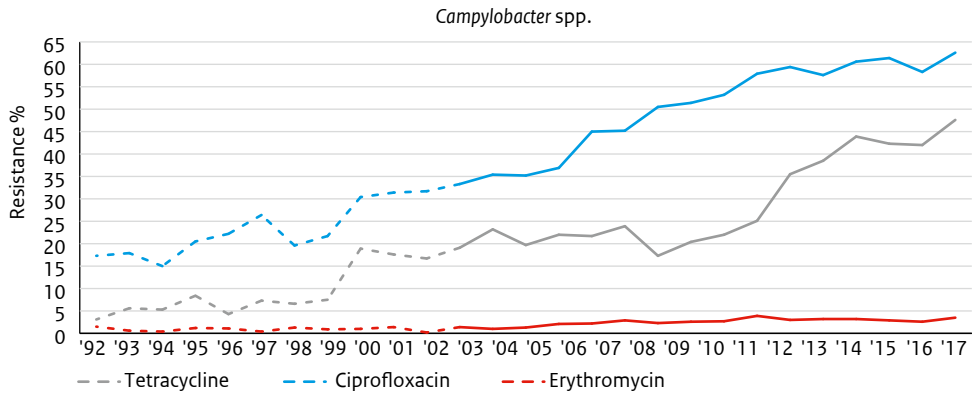
In 2017 *C. jejuni* isolates from beef cattle and *C. coli* isolates from pigs were tested for antimicrobial resistance. Like in the other animal species, resistance proportions were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels in the cattle samples (23.3% for ciprofloxacin, 25.6% for nalidixic acid and 17.8% for tetracycline). Resistance against ciprofloxacin and nalidixic acid were also at lower levels in pig samples (for both 15.7%), but at a high level for tetracycline (88.0%) (Table C02). A high resistance rate (73.5%) was also detected in *C. coli* isolates from pigs.

### Campylobacter in humans

Data on resistance levels are available for ciprofloxacin, tetracycline and erythromycin are summarized in Table C03 and Figure C03. Figure C03 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 again an increase in 2017. Resistance to erythromycin stabilized around 3% since 2011.

Table C03 shows resistance levels for *Campylobacter* spp. isolates, specified according to the most probable infection route, i.e. 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.

**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.



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

**Highlights**

1. Proportions of resistance to ampicillin, sulfamethoxazole and trimethoprim in human STEC O157 isolates were somewhat higher in 2017, compared to 2016 (10.7% to 16.1% for ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). There is an increasing tendency for resistance against these antimicrobials since 2009.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was detected in 3.2% of human STEC O157 isolates.
3. For the first time since seven years one cefotaxime resistant, ESBL-producing isolate was detected.

Shiga-toxin producing *E. coli* O157 (STEC O157) isolates from humans (N = 62) were tested for susceptibility. Table STEC01 shows the MIC results for all *E. coli* O157 isolates from humans; Figure STEC01 presents the trends over time.

**Human STEC O157 isolates**

Resistance proportions of human isolates showed a tendency to increase for ampicillin, tetracycline and trimethoprim since approximately 2009, whereas resistance against sulfamethoxazole was high since 2009, 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 ampicilline, from 14.7% to 16.1% for sulfamethoxazole, and from 8.0% to 14.5% for trimethoprim). Resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016, but was 3.2% for both antimicrobials in 2017. For the first time since seven years, a cefotaxime resistant, ESBL-producing isolate was detected harbouring a $bla_{\text{CTX-M-15}}$ gene.

**Figure STEC01** Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2017.

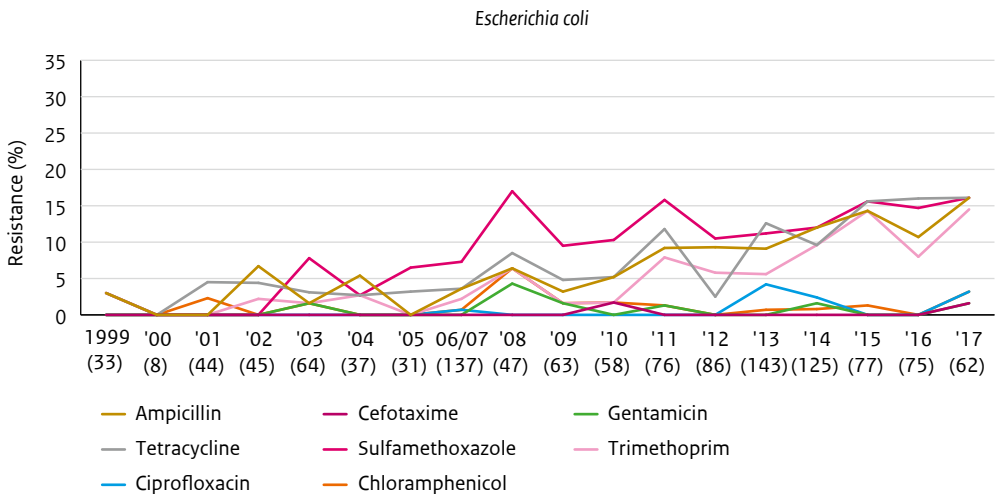


**Table STEC01** MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=62) isolated from humans the Netherlands in 2017.

| *E. coli* | MIC (%) distribution mg/L | | | | | | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N = 62 | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2048 | |
| Ampicillin | | | | | | | | 1.6 | 77.4 | | | | | 16.1 | | | | | 16.1 | 8.0 - 27.7 |
| Cefotaxime | | | | | 98.4 | | | | | 1.6 | | | | | | | | | 1.6 | 0 - 8.7 |
| Ceftazidime | | | | | | 98.4 | | | | 1.6 | | | | | | | | | 1.6 | 0 - 8.7 |
| Gentamicin | | | | | | 75.8 | 22.6 | | | | | 1.6 | | | | | | | 1.6 | 0 - 8.7 |
| Tetracycline | | | | | | | | 32.3 | 51.6 | | | | 3.2 | 12.9 | | | | | 16.1 | 8.0 - 27.7 |
| Sulfamethoxazole | | | | | | | | | | 83.9 | | | | | | | | 16.1 | 16.1 | 8.0 - 27.7 |
| Trimethoprim | | | | | 85.5 | | | | | | | | 14.5 | | | | | | 14.5 | 6.9 - 25.8 |
| Ciprofloxacin | 45.2 | 51.6 | | | | | 1.6 | | 1.6 | | | | | | | | | | 3.2 | 0.4 - 11.2 |
| Nalidixic acid | | | | | | | | | 91.9 | 4.8 | | | | | 3.2 | | | | 3.2 | 0.4 - 11.2 |
| Chloramphenicol | | | | | | | | | | 83.9 | 12.9 | | | | 3.2 | | | | 3.2 | 0.4 - 11.2 |
| Azithromycin* | | | | | | | | | 83.9 | 16.1 | | | | | | | | | 0.0 | 0 - 5.8 |
| Colistin | | | | | | | 100 | | | | | | | | | | | | 0.0 | 0 - 5.8 |
| Meropenem | | 98.4 | 1.6 | | | | | | | | | | | | | | | | 0.0 | 0 - 5.8 |
| Tigecycline | | | | | 88.7 | 11.3 | | | | | | | | | | | | | 0.0 | 0 - 5.8 |

*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 selection pressure as a result of the use of antibiotics in animals, is reflected in the level of antimicrobial resistance in bacteria inhabiting the intestinal tract, especially over time. For this purpose, *E. coli* is included as indicator organisms for the Gram-negative flora. As a result of less priority for including enterococci in the surveillance, no enterococci were tested in 2017.

Isolation of bacteria from the intestine of randomly picked food-producing animals at slaughter aims to detect the occurrence and trends in resistance at the bacterial population level in food animals as prescribed by EFSA [1].
This monitoring is conducted in slaughter pigs and broilers since 1998. Resistance in isolates from both dairy cattle, veal calves and meat samples have been included from 2005 onwards. 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. Monitoring programs in veal calves at farms stopped in 2012. From then, samples from individual veal calves were collected 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 relatively small numbers 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*

This chapter presents information 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.
In 2014, the EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented. This includes susceptibility testing 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).

**Highlights 2017**

1. In 2017, resistance proportions of indicator *E. coli* in caecal samples showed a tendency to decrease in broilers, to stabilize in pigs, and showed a slight increase in veal calves. In dairy cattle the resistance proportions remained at a constant low level.
2. As in former years, resistance proportions in *E. coli* from chicken and turkey meat, were substantially higher than in pork and beef
3. The proportion of *E. coli* isolates resistant to third-generation cephalosporins was low in faecal samples from broilers and pigs and not detected in dairy cattle and veal calves.
4. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
5. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still high in broilers, pigs, veal calves and chicken and turkey meat.
6. Levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

**Resistance levels**

Table Eco01 shows resistance levels of a total of 1194 *E. coli* isolates obtained in caecal samples from broilers, pigs, veal calves and faecal samples of dairy cows , presented as MIC-distributions. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1999 to 2017 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02. Resistance percentages of 452 *E. coli* isolates collected from raw chicken and turkey meat, pork, beef and veal products are presented in Table Eco03. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2017 isolated from raw meat products of poultry, turkey, pork and beef.

For most drugs or drug classes there were notable variations in resistance levels between the different animal species (Table Eco02). Like the years before, highest 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.
Overall, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used in veterinary medicine in The Netherlands.

**Table Eco01** MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1194) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2017.

| *E. coli* | MIC (%) distribution mg/L | | | | | | | | | | | | | | | | | R% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N = 1194 | 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 | | | | | | | 1.9 | 28.5 | 44.3 | 4.4 | 0.2 | | | 20.8 | | | | | 20.9 | 18.7- 23.4 |
| Cefotaxime | | | | | 99.5 | 0.1 | | | | 0.4 | | | | | | | | | 0.5 | 0.2 - 1.1 |
| Ceftazidime | | | | | | 99.5 | 0.1 | 0.2 | | 0.1 | 0.2 | | | | | | | | 0.5 | 0.2 - 1.1 |
| Gentamicin | | | | | | 60.3 | 31.1 | 6.2 | 0.3 | 0.3 | 0.8 | 0.5 | 0.7 | | | | | | 2.4 | 1.6 - 3.5 |
| Tetracycline | | | | | | | | 58.4 | 11.7 | 0.3 | 0.3 | 0.9 | 9.5 | 18.8 | | | | | 29.6 | 27.0 - 32.2 |
| Sulfamethoxazole | | | | | | | | | | 74.5 | 0.2 | | | 0.1 | 0.1 | 0.3 | | 24.9 | 25.4 | 22.9 - 28.0 |
| Trimethoprim | | | | | 29.8 | 46.8 | 3.1 | 0.2 | | | | | 20.2 | | | | | | 20.1 | 17.9 - 22.5 |
| Ciprofloxacin | 73.5 | 16.4 | 0.0 | 0.6 | 5.5 | 2.3 | 0.6 | 0.3 | 0.2 | 0.3 | 0.3 | | | | | | | | 10.1 | 8.4 - 11.9 |
| Nalidixic acid | | | | | | | | | 89.4 | 1.1 | 0.5 | 0.2 | 0.7 | 4.5 | 3.6 | | | | 9.0 | 7.4 - 10.7 |
| Chloramphenicol | | | | | | | | | | 85.8 | 5.2 | 1.2 | 0.6 | 3.3 | 3.9 | | | | 9.0 | 7.4 - 10.7 |
| Azithromycin* | | | | | | | | 2.3 | 37.1 | 56.4 | 3.9 | 0.1 | 0.2 | 0.1 | | | | | 0.3 | 0.1 - 0.9 |
| Colistin | | | | | | | 99.9 | 0.1 | | | | | | | | | | | 0.0 | 0 - 0.3 |
| Meropenem | | 99.8 | 0.2 | | | | | | | | | | | | | | | | 0.0 | 0 - 0.3 |
| Tigecycline | | | | | 78.9 | 17.4 | 3.7 | | | | | | | | | | | | 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 (in %) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2017.

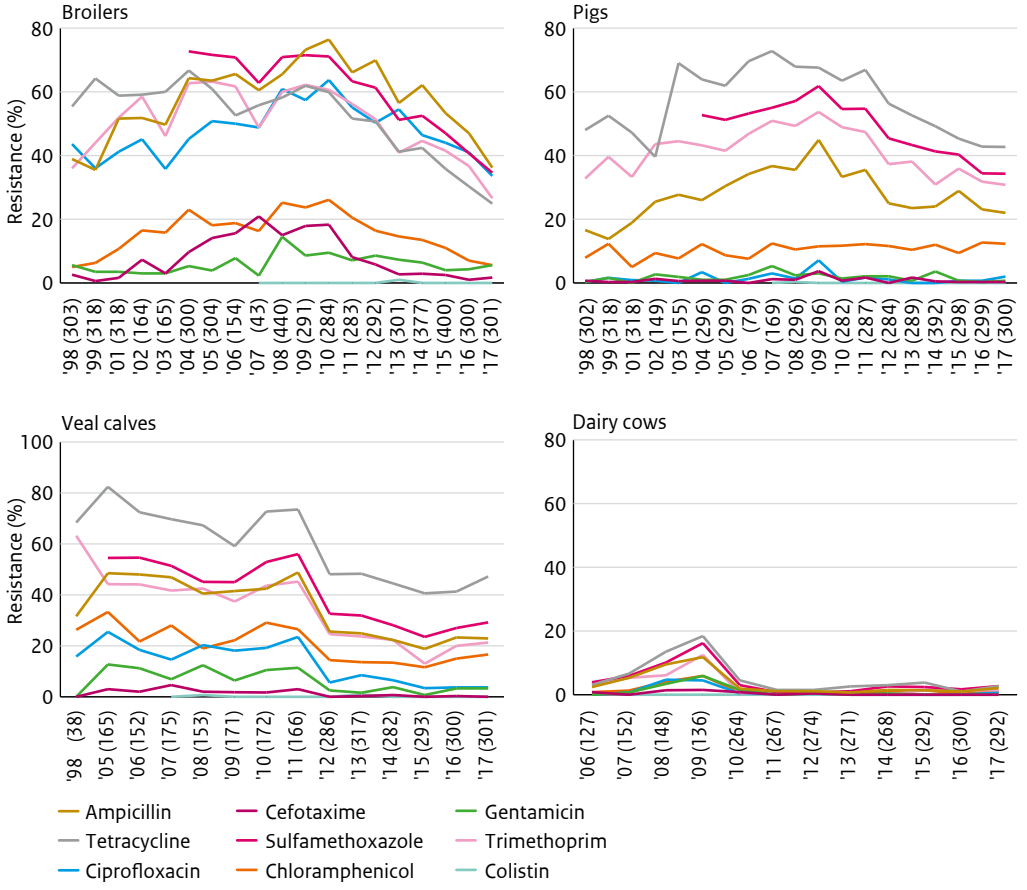
| Faecal samples | Broilers | Pigs | Dairy | Veal calves | |
|---|---|---|---|---|---|
| | N = 301 | N = 300 | N = 292 | White, N = 209 | Rosé, N = 92 |
| Ampicillin | 36.2 | 22,0 | 2,1 | 27,8 | 12,0 |
| Cefotaxime | 1.7 | 0,3 | 0,0 | 0,0 | 0,0 |
| Ceftazidime | 1.7 | 0,3 | 0,0 | 0,0 | 0,0 |
| Gentamicin | 5.6 | 0,7 | 0,0 | 3,8 | 2,2 |
| Tetracycline | 24.9 | 42,7 | 2,7 | 61,2 | 15,2 |
| Sulfamethoxazole | 34.6 | 34,3 | 2,7 | 37,8 | 9,8 |
| Trimethoprim | 26.6 | 30,8 | 1,4 | 28,2 | 5,4 |
| Ciprofloxacin | 33.6 | 2,0 | 0,7 | 5,3 | 0,0 |
| Nalidixic acid | 30.6 | 1,3 | 0,7 | 4,3 | 0,0 |
| Chloramphenicol | 5.6 | 12,3 | 1,0 | 21,5 | 5,4 |
| Azithromycin | 0.7 | 0,7 | 0,0 | 0,0 | 0,0 |
| Colistin | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Meropenem | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Tigecycline | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |

### Quinolones

The proportion of isolates resistant to quinolones were highest in *E. coli* from broilers: 33.6% resistance to ciprofloxacin and 30.6% resistance to nalidixic acid. This was a decrease compared to previous years (going down from 54% resistance for both drugs in 2013), although the resistance levels are still high. High level resistance (MIC >1 mg/L) to ciprofloxacin in broilers was detected in 3.6% (11/301) of the isolates in 2017. In 2017, resistance to ciprofloxacin was 5.3% in *E. coli* isolates from white veal calves, 2.0% in pigs, very low in isolates from dairy cows and not detected in isolates from rosé veal calves.

Resistance to quinolones in *E. coli* from meat was tested for chicken and turkey meat samples and pork, beef and veal, sampled from retail in The Netherlands. In 2017, no samples from imported meat were analysed. Resistance in chicken products at retail was a bit higher than in 2016: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 26.9% (22.4% in 2016) and 23.1% (20.9 % in 2016), respectively. In isolates from turkey products the resistance percentages were a little lower than in 2017 (30.6% and 25.0% for ciprofloxacin and nalidixic acid respectively). Figure Eco03 shows that resistance levels in pork and beef was low, as in former years. The resistance percentages of *E. coli* from meat were somewhat higher for ciprofloxacin than for nalidixic acid. This is most probably due to the increase of plasmid mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid.

**Figure Eco01** Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2017.

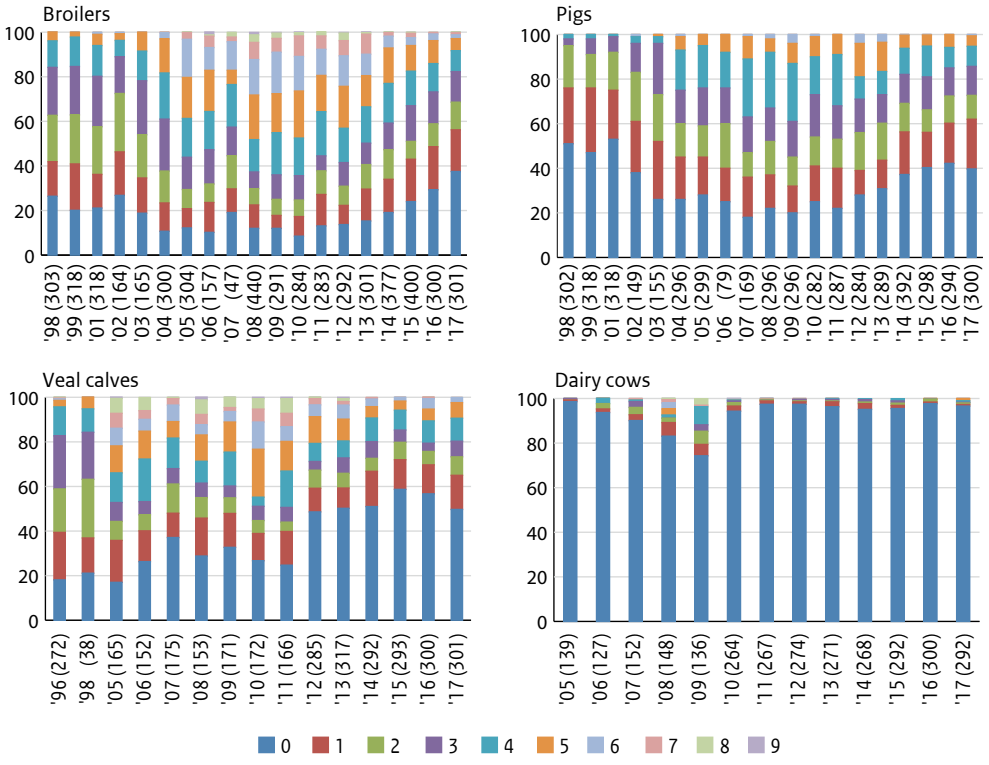



### Cefotaxime resistance

The proportion of isolates resistant to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL/pAmpC producing *E. coli*, was very low in broilers and pigs. Cefotaxime resistance was not detected in isolates from dairy cows and veal calves (white and rosé). Resistance proportions for *E. coli* were 1.7% in broilers and 0.3% in pigs for both cefotaxime and ceftazidime. The 1.7% cefotaxime resistance proportion in broiler isolates was at a similar level as in 2016 (1.0%), which indicates a stabilised low level after a decreasing trend from 2013 to 2016 (Figure Eco01).

For the first time since 2014, resistance to cefotaxime in randomly isolated commensal *E. coli* obtained from chicken meat samples from retail could not be detected (Table Eco03). The proportion of isolates with cefotaxime resistance from turkey meat samples from retail was a bit higher than in 2016 (from

**Figure Eco02** Resistance (%) to 0 - 9 antimicrobial classes among *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2017.




1.5% in 2016 to 5.6% in 2017). As in 2017 no samples from imported meat (outside EU) were collected, the resistance percentages shown in Figure Eco03 for 2017 cannot be compared with the percentages in former years, which were the total result from imported and retail meat samples.

The absence of finding cefotaxime resistant *E. coli* on 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 decreased. However, the proportion of fresh chicken meat samples in which ESC-resistant *E. coli* were found using selective media slightly increased in 2017 to 31.6% after a decreasing trend from 2014 (67%) – 2016 (26.4%) (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 produced in the Netherlands carrying ESC-resistant *E. coli* decreased from 50.3% of the animals sampled in 2016 to 32.6% in 2017 (see chapter 4). The decrease in prevalence and concentrations of ESC-resistant *E. coli* 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 second year with relatively high prevalence of animals positive for ESC-resistant *E. coli* was found. In white veal calves the prevalence was highest with 40.5% of the animals sampled (compared to 28.3% in rosé). The prevalence in 2017 of animals positive for ESC-resistance in pigs and dairy cattle were 11.0 and 10.3% respectively.

**Broiler chickens**

Commensal *E. coli* isolated from caecal samples from broiler chickens showed decreasing resistance to almost all antimicrobials (Table Eco02). Resistance proportions for ampicillin (36.2%), tetracycline (24.9%), sulfamethoxazole (34.6%), trimethoprim (26.6%) and the quinolones ciprofloxacin (33.6%) and nalidixic acid (30.6%) were high, but lower than in 2016. Cefotaxime resistance decreased from 2.5% in 2015 to 1.0% in 2016, but remained stable in 2017 (1.7%) .

**Slaughter pigs**

Resistance proportions for tetracycline, sulfamethoxazole, trimethoprim and ampicillin in *E. coli* isolates from pigs, sampled in 2017, were 42.7%, 34.3%, 30.8% and 22.0%, respectively, which was approximately at the same level as in 2016. The proportion of isolates resistant to these four antibiotics showed a decreasing tendency since 2011, which stabilized from 2015 onwards. (Figure Eco01). Resistance to the 3$^{rd}$ generation cephalosporins did not change compared to 2015 and 2016 (0.3%).

**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 between the two husbandry types. As in the years before, substantially higher resistance levels were measured for 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 have been relatively stable over time, with a clear decrease in 2012, which was the year in which the sampling strategy changed from sampling at farm to sampling at slaughterhouse. This has influenced the results from 2012 and onwards, because most antibiotics are used in the young calves and less in the period before slaughter.

The highest resistance levels in 2017 were against tetracycline (61.2% and 15.2% for white and rosé respectively), sulfamethoxazole (37.8% and 9.8%), trimethoprim (28.2% and 5.4%) and chloramphenicol (21.5% and 5.4%).

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, which better reflected the proportions of slaughtered white and rosé calves in The Netherlands in 2016/2017. 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 not detected in veal calves in 2017 (TableEc002).

### Dairy cattle

Resistance in *E. coli* isolated from dairy cattle is very low compared to resistance proportions observed in pigs, broilers and veal calves (Table Ec002), reflecting the low use of antibiotics in this husbandry system. Resistance proportions were comparable to 2015 and 2016. The overall rates remained below 3%. No resistance to 3rd generation cephalosporins was detected.

### 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. For this reason, trends in multidrug resistance should be interpreted with care. Figure Ec002 shows the data with the determined level of multidrug resistance over the years.

In 2017, a decreasing trend in the proportion of multidrug resistant isolates (resistant to three or more classes of antibiotics) was observed in broilers (from 41.0% in 2016 to 31.4% in 2017), but the proportion of multidrug resistance stabilized at relatively high levels in pigs (27.3%) and veal calves (26.7%). In dairy cattle multidrug resistance in *E. coli* was still rarely detected with 2.1% of the isolates showing resistance to three or more classes of antimicrobials. Multidrug resistance in isolates from pigs was at the same level as in 2016 (27.3%).

The increasing tendency of the percentage of completely susceptible *E. coli* isolates from broilers and pigs (Figure Ec002), which was reported for 2016, is ongoing in broilers, but not in isolates from pigs.

### *E. coli* in raw-meat

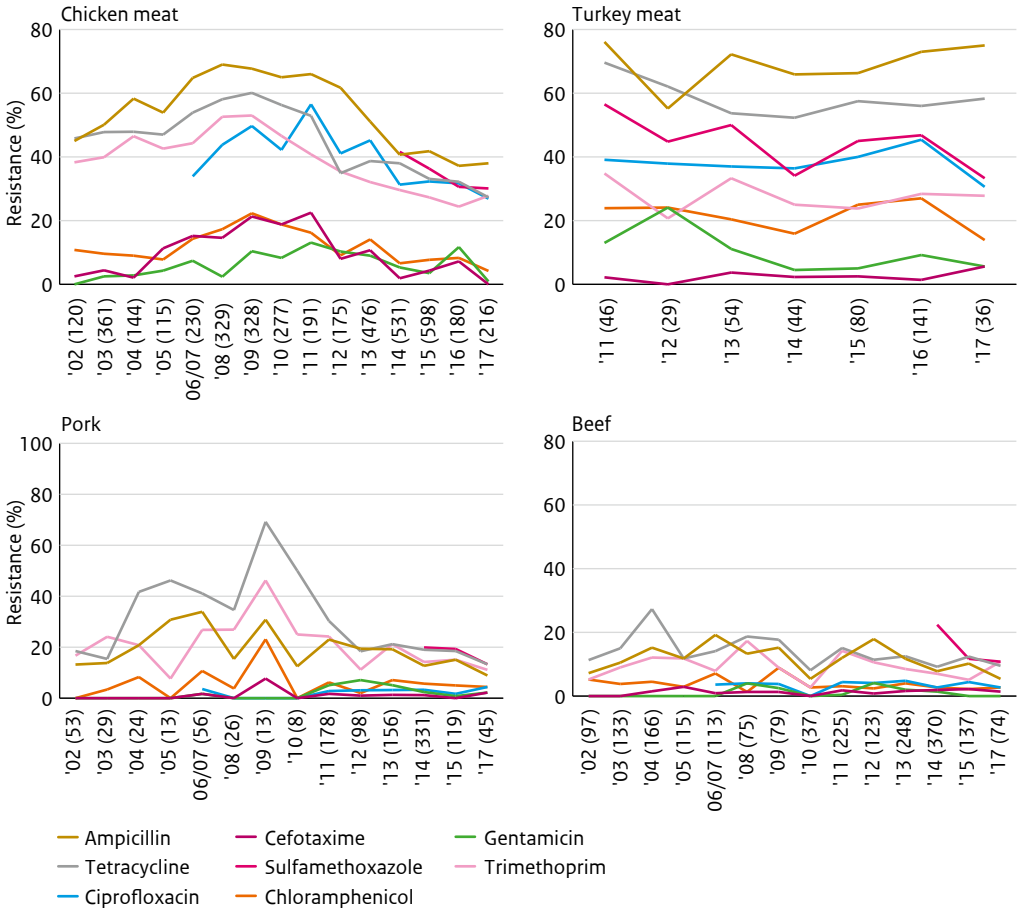
Table Ec003 presents resistance percentages of *E. coli* isolated from raw meat from chicken and turkey, pork, beef and veal, sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail can include meat produced in The Netherlands, but also other EU countries. Meat products imported from outside the EU were not analysed for commensal *E. coli* 2017.

The trends in resistance are presented in Fig Ec003. Resistance rates in chicken meat show a tendency to decrease from 2010 onward, with some slight increases in 2015/2016, but the decreasing trend seems to go on in 2017. In turkey meat, resistance rates have been at a constant high level since 2011, with a decrease in 2017 for sulfamethoxazole, ciprofloxacin and chloramphenicol. In 2017, cefotaxime resistance could not be detected in *E. coli* isolates from chicken meat, but slightly increased in isolates from turkey meat samples, compared to former years. Fluctuations in the resistance rates might be caused by a year-to-year variation in the proportion of retail poultry meat produced within the European Union, but outside of the Netherlands included in the survey.

**Table Eco03** Resistance (in %) of *E. coli* isolated from raw chicken meat, turkey meat, pork, beef and veal at retail in the Netherlands in 2017.

| Meat products | Chicken | Turkey | Pork | Beef | Veal |
|---|---|---|---|---|---|
| | N = 216 | N = 36 | N = 45 | N = 74 | N = 81 |
| Ampicillin | 38.0 | 75.0 | 8.9 | 5.4 | 27.2 |
| Cefotaxime | 0.0 | 5.6 | 2.2 | 1.4 | 0.0 |
| Ceftazidime | 0.5 | 2.8 | 0.0 | 0.0 | 1.2 |
| Gentamicin | 0.9 | 5.6 | 2.2 | 0.0 | 1.2 |
| Tetracycline | 27.3 | 58.3 | 13.3 | 9.5 | 38.3 |
| Sulfamethoxazole | 30.1 | 33.3 | 13.3 | 10.8 | 24.7 |
| Trimethoprim | 27.8 | 27.8 | 11.1 | 10.8 | 22.2 |
| Ciprofloxacin | 26.9 | 30.6 | 4.4 | 2.7 | 4.9 |
| Nalidixic acid | 23.1 | 25.0 | 4.4 | 2.7 | 1.2 |
| Chloramphenicol | 4.2 | 13.9 | 4.4 | 2.7 | 7.4 |
| Azithromycin | 0.5 | 2.8 | 0.0 | 0.0 | 2.5 |
| Colistin | 1.4 | 2.8 | 0.0 | 0.0 | 0.0 |
| Meropenem | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Tigecycline | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

**Figure Eco03** Trends in resistance (%) of *E. coli* isolated from raw chicken meat, turkey meat, pork and beef in the Netherlands from 2002 - 2017.



# 4
# Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2017

**Highlights**

1. Within the randomly isolated indicator *E. coli* in faecal samples from broilers a continuous low proportion of ESBL/AmpC-producing *E. coli* was observed in the last five years (<3%) and this was confirmed in 2017 (1.7%). No ESBL/AmpC-producing *E. coli* were detected in faecal samples from pigs, veal calves and dairy cattle.
2. Selective culturing in livestock faeces showed a further decrease in the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* in broilers.
3. For the second year in a row, an increase was observed in white and rosé veal calves carrying ESBL/AmpC-producing *E. coli*. 2017 was the first year a higher prevalence was recorded in veal calves than in broilers (36.7% vs 32.6%).
4. The most prevalent ESBL/AmpC gene was $bla_{\text{CTX-M-1}}$ in all animal species. $bla_{\text{CTX-M-15}}$ was found frequently in veal calves and dairy cows (30%). $bla_{\text{CMY-2}}$ in broilers (25%), $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52c}}$ and $bla_{\text{CTX-M-14}}$ followed in prevalence. A comparable gene distribution was observed in corresponding meat samples.
5. Prevalence of ESBL/AmpC-producing *E. coli* in meat in 2017 was 9.6%. After three years of decreasing prevalence (67% to 24% in 2014-2016), in 2017 31.6% of fresh chicken meat samples were found positive, resulting in a similar prevalence as in broilers (32.6%). Imported chicken meat was more frequently positive (56.1%). Also lamb and veal meat were found more frequently positive than in previous years.
8. The proportion of human ESBL/AmpC-producing *Salmonella* in 2017 was 1.8%, confirming a continuous low level (≤2%) since 2014. Most represented ESBL/AmpC genes were $bla_{\text{CTX-M-14b}}$, generally associated with S. Kentucky, $bla_{\text{CTX-M-9}}$ in S. Typhimurium, and $bla_{\text{CMY-2}}$ in S. Typhimurium and S. Agona.
1. The majority (84%) of ESBL/AmpC-producing *Salmonella* from humans were highly multidrug resistant (5-8 antibiotics).
2. No carbapenemase-producing *Enterobacteriaceae* were detected in livestock.
3. *E. coli* carrying $bla_{\text{OXA-48}}$ was detected in dog faeces in the Netherlands for the first time.
4. Colistin resistance gene *mcr-1* was identified at low-level in *E. coli* from livestock (1.2%) and at higher levels in retail meat from chicken (7.7%), but not in *Salmonella*.

## 4.1 ESBL/AmpC-producing bacteria

### 4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2017

Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is routinely done by random isolation of a minimum of 170 *Escherichia coli*, each representing one epidemiological unit, from faecal samples of food-producing animals, as prescribed by EFSA guidelines.[2] Isolates are tested for susceptibility to cefotaxime and ceftazidime and reduced susceptible isolates are determined based on EUCAST epidemiological cut-off values, as described in Chapter 3. Since 1998, cefotaxime resistance is observed at low levels in all animal species. Figure ESBL01 shows the percentage of cefotaxime resistant *E. coli* randomly picked from non-selective media derived from broilers, slaughter pigs (1998 – 2017), veal calves and dairy cows (2005 – 2017). In broilers, after 2003 an apparent increase in cefotaxime resistance was observed up to levels that varied between 15 – 20%, with the highest peak observed in 2007. The strong decline observed in 2011, from 18.3% to 8.1%, was most likely due to decreased usage of antibiotics since the spring of 2010 when the (off label) use of ceftiofur was ceased at Dutch hatcheries. A continuous low proportion of ESBL/AmpC-producing *E. coli* in broilers was observed in the last five years (<3%) and this was confirmed by the low level of cefotaxime resistance in 2017 (1.7%).

From a total of 1194 randomly selected *E. coli* isolates tested in 2017, five isolates from broilers and one isolate from pig (Table ESBL01) displayed reduced susceptibility (MIC > 0.25 mg/L) to cefotaxime (see also 3.2.1). No ESBL/AmpC-suspected *E. coli* isolates were found in veal calves and dairy cattle. Cefotaxime resistant isolates were screened for beta-lactamase gene families using an in-house developed RT-PCR (Geurts *et al,* 2017) or the Check-Points CT101 miniaturised micro-array (Check-Point, Wageningen, NL). Genes were confirmed by dedicated PCR and sequencing. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutations in the chromosomal *ampC* genes. The results of this molecular typing are displayed in Table ESBL01.
Three different plasmid mediated ESBL/AmpC gene types were detected in the isolates from broilers: $bla_{\text{CTX-M-1}}$ (n=2), $bla_{\text{TEM-52c}}$ (n=1), and $bla_{\text{SHV-2}}$ (n=2). After two years of absence (2015-2016), $bla_{\text{TEM-52c}}$ was again detected as well as $bla_{\text{SHV-2}}$ missing since 2011 in broilers. The *E. coli* isolate from slaughter pig exhibited an atypical resistance pattern with borderline resistance to both cefotaxime and ceftazidime, but susceptibility to ampicillin. No ESBL/AmpC gene or chromosomal mutation were identified. For this reason the slaughter pig isolate was not considered to be an ESBL/AmpC-producer and therefore was not included in Table ESBL01 and Figure ESBL01. In general, no chromosomal *ampC* genes or $bla_{\text{CMY-2}}$ were detected in 2017. It can be concluded that by random isolation, only five cefotaxime resistant isolates (0.4%) associated to three plasmid mediated ESBL/AmpC genes were found in 2017, keeping the resistance proportion of the last three years under 1%. This confirms the major improvement compared to 2009 when ESBL/AmpC-producing isolates added up to 7.6%, before antibiotic usage reduction started in Dutch livestock.

[2] 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.

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

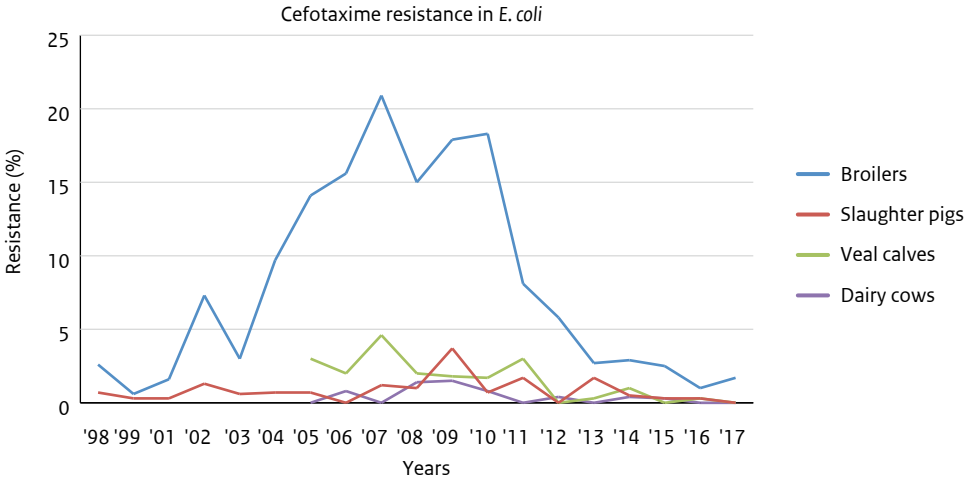


**Table ESBL01** ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2017.

| | ESBLs isolated from | | | | | | ESBL-genes detected | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Broilers[c] | Veal calves | Slaughter pigs | Dairy cows[d] | Turkeys | Total ESBL suspected (n) | CTX-M-1-group[a] | CTX-M-2 | CTX-M-9-group! | TEM-52c | TEM-20 | SHV-12[b] | 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 |
| Total | 271 | 27 | 31 | 8 | 6 | 343 | 135 | 19 | 3 | 39 | 2 | 42 | 11 | 44 | 20 | 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 2017

As of 2014, selective culturing for ESBL/AmpC-producers in broilers was implemented together with the ongoing active surveillance in slaughter pigs and veal calves started in 2011. In 2017, caecal samples were taken at slaughter (white and rosé veal calves, slaughter pigs and broilers) and faecal samples at farms (dairy cows). Screening was done by overnight incubation of faecal samples (1 gram) in 9 ml Buffered Peptone Water (BPW) followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime, according to EURL-AR protocols: http://www.eurl-ar.eu/233-protocols.htm.
Selective culturing for ESBL/AmpC-producers of meat samples was also implemented as of 2014. Meat samples (25 gram) were pre-enriched in 225 ml BPW followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime and on Brilliance ESBL Agar (Oxoid, part of Thermo Fischer Scientific). From each plate, colonies with typical *E. coli* morphology were selected for bacterial species identification and confirmed *E. coli* isolates were screened for the identification of beta-lactamase gene families, as described above.

**Results of selective isolation of ESBL/AmpC-producing *E. coli* in faeces**

In 2017, 1203 faecal samples were screened for the presence of ESBL/AmpC-producing *E. coli*, each sample representing one slaughter batch of animals from one farm. Suspected ESBL/AmpC isolates comprised all *E. coli* growing on MacConkey with 1 mg/L cefotaxime including isolates carrying mutations in the chromosomal *ampC* gene promoter. Confirmed ESBL isolates encoding ESBL or AmpC genes, most likely located on a horizontally transmissible plasmid, are reported in Table ESBL02.
Of 1203 analysed samples, 22.6% were positive for ESBL/AmpC-producing *E. coli*, mainly due to the high prevalence in veal calves (36.7%, combining white and rosé). The increase already observed in 2016 in both white and rosé veal calves was confirmed in 2017 (40.5% and 28.3%, respectively), recording for the first time a higher prevalence in veal calves than in broilers (32.6%). As noted in the past, prevalence in white veal calves was higher than in rosé veal calves.
ESBL/AmpC-producing *E. coli* prevalence in broilers registered further decrease in 2017 compared to 2015-2016 (from 56.5-50.3% to 32.6%). The prevalence in slaughter pigs and in dairy cows decreased and stabilized at around 10%. In conclusion, 2017 marked an inversion in ESBL/AmpC-producing *E. coli* carriership in livestock, with increased prevalence in veal calves versus a reduction in broilers (Figure ESBL02). An explanation for this phenomenon is not available yet.

**Figure ESBL02** Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows.

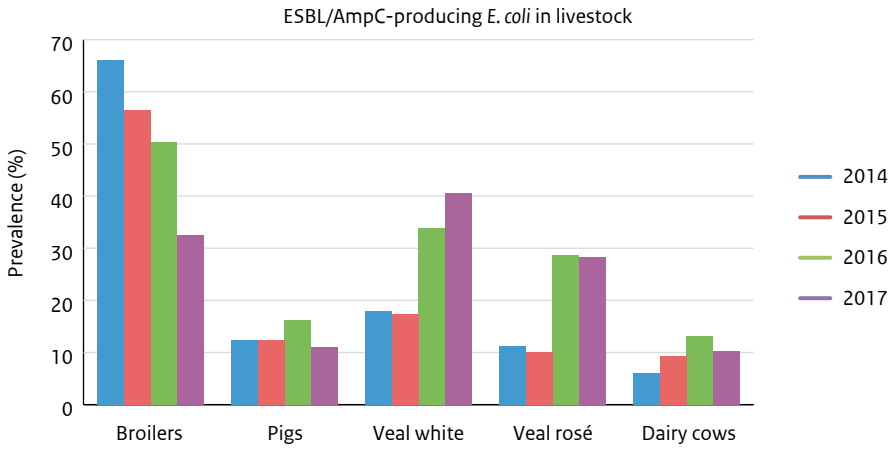



**Table ESBL02** Prevalence of *E. coli* isolates showing reduced susceptibilty to cefotaxime derived from selective culturing of faecal samples from broilers, layers, ducks, slaughter pigs, veal calves and dairy cows taken at slaughter in 2017.

| | N samples | N suspected ESBL | N confirmed ESBL | Prevalence(%) ESBL confirmed |
|---|---|---|---|---|
| Broilers | 301 | 98 | 98 | 32.6 |
| Pigs | 300 | 47 | 33 | 11.0 |
| Veal calves | | | | |
| white | 210 | 86 | 85 | 40.5 |
| rosé | 92 | 28 | 26 | 28.3 |
| Dairy cows | 300 | 36 | 30 | 10.0 |
| Total | 1203 | 295 | 272 | 22.6 |

ESBL/AmpC genes detected in animal faeces are reported in Table ESBL03. Comparable to former years (MARAN 2015, 2016 and 2017), high ESBL gene type variability was confirmed in 2017. $bla_{CTX\text{-}M\text{-}1}$ was still the dominant gene variant in all animal species (126 out of 295 genes detected), followed by $bla_{CTX\text{-}M\text{-}15}$ (n=46), $bla_{CMY\text{-}2}$ (n=28), $bla_{SHV\text{-}12}$ (n=20) and $bla_{TEM\text{-}52c}$ (n=14). The increased ESBL/AmpC-producing *E. coli* prevalence observed in veal calves was associated with the highest gene variability (12 different ESBL genes, mostly in white veal calves), comparable to the results of 2016. For the first time, prevalence of $bla_{CTX\text{-}M\text{-}15}$ (typically associated with humans) was higher than $bla_{CMY\text{-}2}$ (15.6% vs 9.5%, respectively), with a dominant distribution of $bla_{CTX\text{-}M\text{-}15}$ in cattle (veal calves and dairy cows) and $bla_{CMY\text{-}2}$ in broilers (Table ESBL03). Genes $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}14}$, also frequently associated with human isolates, were mostly detected in veal calves (with the exception of three $bla_{CTX\text{-}M\text{-}14}$ in slaughter pigs and one $bla_{CTX\text{-}M\text{-}14}$ in dairy cattle) comparable to the prevalence observed in 2016. Slaughter pig and dairy cow isolates didn't show significant differences compared to previous years. Chromosomal *ampC* types confirmed their role in conferring cefotaxime resistance as already observed in 2015-2016 with relatively high numbers in slaughter pig and dairy cow isolates (29.8% and 16.7%, respectively). As in previous years, no combination of ESBL genes was detected with the exception of one *E. coli* isolate from dairy cow exhibiting the AmpC beta-lactamase $bla_{CMY\text{-}42}$ gene together with beta-lactamase gene $bla_{TEM\text{-}190}$.

**Results of selective isolation of ESBL/AmpC-producing *E. coli* in raw meat**

Prevalence of ESBL suspected isolates in fresh raw meat was investigated and results are shown in Table ESBL04. Meat preparations (except for imported frozen poultry meat with approximately 1% salt) were not screened in 2017. Out of 1626 fresh meat samples, 156 were confirmed positive for ESBL/AmpC-producing *E. coli* (9.6%), with the highest prevalence in imported chicken meat (56.1%). This result was comparable to previous years (67-61%, 2014-2016) and in line with the decreasing prevalence started in 2012 (83%). Fresh turkey meat registered a prevalence of 17,6%, comparable to the reduction observed in fresh turkey meat in recent years, from 50.9% in 2014. While beef showed a reduction compared to 2016 (from 2.0% to 0.9%), a slightly higher ESBL/AmpC prevalence was observed in lamb (from 2.7% to 5.1%) and veal (from 4.4% to 7.5%) compared to 2016. ESBL/AmpC-producing *E. coli* prevalence in veal has been increasing from 2014 onwards (from 0% to 3.8% in 2014 and 2015, respectively). Yet, for some types of meat the number of samples was relatively low, so the figures should be interpreted with care.

All 156 isolates were confirmed by MALDI-TOF as *E. coli* and molecularly characterized. Table ESBL05 shows the different ESBL/AmpC gene types detected in meat. Most of the genes found in beef and veal ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}32}$ and $bla_{CTX\text{-}M\text{-}55}$ ) were also present in faecal samples of veal calves indicating possible faecal contamination during slaughter and/or meat processing. Chicken meat displayed more ESBL/AmpC gene variability than Dutch broiler faecal samples, with $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{SHV\text{-}2}$ not detected in the latter. This difference in gene variation might be explained by the fact that fresh retail chicken meat in Dutch supermarkets is not exclusively produced in the Netherlands, but can also originate from neighbouring countries within the EU. Moreover, the inclusion of imported frozen chicken meat from outside the EU (mainly from South America and Asia) inevitably increases the variability of ESBL/AmpC genes detected. Coexistence of different ESBL genes was observed in two *E. coli* isolates from chicken meat: $bla_{CTX\text{-}M\text{-}2}$ with $bla_{CTX\text{-}M\text{-}8}$, and $bla_{CMY\text{-}2}$ with $bla_{TEM\text{-}52c}$. The dominant human associated $bla_{CTX\text{-}M\text{-}15}$ gene was detected in higher prevalence than 2015-2016 (from 5.6-5.7% to 11.5%) in almost all meat types, with the highest prevalence observed in fish and shrimps (88%) and veal (29%), in line with the prevalence observed in veal calf faecal samples (Table ESBL03). Other frequent gene

types were $bla_{CMY-2}$ and $bla_{SHV-12}$ typically found in broiler faecal samples too. Chromosomal *ampC* types were detected mainly in *E. coli* isolates from lamb meat.

**Table ESBL03** Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves, and dairy cows in 2017. 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 | 46 | 23 | 35 | 13 | 9 | 126 |
| | CTX-M-3 | | 1 | | | 1 | 2 |
| | CTX-M-15 | | | 25 | 9 | 12 | 46 |
| | CTX-M-32 | 1 | | 5 | 1 | | 7 |
| | CTX-M-55 | 1 | | 2 | | | 3 |
| **CTX-M-2 group** | | | | | | | |
| | CTX-M-2 | | | 1 | | 1 | 2 |
| **CTX-M-8/25 group** | | | | | | | |
| | CTX-M-8 | | | | | 1 | 1 |
| **CTX-M-9group** | | | | | | | |
| | CTX-M-9 | | | 1 | | | 1 |
| | CTX-M-14 | | 3 | 5 | | 1 | 9 |
| | CTX-M-65 | | | 3 | 1 | 3 | 7 |
| **TEM** | | | | | | | |
| | TEM-52c | 6 | 3 | 4 | 1 | | 14 |
| | TEM-52cVar | 4 | 2 | | | | 6 |
| | TEM-225 | | | | | | 1 |
| **SHV** | | | | | | | |
| | SHV-12 | 15 | 1 | 3 | | 1 | 20 |
| **CMY** | | | | | | | |
| | CMY-2 | 25 | | 1 | 1 | 1 | 28 |
| | CMY-42/TEM-190 | | | | | 1 | 1 |
| **Chromosomal ampC** | | | | | | | |
| | ampC-type-3 | | 14 | 1 | 2 | 5 | 22 |
| | ampC-type-3-like | | | | | | |
| Total | | 98 | 47 | 86 | 28 | 36 | 295 |

**Table ESBL04** Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat products in the Netherlands in 2017.

| Animal source | | N screened | N ESBL/AmpC positive (confirmed) | % ESBL/AmpC positive |
|---|---|---|---|---|
| **Beef** | | | | |
| | fresh meat | 528 | 5 | 0,9 |
| **Veal** | | | | |
| | fresh meat | 226 | 17 | 7,5 |
| **Pork** | | | | |
| | fresh meat | 275 | 4 | 1,5 |
| **Chicken** | | | | |
| | fresh meat [a] | 228 | 72 | 31,6 |
| | import [b] | 57 | 32 | 56,1 |
| **Turkey** | | | | |
| | fresh meat [c] | 38 | 6 | 15,8 |
| | import [d] | 4 | 1 | 25,0 |
| **Lamb** | | | | |
| | fresh meat | 198 | 10 | 5,1 |
| **Sheep** | | | | |
| | fresh meat | 4 | 0 | 0,0 |
| **Goat** | | | | |
| | fresh meat | 8 | 1 | 12,5 |
| **Fish and shrimps** | | | | |
| | fresh meat | 56 | 7 | 12,5 |
| **Crocodile** | | | | |
| | fresh meat | 3 | 1 | 33,3 |
| **Frog** | | | | |
| | fresh meat | 5 | 1 | 20,0 |
| Total | | 1630 | 157 | 9,6 |

*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 2017.

| ESBL gene | | Chicken | Turkey | Beef | Veal | Pig | Lamb | Goat | Crocodile | Frog | Fish and shrimpls | Totaal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CTX-M-1 group** | | | | | | | | | | | | |
| | CTX-M-1 | 20 | 4 | 2 | 3 | 1 | 1 | | | | | 31 |
| | CTX-M-15 | 1 | 1 | 1 | 5 | 1 | 2 | | | 1 | 6 | 18 |
| | CTX-M-32 | 1 | | | 4 | | | | | | | 5 |
| | CTX-M-55 | 2 | | 1 | 2 | | | | | | 1 | 6 |
| **CTX-M-2 group** | | | | | | | | | | | | |
| | CTX-M-2 | 9 | | 1 | 1 | | | | | | | 11 |
| | CTX-M-2/ CTX-M-8 | 1 | | | | | | | | | | 1 |
| **CTX-M-8/25 group** | | | | | | | | | | | | |
| | CTX-M-8 | 4 | 1 | | | | | | | | | 5 |
| **CTX-M-9 group** | | | | | | | | | | | | |
| | CTX-M-14 | | | | | | 2 | | | | | 2 |
| | CTX-M-65 | | | | 1 | | | | | | | 1 |
| **TEM** | | | | | | | | | | | | |
| | TEM-52 | 1 | | | 1 | | | | | | | 2 |
| | TEM-52c | 4 | | | | | | | | | | 4 |
| | TEM-52cVar | 5 | | | | | | | | | | 5 |
| **SHV** | | | | | | | | | | | | |
| | SHV-12 | 14 | | | | | 1 | 1 | | | | 16 |
| | SHV-2 | 1 | | | | | | | | | | 1 |
| | SHV-2a | 1 | | | | | | | | | | 1 |
| **CMY** | | | | | | | | | | | | |
| | CMY-2 | 38 | | | | 1 | | | 1 | | | 40 |
| | CMY-2/TEM-52c | 1 | | | | | 1 | | | | | 2 |
| **Chromosomal ampC** | | | | | | | | | | | | |
| | ampC-type-3 | | | | | 1 | 3 | | | | | 4 |
| | ampC-type-11 | 1 | | | | | | | | | | 1 |
| Total | | 104 | 6 | 5 | 17 | 4 | 10 | 1 | 1 | 1 | 7 | 156 |

**ESBL/AmpC-producing Salmonella**

Surveillance of resistance to extended spectrum cephalosporins is also routinely performed in *Salmonella enterica* isolated in the Netherlands from both human and animal (meat) source.

In 2017, the NVWA tested 187 *Salmonella* isolates mostly originating from imported meat samples, also including imported seafood and herbs. A high proportion of the isolates exhibited resistance to cefotaxime (n=69) in four different serovars: Heidelberg (=65), Minnesota (n=2), Abony (n=1), and Schwarzengrund (n=1), exclusively originating from imported chicken meat from outside the EU. No further molecular characterization was performed on these cefotaxime resistant *Salmonella* isolates from meat.

A total of 1697 *Salmonella* of human origin isolated in 2017 were sent by RIVM to WBVR to be tested for susceptibility to cefotaxime and ceftazidime. In total, 31 cefotaxime or ceftazidime resistant *Salmonella* were identified (Table ESBL06). The prevalence of ESBL/AmpC-producing *Salmonella* was 1.8%, comparable to that observed in 2015 and 2016 (1.9% and 1.7%, perspectively) and almost half of 2013 (4%). The predominance of *S.* Kentucky observed in 2016 was confirmed in 2017 (n=18), followed by Typhimurium (n=8), and four other serovars identified to carry ESBL/AmpC genes (material and methods are the same as described for *E. coli* in the previous section).

ESBL/AmpC genes detected in *Salmonella* of human origin are reported in Table ESBL06. The most represented gene types were: i) $bla_{\text{CTX-M-14b}}$, generally associated with *S.* Kentucky; ii) $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium; and iii) $bla_{\text{CMY-2}}$ in *S.* Typhimurium and *S.* Agona. Comparable to previous years' results, prevalence of $bla_{\text{CMY-2}}$ in 2017 was assessed at 10%. Similarly, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ were less represented than previous years. $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-14b}}$ confirmed their predominant role compared to previous years with an increase from 3-8% (2016) to 19-52% (2017), respectively. No ESBL/AmpC gene combination was detected in *Salmonella*.

All cefotaxime resistant *Salmonella* isolates of human origin were highly multidrug resistant, as shown in Table ESBL07. The increased multi-resistance observed in the last years compared to 2014 (23%) was similar to that of 2017 with most of the isolates resistant to 5-8 antibiotics (84%). 3% of the isolates were resistant to 9 out of 10 antibiotics but no resistance was detected against meropenem. For the first time one *Salmonella* isolate was resistant to azithromycin. As for 2016, colistin resistance was 0% in 2017, compared to 8.8% in 2015.

ESBL/AmpC gene types found in *Salmonella* since 2007 are summarized in Table ESBL08. Every year $bla_{\text{CMY-2}}$ and genes belonging to the $bla_{\text{CTX-M-1}}$ -group have been associated to *Salmonella* isolates from diverse sources. After detection in 2015, $bla_{\text{CTX-M-2}}$ was not detected in 2016 and in 2017. The most prevalent group was $bla_{\text{CTX-M-9}}$ (23 out of 31 genes) confirming the increase registered in the last three years. $bla_{\text{DHA-1}}$ was identified for the first time in 2016 in a human isolate of *S.* Bovismorbificans and in *S.* Kentucky in 2017. Overall, *Salmonella* isolates showed different ESBL/AmpC gene variability than in 2016.

In conclusion, ESBL/AmpC-producing *E. coli* are common in Dutch food-producing animals and in raw meat mainly of poultry origin. ESBL/AmpC genes were detected in 0.4% of randomly isolated *E. coli*. For the first time selective culturing of livestock faecal samples showed higher prevalence in veal calves than in broilers (36.7% vs 32.6%, respectively). The dominant ESBL/AmpC gene type was confirmed to be $bla_{\text{CTX-M-1}}$ in all animal species. The human ESBL gene $bla_{\text{CTX-M-15}}$ was frequently found in veal calf and dairy cow faecal samples and derived meat products, and only rarely found in broilers and chicken products confirming the observations of 2015-2016. Human *Salmonella* isolates were mostly associated with $bla_{\text{CTX-M-9}}$ -group genes and showed a multidrug resistant phenotype.

**Table ESBL06** Beta-lactamases in *Salmonella* isolated from humans in 2017.

| | CTX-M-1 group | | | CTX-M-9 group | | | SHV | CMY | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Serovar** | **CTX-M-1** | **CTX-M-15** | **CTX-M-55** | **CTX-M-9** | **CTX-M-14b** | **CTX-M-65** | **SHV-12** | **CMY-2** | **DHA-1** | **Total** |
| 1,4,5,12:i:- | | | 1 | | | | | | | 1 |
| Agona | | | | | | | | 1 | | 1 |
| Infantis | 1 | | | | | 1 | | | | 2 |
| Kentucky | | 1 | | | 16 | | | | 1 | 18 |
| Typhimurium | | | | 6 | | | | 2 | | 8 |
| Virchow | | | | | | | 1 | | | 1 |
| **Total** | **1** | **1** | **1** | **6** | **16** | **1** | **1** | **3** | **1** | **31** |

**Table ESBL07** Resistance and multidrug resistance percentages of human ESBL-producing *Salmonella* in the Netherlands in 2017.

| Antimicrobials | R% |
|---|---|
| Ampicillin | 100,0 |
| Cefotaxime | 100,0 |
| Ceftazidime | 77,4 |
| Gentamicin | 80,6 |
| Tetracycline | 96,8 |
| Sulfamethoxazole | 90,3 |
| Trimethoprim | 16,1 |
| Ciprofloxacin | 93,5 |
| Nalidixic acid | 64,5 |
| Chloramphenicol | 35,5 |
| Azithromycin | 3,2 |
| Colistin | 0,0 |
| Meropenem | 0,0 |
| Tigecycline | 19,4 |

| Multi drug resistance | N = 31 |
|---|---|
| 0 | 0% |
| 1 | 0% |
| 2 | 0% |
| 3 | 3% |
| 4 | 10% |
| 5 | 3% |
| 6 | 29% |
| 7 | 45% |
| 8 | 6% |
| 9 | 3% |
| 10 | 0% |

**Table ESBL08** ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2017.

| Year | CTX-M-1-group[a] | CTX-M-2[b] | CTX-M-8 | CTX-M-9-group[c] | TEM-52 | TEM-20 | SHV-12[d] | CMY-2[e] | 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 |
| 2016[f] | 7 | | | 15 | 2 | | | 10 | | 1 | 36 | 2117 | 1,7 |
| 2017[g] | 3 | | | 23 | | | 1 | 3 | | 1 | 31 | 1697 | 1,8 |
| Total | 103 | 45 | 8 | 58 | 47 | 4 | 11 | 126 | 3 | 2 | 408 | 19481 | 2,1 |

a. *contains $bla_{CTX-M-1}$ (n=70, in all years), $bla_{CTX-M-55}$ (n=8, 2008-2010,2012, 2015), $bla_{CTX-M-15}$ (n=10, 2011-2013 ), $bla_{CTX-M-3}$ (n=3 , 2010, 2012) 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 $bla_{CTX-M-9}$ (n=8, 2008-2009, 2012-2015), $bla_{CTX-M-14}$ (n=6, 2009-2012, 2015) and $bla_{CTX-M-65}$ (n=6, 2013-2015).*

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 sent by NVWA to WBVR for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in BPW and after incubation the culture was centrifuged and DNA isolated from pellet. Five individual samples were pooled and analysed together. 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. RT-PCR was 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 2017 (n=1200) resulted in six *bla*$_{OXA-48}$ -like positive faecal samples in the RT-PCR (three broilers, two slaughter pigs and one dairy cow). *bla*$_{OXA-48}$ -like genes are known to be chromosomally associated with *Shewanella* spp. In three samples the presence of *bla*$_{OXA-48}$ -carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: *bla*$_{OXA-48b}$ (n=2) and *bla*$_{OXA-199}$ (n=1). 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 and 2016 (MARAN 2016 and 2017). 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 2018.

### 4.2.2 Monitoring in companion animals

The prevalence of carbapenemase producing *Enterobacteriaceae* (CPE) in companion animals in Europe is relatively low. CPE have been observed in pet dogs from Germany (Stolle *et al,* 2013), Spain (González-Torralba *et al,* 2016) and France (Melo, *et al,* 2017). Monitoring to detect introduction of CPE in companion animals in the Netherlands was initiated in 2015. The screening for CPE comprised an initial retrospective study and a prospective study. Until 2016, CPE have not been detected in the Netherlands (MARAN 2017). The prospective study was continued in 2017.

Fecal samples of cats and dogs were obtained through the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University. Because the expected prevalence of CPE is low and reported CPE are frequently multi-resistant, the inclusion criterion for dog fecal samples was antibiotic treatment of the animal. Since cats are not frequently treated with antimicrobials, no inclusion criterion was defined and all available fecal samples from cats submitted to VMDC were included in the study. In 2017, 200 fecal samples from cats and 138 fecal 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), $bla_{OXA}$ -groups 23, 24, 51 and 58. (Voets *et al,* 2011) and $bla_{OXA}$ -group-48 (Poirel *et al,* 2004).

In 2017, all fecal samples from cats were negative for CPE. One isolate from dog was positive for *E. coli* harboring $bla_{OXA-48}$. This was the first CPE isolated from a live animal in the Netherlands. The sample was submitted to the diagnostic laboratory of the veterinary faculty (VMDC) for parasitological diagnostics because of chronic diarrhea and the dog had been treated with metronidazole for 10 days. Molecular analysis of the isolate is ongoing but preliminary analysis suggests that the $bla_{OXA-48}$ gene is transferable because located on a mobile element (J. Hordijk, personal communication).

### 4.2.3 Monitoring in imported seafood

In 2017, 56 batches of frozen fish and shrimps originating from fish farms in South-East Asia were screened for the presence of CPE. Two isolates of carbapenemase-producing *Enterobacter cloacae* complex were detected in different batches of frozen shrimps, both exhibiting resistance to carbapenems but not to third generation cephalosporins. The first isolate originated from India (April 2017) and preliminary analysis suggests the presence of a new plasmid located carbapenemase gene (M. Brouwer, personal communication). The second isolate originated from Vietnam (August 2017) and harboured a chromosomally located $bla_{IMI-1}$ embedded in an insertion element (EcloIMEX) (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 fecal contamination. Therefore detection 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 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. Purified DNA of pooled PBW cultures (five samples per pool) from a total of 1200 faecal samples were tested with conventional PCR for the presence of *mcr-1* and *mcr-2* according to EURL-AR protocols (http://www.eurl-ar.eu/233-protocols.htm). 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 fourteen faecal samples (1.2%) from selective culturing in several animal species: veal calves (n=9, 3.0%), broilers (n=3, 1.0%) and slaughter pigs (n=2, 0.7%). Despite an increased test sensitivity, the proportion of *mcr*-positive faecal samples remained low.
In retail meat three randomly isolated colistin resistant *E. coli* [chicken (n=2) and turkey meat (n=1)] were confirmed as *mcr-1* carriers which is indicative for a higher prevalence in poultry meat than in broilers. BPW enrichment from fresh chicken meat indicated 7.7% of the samples (3/39) was positive for *mcr-1* by PCR screening. The higher prevalence in chicken meat compared to faecal broiler samples can be explained by the fact that part of fresh retail meat in Dutch supermarkets originates from other countries where use of colistin in livestock might be the reason for higher *mcr* prevalence in meat. Finally, *mcr-1* was not identified in *Salmonella*.
In summary, *mcr-1* was identified at low-level in *E. coli* from different livestock species and at higher levels in retail meat from chicken and turkey, but not in *Salmonella*.

## References


Bradford *et al;* Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-Lactamases in New York city. Clin Infect Dis. 2004 Jul 1;39(1):55-60

Brouwer M.S.M. *et al; Enterobacter cloacae* complex isolated from shrimps from Vietnam encoding $bla_{IMI-1}$, resistant to carbapenems but not cephalosporins. Antimicrob Agents Chemother. 2018, doi: 10.1128/AAC.00398-18.

Cohen-Stuart *et al;* Guideline for phenotypic screening and confirmation of carbapenemases in *Enterobacteriaceae*. Int J Antimicrob Agents, 2010: 36(3):205-10

Ellington *et al;* Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother. 2007 Feb;59(2):321-2

Geurts Y. *et al*. Development of sensitive and cost-effective Real-time PCR assays for rapid detection of the beta-lactamase genes CTX-M-1, SHV, TEM and AmpC gene CMY-2 in *Enterobacteriaceae*. 2017, 27th ECCMID, Vienna (Austria).

González-Torralba *et al;* Survey of carbapenemase-producing *Enterobacteriaceae* in companion dogs in Madrid, Spain. Antimicrob Agents Chemother, 2016: 60(4):2499-2501

Manchanda *et al;* Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Indian J Med Microbiol. 2011 Jul-Sep;29(3):249-53

MARAN Reports (2002-2017): https://www.wur.nl/nl/Expertises-Dienstverlening/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-Rapporten.htm

Melo *et al;* OXA-48-producing ST372 *Escherichia coli* in a French dog. Antimicrob Chemother. 2017; 72(4):1256-1258

Poirel *et al;* Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004 Jan;48(1):15-22.

Stolle *et al;* Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. J Antimicrob Chemother, 2013; 68(12);2802-2808

Voets *et al;* A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents. 2011 Apr;37(4):356-9

Zong Z. Discovery of $bla_{OXA-199}$, a chromosome-based $bla_{OXA-48}$ -like variant, in *Shewanella xiamenensis*. PLoS ONE 2012; 7: e48280.