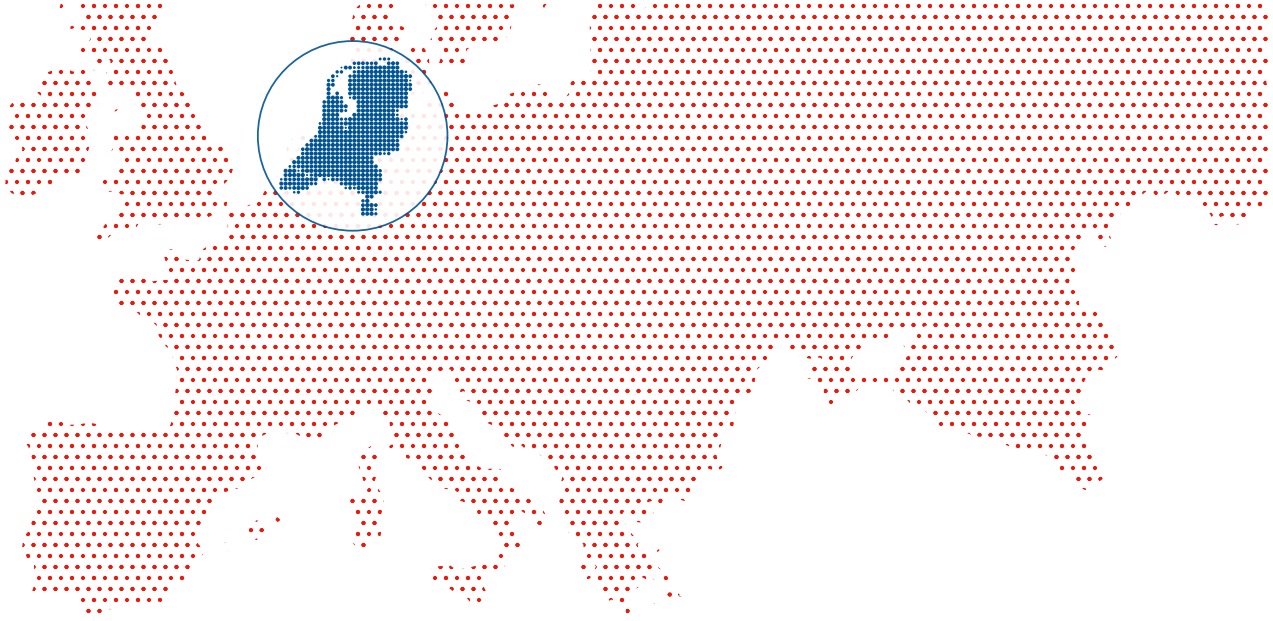
NethMap 2017

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

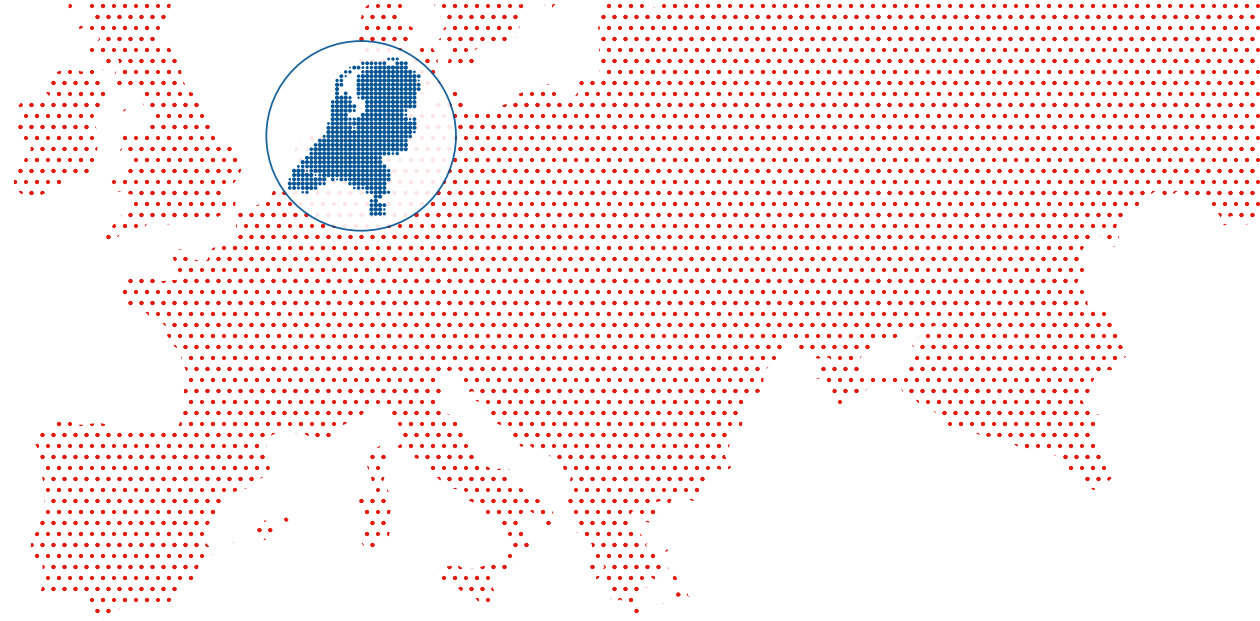


National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

SWAB

MARAN 2017

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2016



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

Food and Consumer Product Safety Authority
Ministry of Economic Affairs, Agriculture and Innovation

WAGENINGEN UNIVERSITY & RESEARCH

Universiteit Utrecht

SDa Autoriteit Diergeneesmiddelen

NethMap 2017

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2016

June 2017

Synopsis

NethMap/MARAN-report

The number of bacteria that are resistant to antimicrobials is increasing worldwide. In the Netherlands, the number of resistant bacteria that can cause infections in humans has remained broadly stable. Nevertheless there is cause for concern and caution. Compared to 2015, in 2016 more 'outbreaks' in healthcare institutions of bacteria that are resistant to last-resort antimicrobials were reported. There is a chance that these bacteria will become more and more common. Although healthy people are not affected, these bacteria can make vulnerable people very sick. If more and more bacteria become resistant to antimicrobials, the treatment options will eventually become limited and it will also become more difficult to treat less serious conditions such as urinary tract infections.

The more antimicrobials are used, the greater the chance that bacteria will develop resistance. In 2016, general practitioners wrote approximately two percent fewer prescriptions for antimicrobials than in 2015. The total use of antimicrobials in Dutch hospitals remained stable in 2015, compared to an increase in antimicrobial use in the previous year. The use of antimicrobials for animals decreased further in 2016 compared to 2015, but has been decreasing more slowly in recent years than it did previously. The degree of bacterial resistance in animals also decreased further.

This is shown in the annual NethMap/MARAN 2017 report, in which various organisations present their data on antimicrobial use and resistance in the Netherlands, for humans as well as animals.

Firstly, to combat resistance, it is important to base the choice to prescribe antimicrobials on the individual patient and the infection concerned. Secondly, it is important that it quickly becomes clear when resistant bacteria are involved and that proper tests are used to determine this. Thirdly, it is important that healthcare providers carefully follow existing hygiene procedures, such as handwashing, in order to prevent resistant bacteria from spreading. For example, thanks to these measures, the number of MRSA bacteria in hospitals has remained low in recent years. This type of 'hospital bacteria' is transmitted via skin-to-skin contact, particularly via the hands, and is insensitive to many types of antimicrobials.

Key words:
Antimicrobial resistance, bacteria, antimicrobial use, infection

Publiekssamenvatting

NethMap/MARAN-rapport

Wereldwijd neemt het aantal bacteriën die resistent zijn tegen antibiotica toe. In Nederland is het aantal resistente bacteriën die bij mensen infecties kunnen veroorzaken, ongeveer stabiel gebleven. Toch blijft er reden voor zorg en oplettendheid. In 2016 zijn er ten opzichte van 2015 meer 'uitbraken' in zorginstellingen gemeld van bacteriën die resistent zijn tegen de antibiotica die als laatste redmiddel worden gebruikt. De kans bestaat dat deze bacteriën nog vaker gaan voorkomen. Gezonde mensen hebben daar geen last van, maar kwetsbare mensen kunnen er ziek van worden. Als steeds meer bacteriën resistent worden tegen antibiotica, worden de behandelmogelijkheden op den duur beperkt en wordt het moeilijker om ook onschuldige kwalen als een blaasontsteking te kunnen behandelen.

Hoe meer antibiotica worden gebruikt, hoe groter de kans dat bacteriën resistent worden. In 2016 hebben huisartsen ongeveer 2 procent minder antibioticakuren voorgeschreven dan in 2015. In Nederlandse ziekenhuizen is het totale gebruik in 2015 stabiel gebleven, in tegenstelling tot een stijging van antibioticagebruik in het jaar ervoor. Het gebruik van antibiotica voor dieren is in 2016 verder gedaald ten opzichte van 2015, maar neemt de laatste jaren minder snel af dan daarvoor. De mate waarin resistente bacteriën bij dieren voorkomen bleek ook verder te zijn afgenomen.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2017, waarin diverse organisaties de gegevens over het antibioticagebruik en -resistentie in Nederland, zowel voor mensen als voor dieren, gezamenlijk presenteren.

Om resistentie tegen te gaan is het van belang de keuze om antibiotica voor te schrijven af te stemmen op de individuele patiënt en de infectie. Ten tweede is het belangrijk dat snel duidelijk wordt wanneer er sprake is van resistente bacteriën en dat goede tests worden gebruikt om dat te bepalen. Ten derde is het van belang dat zorgverleners zorgvuldig de bestaande (hygiëne)maatregelen, zoals handen wassen, naleven om te voorkomen dat resistente bacteriën zich verspreiden. Door op deze manieren te handelen is bijvoorbeeld het aantal MRSA-bacteriën in ziekenhuizen de afgelopen jaren laag gebleven. Deze 'ziekenhuisbacterie' wordt overgedragen via direct huidcontact, vooral via handen, en is ongevoelig voor veel soorten antibiotica.

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 2017 and earlier versions are also available from the website of the SWAB: www.swab.nl.

Members of SWAB's working group on antibiotic stewardship
Prof Dr BJ Kullberg (chair)
Dr E Boel
Prof Dr AW Friedrich
Dr R van Hest
Prof Dr M Hulscher
Dr PD van der Linden
Dr. J ten Oever
Prof Dr J Prins
Dr M Rommers
Prof Dr B Sinha
Prof Dr A Verbon
Dr CM Verduin
Prof Dr H Wertheim
Dr T 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 Prof Dr MC Vos, Dr R Willems, all participants of ISIS-AR, GRAS, *C. difficile* surveillance, anaerobic pathogen surveillance, azole resistance surveillance, the Netherlands Reference laboratory for meningitis in Amsterdam, 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; Amersfoort, Meander MC; Amstelveen, ziekenhuis Amstelland; Amsterdam, AMC; Amsterdam, BovenIJ; Amsterdam , MC Slotervaart; Amsterdam, OLVG Oost; Amsterdam, OLVG West; Amsterdam, VUMC; Apeldoorn, Gelre ziekenhuizen; Arnhem, Rijnstate ziekenhuis; Assen, Wilhelmina ziekenhuis; Bergen op Zoom, Bravis ziekenhuis (locatie Bergen op Zoom); Boxmeer, Maasziekenhuis Pantein; Breda, Amphia ziekenhuis; Den Bosch, Jeroen Bosch ziekenhuis; Den Haag, Haaglanden Medisch Centrum; Den Haag, HAGA ziekenhuizen; Den Helder, Gemini ziekenhuis; Deventer, Deventer ziekenhuis; Doetinchem, Slingerland ziekenhuis; Dordrecht, Albert Schweizer ziekenhuis; Dordrecht; Nij Smellinghe ziekenhuis; Ede, Gelderse vallei; Eindhoven, Catharina ziekenhuis; Eindhoven, Maxima MC; Emmen, Scheperziekenhuis; Enschede, Medisch Spectrum Twente; Geldrop, St. Anna ziekenhuis; Gorinchem, Beatrix ziekenhuis; Gouda, Groene hart ziekenhuis; Groningen, Martini ziekenhuis; Groningen, Refaja; Groningen, UMCG; Haarlem, Spaarne ziekenhuis; Harderwijk, St.Jansdal; Heerenveen, De Tjongerschans; Helmond, Elkerliek; Hoorn, Westfries gasthuis; Leeuwarden, Medisch centrum Leeuwarden; Leiden, Alrijne ziekenhuis; Leiden, LUMC; Lelystad, MC Zuiderzee; Maastricht, MUMC; Nieuwegein, St.Antonius ziekenhuis; Nijmegen, CWZ; Nijmegen, Radboudumc; Oss/Veghel, Bernhoven; Roermond, Laurentius ziekenhuis; Rotterdam, ErasmusMC; Rotterdam, Ikazia ziekenhuis; Rotterdam, Maasstad ziekenhuis; Rotterdam, St.Franciscus gasthuis; Sittard, Zuyderland (locatie Sittard); Terneuzen, ZorgSaam; Tilburg, ETZ (locatie Elisabeth en TweeSteden); Tiel, Ziekenhuis Rivierenland; Utrecht, Diakonessenhuis; Utrecht, UMCU; Venlo, Vie Curi MC; Winterswijk, Streekziekenhuis Koningin Beatrix; Woerden, Zuwe Hofpoort; Zeeland, ADRZ; Zoetermeer, Lange Land ziekenhuis; Zutphen, Gelre ziekenhuizen; Zwolle, Isala klinieken.

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; 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; Enschede, Laboratorium voor Medische Microbiologie Twente/ Achterhoek; Goes, Admiraal De Ruyter Ziekenhuis; Gouda, Groene Hart Ziekenhuis; Groningen, Certe; Haarlem, Streeklaboratorium voor de Volksgezondheid; Heerlen, Zuyderland MC Heerlen; Hilversum, Centraal Bacteriologisch en Serologisch Laboratorium; Leeuwarden, Izore, Centrum Infectieziekten Friesland; Leiden, LUMC; Nieuwegein, St. Antonius Ziekenhuis; Nijmegen, Canisius Wilhelmina Ziekenhuis; Nijmegen, UMC St. Radboud; Roosendaal, Bravis ziekenhuis, locatie Roosendaal; Schiedam, Franciscus Vlietland Ziekenhuis; '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; Woerden, Zuwe Hofpoort Ziekenhuis; Zwolle, Isala

Contents

1 Introduction

This is NethMap 2017, the SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in the Netherlands in 2016 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 2017 extends and updates the information of the annual reports since 2003. Since the introduction of a revised format three 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.

In February 2017, 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 are being set up to improve regional collaboration to control 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 2017) and the veterinary sector (MARAN 2017).

2.1 Most important trends in antimicrobial use

In outpatients

- Compared to 2015, total antibiotic use in outpatients decreased from 10.67 to 10.39 DDD/1000 inhabitant days (DID).
- After an increase last year, the use of amoxicillin stabilised at 2.09 DID.
- The use of tetracyclines decreased substantially with 0.14 DID to 2.11 DID.
- The use of nitrofurantoin, ciprofloxacin and azithromycin remained stable in 2016.

In hospitals

- The inpatient use of antibiotics remained stable in 2015.
- Some remarkable shifts in the use of drugs are seen.
- The use of beta-lactamase resistant penicillins decreased with 0.9 DDD/100 patient-days.
- The use of penicillins with extended spectrum increased with 0.8 DDD/100 patient-days.
- There are large differences in total antibiotic drug use between Dutch hospitals.
- General hospitals used the least antibiotics (77.1 DDD/100 patient-days), whereas university hospitals reported the most (79.2 DDD/100 patient-days).
- Use of second generation cephalosporins increased by 0.4 DDD/100 patient-days, especially in general hospitals.
- Total use of carbapenems remained stable at 1.7 DDD/100 patient-days. However, use of meropenem in university hospitals increased.

In nursing homes

- The mean use of antibiotics increased to 65.3 DDD/1000 residents but varied widely (range 13-130 DDD/1000 residents/day).
- The most frequently used antibiotics remained combinations of penicillins (mainly amoxicillin with clavulanic acid), nitrofurantoin derivatives, and fluoroquinolones with 30%, 18% and 15%, 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 2017.

In GPs

- For most antimicrobials, there are no statistically significant and clinically relevant shifts in resistance levels since 2012.
- 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 *P. mirabilis*, there was a significant and clinically relevant decrease in resistance to amoxicillin/ampicillin in patients aged ≤12 years and to co-amoxiclav in patients aged >12 years to 5% in 2016.
- The percentage of highly resistant microorganisms (HRMO) and multidrug-resistance remained low in all Enterobacteriaceae (≤5%).
- In *S. aureus*, a significant and clinically relevant increase was found for clindamycin including inducible resistance to 9% in 2016.
- Resistance levels for *E. coli* were comparable between geographical regions for most antimicrobials.
- Fosfomycin resistance in *E. coli* in patients >12 years, although still low at 1.4%, increased for the 5th consecutive year.
- Resistance to the antibiotics to treat tuberculosis remained stable over the last 4 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 14% in 2016.

Table 2.2.1 Overview of surveillance programs in the Netherlands in 2016.

Surveillance program[1]	Origin of isolates	availability	Sources 2016	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-	41 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-	77% of STI center attendees	Decentral testing	Gradient testing
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Agar dilution and BACTEC-Mgit 960 (liquid)
					BACTEC-Mgit 960 (liquid breakpoint)
Influenza antiviral drugs	community, GP, nursing home, hospital	2005-	NIVEL GP sentinels, SNIV nursing home sentinels, hospital/ regional laboratories	central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Neuraminidase enzym inhibition assay; for established molecular markers sequencing and/or single nucleotide polymorphism (SNP) PCR
Resistance among anaerobic pathogens	Hospital	2010-	1 lab	Central testing	Etest
Clostridium difficile	Hospital, nursing homes	2005-	23 hospitals	(de)central testing	Gradient testing and 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*

GP: General practitioner; NIVEL: Netherlands Institute for health services research; SNIV: National sentinel surveillance network for infectious diseases in nursing homes; STI: Sexually Transmitted Infections; WHO-CC: World Health Organisation Collaborating Centre

In hospitals

- Compared to 2012, overall resistance rates for many antimicrobials were similar, with a few exceptions, for which statistically significant and clinically relevant increasing or decreasing trends were observed:
  - Outpatient departments: In *K. pneumoniae*, a significant increase was seen in resistance to cefotaxime/ceftriaxone and HRMO.
  - Unselected hospital patient departments: In *Pseudomonas aeruginosa*, a significant decrease in resistance was observed for piperacillin-tazobactam and gentamicin. However, resistance to tobramycin, more active against *P. aeruginosa* was maintained at 1%. In *Acinetobacter* spp. a significant decrease in resistance was observed for co-trimoxazole.
  - Intensive Care Units: In *K. pneumoniae*, resistance to piperacillin-tazobactam and gentamicin decreased significantly. In *E. cloacae*, significant decreases were found for ciprofloxacin, gentamicin, tobramycin, co-trimoxazole and percentage HRMO. In *S. aureus*, a significant decreasing trend was observed for ciprofloxacin and also in coagulase-negative staphylococci resistance to flucloxacillin, ciprofloxacin, and rifampicin decreased significantly.
  - Blood isolates from inpatient departments: In *E. coli*, resistance to co-amoxiclav increased significantly, most likely based on a new test panel for Gram-negative bacteria in VITEK 2 automated systems. In *E. cloacae*, significant decreasing trends were observed for resistance to gentamicin, tobramycin, and co-trimoxazole.
- The percentage of HRMO was highest among *E. coli* and *K. pneumoniae*.
- In 2016, the prevalence of ESBLs was 5.8% in in-patient departments excluding Intensive Care Unit (ICU) and 8.5% in ICU's compared to 5.5% and 7.8% in 2015, respectively.
- The MRSA prevalence in blood culture isolates remained low, 1%.
- Of the 151 CPE producing isolates submitted to the CIb, the most frequently identified carbapenemase encoding genes in Enterobacteriaceae were $bla_{\text{OXA-48}}$, bla_{NDM} and bla_{KPC}. In *P. aeruginosa* this was the bla_{VIM} gene.
- Resistance to azoles in *Aspergillus fumigatus* increased to 12.9% on average and 20.9% in one hospital. This requires serious attention.
- In meningococci penicillin resistance is still sporadic and was not found in the last three years. Likewise, the proportion of moderately susceptible strains did not alter over the last three years (around 12%) and resistance to ceftriaxone was not found.

2.3 Antibiotic use and resistance in veterinary sector

Antibiotic use

- Sales of antimicrobial veterinary medicinal products in 2016 (176 tonnes) showed a remarkable reduction (15%) compared to 2015 (206 tonnes).
- In relation to 2009, the index year used by the Ministry of Economic Affairs, in 2016 total sales decreased by 64.5%. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 69%.
- The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum.

Antimicrobial resistance

- Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S. Enteritidis* (23%), *S. Typhimurium* (18%) and *S. Kentucky* (11%).
- In 2016, the percentage ESBL suspected (cefotaxime MIC > 0.5 mg/L) Salmonella isolates was 1.7%, among eleven different serovars, predominantly isolated from human and poultry sources.
- Ciprofloxacin resistance in Campylobacter isolates from human patients is still high (with a slight decrease in 2016), which is a concern for public health. Resistance to erythromycin, representing macrolides as a first choice antibiotic in human medicine for campylobacteriosis, remained low.
- For *C. jejuni* and *C. coli* from human patients, resistance levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.
- After a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim since 2009 in STEC O157 isolates from humans, in 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15.6% to 14.7%) and trimethoprim (from 14.3% to 8.0%).
- Resistance for the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates.
- In 2016, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers, pigs and dairy cattle and stabilized in veal calves.
- Resistance levels for almost all tested antibiotics were much higher in samples of imported chicken and turkey meat than in samples from retail.
- Resistance levels in *E. coli* were 1.0% in broilers, 0.3% in pigs and 0.8% in rosé veal calves for both cefotaxime and ceftazidime. The 1.0% cefotaxime resistance in broilers was a further decrease in occurrence compared to 2013, 2014 and 2015 (2.7%, 2.9%, and 2.5% respectively).
- ESBL/AmpC-producing *Escherichia coli* represented 0.3% of the randomly isolated *E. coli*, the lowest proportion observed since 2007.
- A follow up of the 2009 study on within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers showed a significant decrease from 66% in 2009 to 38% in 2016.
- The most prevalent ESBL/AmpC gene in *E. coli* from livestock and meat was $bla_{\text{CTX-M-1}}$ in almost all animal species followed by $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-14}}$.
- The colistin resistance gene *mcr-1* was present at low level in *E. coli* from livestock (0.5%) and in retail meat from turkeys (8.3%) and chicken (0.7%).

2.4 Implications for therapy

Overall, no major shifts in resistance rates have occurred in the Netherlands over the last five years, with one exception (see below). The resistance rates in 2016 did not increase further for most antibiotics or even decreased. Yet, there is a continuing concern, in particular for patients on the ICU where 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, and 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 below 2% in *E. coli* indicating suitable use for urinary tract infections.
- The worrisome annual increase in fosfomycin resistance in *E. coli* over the last 5 years, although still low at 1.4%, may reflect a potential problem in the future.
- Clindamycin (inducible) resistance in *S. aureus* has risen to 9%, a value that should be considered relevant when considering clindamycin therapy without culture.
- Resistance to penicillin was 0% in pneumococci and reduced susceptibility 2%, reflecting no major change and no changes in treatment strategy.

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

- The main change is the increase in resistance in *K. pneumoniae*, resistance now 7% to cefotaxime/ceftriaxone and the %HRMO's close to 10%. Patients suspected for *K. pneumoniae* infection are at specific risk of non-adequate treatment.
- Aminoglycoside resistance appears to have decreased slightly again but this does not have implications for therapy at present. High levels of resistance to amoxicillin, co-amoxiclav, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections.
- Piperacillin/tazobactam, cefotaxime/ceftriaxone, ceftazidime and aminoglycoside resistance rates are all between 5 and 10% and in the range that is generally considered to be acceptable for patients not severely ill.
- 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.

Intensive care patients

- Similar to other wards, increase in resistance in *K. pneumoniae* is the main treatment challenge. The %HRMO in this group was 12%. Resistance in Enterobacteriaceae in general were mostly similar or lower than in the previous year.
- Local resistance levels vary significantly, including by time. Tailored therapy and culture remain the mainstay of therapy.

Specific micro-organisms

- The most worrisome development is the increase in azole resistance in *Aspergillus fumigatus* now averaging 12.9% and in one centre exceeding 20%. This indicates that monotherapy of azoles is no longer an option, in particular in high risk patients. Alternatives include combination therapy with an echinocandin, although not proven to be more effective, or liposomal amphotericin B monotherapy.

2.5 Antimicrobial stewardship

After the report of the first pilot study last year, a more extensive report is provided this year. 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 at least 42% of the hospitals. 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. A survey among hospitals indicate that A-teams dedicate significant time to antimicrobial stewardship related activities (mean 0.68 FTE), and significant progress has been made in IV-oral switch and other programs. However, the respondents in the survey also clearly indicate that for full implementation of the program more resources from the hospitals are required to sustain activities.

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. Although carbapenem resistance in *K. pneumoniae* remained at relatively low levels for most countries in 2015, data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that resistance to carbapenems at EU/EEA level significantly increased from a population-weighted mean of 6.2% in 2012 to 8.1% in 2015 in invasive isolates. For *E. coli*, EARS-Net data for 2015 show a much lower EU/EEA population-weighted mean percentage (<0.1%) of carbapenem resistance in invasive isolates. Furthermore, in Europe, third-generation cephalosporin resistance in gram negatives was often seen in combination with fluoroquinolone and aminoglycoside resistance. The EU/EEA trend for this type of combined resistance increased significantly between 2012 and 2015 for both *E. coli* and *K. pneumoniae*.

In the Netherlands, CRE remained a rare occurrence in 2016, but four outbreaks in healthcare settings were described compared to one in the previous year. In 2016, 0.01% of *E. coli* and 0.15% of *K. pneumoniae* were non-susceptible to carbapenems, which was stable in the last years. In general, with a few exceptions, no major shifts in resistance rates have occurred over the last five years in this country. The resistance rates in 2016 did not increase further for most antibiotics. Still, in certain healthcare settings, a rise in third-generation cephalosporin resistance in *K. pneumoniae* and/or combined resistance against fluoroquinolones and aminoglycosides was observed, which requests ongoing attention.

To control the occurrence and spread of HRMO, an integrated approach aimed at antimicrobial resistance, healthcare-associated infections and antimicrobial use at regional, local and national level, is needed. In February 2017, the Ministry of Health sent out a letter on the progress of the actions initiated in 2015 to control antimicrobial resistance. Three major targets have been set up in human healthcare and will be developed further in the coming years. First, the project "Eenheid van Taal" was kicked off in a pilot phase including five healthcare centers, aiming at the development of standardized communication on microbiological, clinical and epidemiological data between stakeholders. Second, the setup of ten Regional Cooperative Networks concerning antimicrobial resistance was initiated at the Ministry's request. The aim of the networks is to fight the spread of antimicrobial resistance at a regional level through communication and cooperation between the involved healthcare settings. Lastly, the national surveillance of antimicrobial resistance, healthcare-associated infections, and antimicrobial use will be further improved. In humans a further reduction in antibiotic use is being pursued.

Conclusions

The data presented in NethMap 2017 demonstrate the importance of adequate surveillance systems to gain insight in 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. However, to target interventions for controlling this global threat the current systems should be developed further and cooperation at a regional and national level is warranted.

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 1000 inhabitants per day (DID).

Results
Total outpatient antibiotic use in 2016 decreased from 10.67 in 2015 to 10.39 DID (Table 3.1.1). The use of tetracyclines decreased with 0.14 DID, to a level of 2.11 DID. After an increase in the use of amoxicillin in 2015, its use stabilised at 2.09 DID in 2016. Concomitantly, the use of amoxicillin with clavulanic acid stabilised in 2016. The total use of macrolides decreased further, while a shift was seen towards using azithromycin to 0.81 DID (+0.02 DID). Within the group of the fluoroquinolones, the use of ciprofloxacin was again stable at 0.6 DID, whereas the use of levofloxacin and other fluoroquinolones decreased further (Figure 3.1.1). Nitrofurantoin use was stable again in 2016.

The total amount of antibiotic use expressed as DID in 2016 was similar to the use back in 2013 (Table 3.1.1). Although use of lincosamides, i.e. clindamycin, is low (0.2 DID), its use has increased over the years and has doubled compared to the situation in 2007.

Discussion
After a marginal increase in 2015, the slight but steady decrease in outpatient antibiotic use in the Netherlands since 2012 continued in 2016. Decreased tetracycline prescribing probably reflects a delayed change caused by adaptation of the national treatment guideline ‘acute cough’. Since 2012, amoxicillin is the preferred antibiotic, because of increasing resistance of *S. pneumoniae* to doxycycline.

Stabilisation in the use of nitrofurantoin and ciprofloxacin is promising, as they are valuable first-line treatments for uncomplicated and complicated urinary tract infection, respectively.

Figure 3.1.1 a-d. Use of antibiotics for systemic use in outpatients, 2007-2016 (Source: SFK).

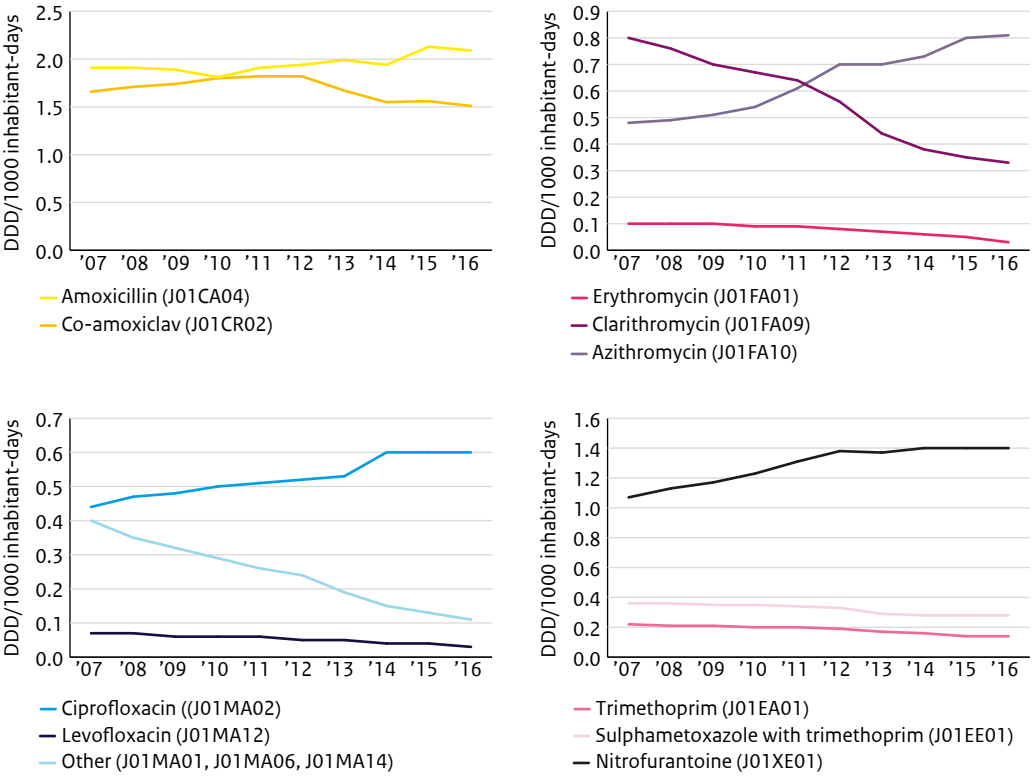
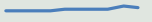


Table 3.1.1 10-years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1000 inhabitant-days), 2007-2016 (Source: SFK).

ATC Group*	Therapeutic group	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
J01AA	Tetracyclines	2.57	2.66	2.67	2.67	2.60	2.49	2.33	2.23	2.25	2.11	
J01CA	Penicillins with extended spectrum	1.91	1.91	1.89	1.81	1.91	1.94	1.99	1.94	2.13	2.09	
J01CE	Beta-lactamase sensitive penicillins	0.46	0.42	0.39	0.37	0.35	0.33	0.31	0.30	0.23	0.24	
J01CF	Beta-lactamase resistant penicillins	0.32	0.36	0.38	0.38	0.39	0.41	0.41	0.44	0.43	0.45	
J01CR	Penicillins + beta-lactamase-inhibitors	1.66	1.71	1.74	1.80	1.82	1.82	1.67	1.55	1.56	1.51	
J01D	Cephalosporins	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	
J01EA	Trimethoprim and derivatives	0.22	0.21	0.21	0.20	0.20	0.19	0.17	0.16	0.14	0.14	
J01EE	Sulphonamides + trimethoprim	0.36	0.36	0.35	0.35	0.34	0.33	0.29	0.28	0.28	0.28	
J01FA	Macrolides	1.39	1.36	1.33	1.31	1.34	1.34	1.22	1.18	1.20	1.17	
J01FF	Lincosamides	0.10	0.11	0.12	0.14	0.15	0.16	0.17	0.18	0.19	0.20	
J01GB	Aminoglycosides	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.00	
J01MA	Fluoroquinolones	0.91	0.89	0.86	0.85	0.82	0.80	0.76	0.79	0.77	0.74	
J01XE	Nitrofuran derivatives	1.07	1.13	1.17	1.23	1.31	1.38	1.37	1.40	1.40	1.40	
J01XX05	Methenamine	0.03	0.02	0.03	0.04	0.03	0.04	0.03	0.03	0.02	0.01	
J01	**Antibiotics for systemic use (total)**	**11.10**	**11.24**	**11.21**	**11.23**	**11.37**	**11.34**	**10.80**	**10.53**	**10.67**	**10.39**	

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

3.2 Hospital care

Methods

Data on the use of antibiotics in Dutch hospitals in 2015 were collected by means of a questionnaire distributed to all Dutch hospital pharmacists. Data were received from 70 hospitals, together with the annual number of bed-days and admissions. 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/1000 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

The inpatient use of antibiotics remained stable in 2015. Total inpatient use of antibiotics, when calculated as DDD/100 patient-days, showed only a slight decrease of -0.8% (from 78.5% to 77.9%). When calculated as DDD/100 admissions, data on hospital antibiotic consumption showed a slight increase of +1.3% (from 326.0% to 330.1%) (Table 3.2.1 and 3.2.2).

Looking in more detail, some notable changes in inpatient use of antibiotics can be seen. The use of beta-lactamase resistant penicillins showed a large decrease (-0.9 DDD/100 patient days), followed by a decrease of -0.6 DDD/100 patient days for fluoroquinolones. In contrast, the use of penicillins with extended spectrum increased further to 9.2 DDD/100 patient days (+0.8 DDD/100 patient days) along with first- and second-generation cephalosporins, which use increased with 0.2 and 0.4 DDD/100 patient days. Meanwhile, use of third generation cephalosporins decreased with -0.2 DDD/100 patient days. In addition, the total use of carbapenems and nitrofurantoin derivates remained stable. Figure 3.2.1 and 3.2.2 show the use per antibiotic subgroup in 2006-2015 in more detail.

Although total antibiotic drug use in the Netherlands is low in general, large variation is seen between Dutch hospitals (Figure 3.2.3 and 3.2.4). Considering site of care, in 2015, general hospitals used the lowest amount of antibiotics (77.1 DDD/100 patient-days), whereas university hospitals reported the highest overall antibiotic use (79.2 DDD/100 patient-days). Differences in total antibiotic consumption between types of hospitals were smaller than last year. Figure 3.2.5 and 3.2.6 show the use per antibiotic subgroup for these different types of hospitals in 2015. The use of combinations of penicillins, mainly amoxicillin with clavulanic acid, is still the highest in general hospitals, with 21.9% versus 17.3%

and 14.5% in large teaching hospitals and university hospitals, respectively. Use of fluoroquinolones, carbapenems and glycopeptides is most situated in university hospitals, whereas most use of penicillins with extended spectrum and nitrofuran derivates comes from general hospitals. Although overall use of carbapenems remained stable, the use of meropenem increased, especially in university hospitals (figure 3.2.6B).

University hospitals use most third generation cephalosporins and large teaching hospitals reported the most first- and second-generation cephalosporin use (Figure 3.2.6 and 3.2.7). In general hospitals the use of second generation cephalosporins increased with 1.17 DDD/100 patient-days to 5.83 DDD/100 patient-days (Figure 3.2.6A). First- and second generation cephalosporins are mainly represented by cefazolin and cefuroxime. Ceftriaxone is the antibiotic of the third generation cephalosporins that is most used, although within this group more different agents are used, especially in university hospitals (Figure 3.2.7).

More than three quarter of the antimycotics (J02), antimycobacterials (J04) and antivirals (J05) for systemic use were used in university hospitals (data not shown). In table 3.2.3 use of antimycotics (J02), antimycobacterials (J04) and antivirals (J05) in university hospitals is provided from the years 2007 to 2015, expressed in DDD/100 patient-days. As in 2014, the use of antimycotics increased further, mainly due to increased use of amphothericin B. The use of antimycobacterials remained stable. Since 2012, the use antivirals was relatively stable, but increased in 2015. Increases were seen in use of neuraminidase inhibitors, nucleoside reverse transcriptase inhibitors and other antivirals (a group encompassing also integrase inhibitors and antivirals for treatment of hepatitis C).

In 2016 PREZIES data were received from 31 hospitals, including 8646 patients of which 2902 received antibiotics, with a total of 3774 prescriptions. Antibiotic use divided by surgical versus medical prophylaxis and hospital versus community acquired infections is depicted in Figure 3.2.8. Most often used antibiotics were amoxicillin with clavulanic acid (18%), ciprofloxacin (12%) and cefuroxime (10%). For treatment of nosocomial infections use of cefuroxime doubled compared to its use in 2015 (from 7% to 14%) and also the use of meropenem increased from 2% to 3%. Cefazolin was used in 54% of cases in surgical prophylaxis. Use for medical prophylaxis was more diverse.

Discussion

In 2015, antibiotic use in hospitals remained stable compared with 2014, although further intensification of the use of antibiotics based on DDDs per admission continues. Moreover, we observed a large variation in total antibiotic use between Dutch hospitals. Despite the stable consumption, there are significant shifts between different subgroups of antibiotics. Mainly, the use of cephalosporins continues to rise. Although we observed an overall decrease in use of third-generation cephalosporins, again more first- and especially more second-generation cephalosporins were used. In addition, this year we saw a slight increase in the use of meropenem. Encouraging however is the overall decrease in beta-lactamase resistant penicillins and fluoroquinolones.

Table 3.2.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2006-2015 (Source: SWAB).

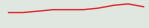
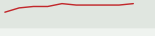
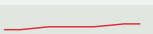
ATC group*	Therapeutic group	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
J01AA	Tetracyclines	1.6	1.4	1.7	1.6	1.7	1.8	1.7	1.7	1.9	1.9	
J01CA	Penicillins with extended spectrum	7.6	7.3	6.5	7.6	7.3	7.3	7.6	8.0	8.4	9.2	
J01CE	Beta-lactamase sensitive penicillins	1.4	1.2	1.3	1.6	1.5	1.5	1.7	1.9	2.4	2.4	
J01CF	Beta-lactamase resistant penicillins	5.9	5.7	6.4	6.6	6.8	6.7	7.1	8.1	8.7	7.7	
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	15.1	14.5	16.2	16.5	16.0	15.8	15.0	14.8	14.5	14.3	
J01DB	First-generation cephalosporins	2.0	2.6	2.6	3.0	3.0	3.5	3.6	3.7	4.4	4.6	
J01DC	Second-generation cephalosporins	3.8	2.8	3.0	3.6	3.4	3.7	4.1	4.7	5.0	5.3	
J01DD	Third-generation cephalosporins	2.7	3.0	3.2	3.5	3.7	3.9	4.4	5.0	5.7	5.5	
J01DH	Carbapenems	0.6	0.8	1.0	1.1	1.2	1.4	1.5	1.7	1.6	1.7	
J01EA	Trimethoprim and derivatives	0.8	0.5	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.3	
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.1	2.3	2.4	2.0	2.0	1.9	1.8	1.9	1.9	1.8	
J01FA	Macrolides	2.5	2.8	2.7	2.6	2.7	2.9	2.8	2.6	2.9	2.7	
J01FF	Lincosamides	2.0	2.1	2.1	2.4	2.3	2.3	2.2	2.3	2.3	2.4	
J01GB	Aminoglycosides	2.5	2.6	3.9	4.2	4.1	3.9	3.3	3.5	3.6	3.7	
J01MA	Fluoroquinolones	8.0	7.6	8.8	9.3	9.0	9.2	8.9	8.6	9.0	8.4	
J01XA	Glycopeptides	0.7	1.0	1.1	1.3	1.3	1.3	1.4	1.5	1.6	1.6	
J01XB	Polymyxins	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.2	0.2	0.2	

Table 3.2.1 (continued) Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2006-2015 (Source: SWAB).

ATC group*	Therapeutic group	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
J01XD	Imidazole derivatives	1.7	1.8	1.7	1.8	1.9	2.2	2.3	2.6	2.6	2.6	
J01XE	Nitrofuran derivatives	1.0	1.1	1.2	1.1	1.2	1.2	1.2	1.3	1.6	1.4	
J01XX08	Linezolid	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
	other antibacterials	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	
J01	**Antibiotics for systemic use (total)**	**62.3**	**61.6**	**66.8**	**70.9**	**70.2**	**71.3**	**71.3**	**74.7**	**78.5**	**77.9**	
	expressed in DDD/100 admissions:											
J01	**Antibiotics for systemic use (total)**	**335.9**	**337.5**	**344.7**	**321.3**	**315.9**	**306.4**	**295.7**	**307.8**	**326.0**	**330.1**	

* *From the 2015 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.2.1 Use of beta-lactams in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2006-2015 (Source: SWAB).

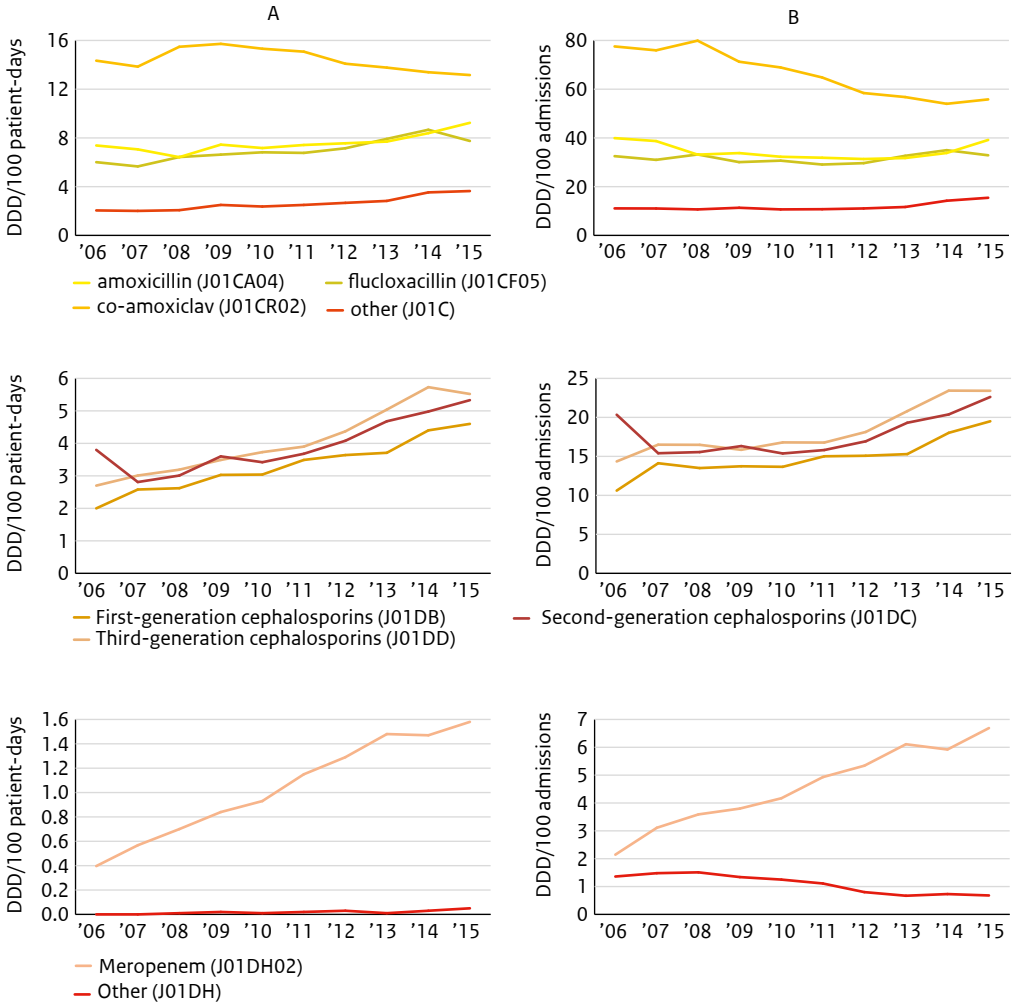


Table 3.2.2 10-years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1000 inhabitant-days), 2006-2015 (Source: SWAB).

ATC Group*	Therapeutic group	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	0.027	0.025	0.023	0.025	0.027	0.026	0.024	0.022	0.023	0.025
J01CA	Penicillins with extended spectrum	0.113	0.110	0.101	0.111	0.110	0.103	0.100	0.099	0.101	0.118
J01CE	Beta-lactamase sensitive penicillins	0.022	0.020	0.019	0.023	0.023	0.020	0.023	0.023	0.028	0.028
J01CF	Beta-lactamase resistant penicillins	0.091	0.087	0.086	0.093	0.097	0.089	0.093	0.100	0.105	0.097
J01CR	Penicillins + beta-lactamase-inhibitors	0.239	0.233	0.229	0.241	0.256	0.223	0.211	0.199	0.187	0.186
J01DB	First-generation cephalosporins	0.032	0.035	0.034	0.040	0.042	0.045	0.049	0.047	0.052	0.055
J01DC	Second-generation cephalosporins	0.056	0.051	0.045	0.051	0.055	0.050	0.052	0.055	0.058	0.065
J01DD	Third-generation cephalosporins	0.039	0.037	0.040	0.047	0.050	0.050	0.057	0.062	0.066	0.067
J01DH	Carbapenems	0.009	0.010	0.011	0.014	0.015	0.018	0.019	0.020	0.019	0.021
J01EA	Trimethoprim and derivatives	0.009	0.009	0.007	0.007	0.009	0.006	0.005	0.004	0.003	0.003
J01EE	Sulphonamides + trimethoprim	0.034	0.033	0.029	0.030	0.030	0.026	0.024	0.024	0.022	0.021
J01FA	Macrolides	0.040	0.040	0.037	0.039	0.041	0.037	0.038	0.034	0.034	0.034
J01FF	Lincosamides	0.031	0.031	0.029	0.033	0.035	0.032	0.031	0.032	0.028	0.030
J01GB	Aminoglycosides	0.039	0.041	0.048	0.055	0.058	0.054	0.044	0.045	0.044	0.046
J01MA	Fluoroquinolones	0.121	0.124	0.139	0.129	0.138	0.127	0.124	0.116	0.112	0.112
J01XA	Glycopeptide antibacterials	0.011	0.011	0.012	0.015	0.016	0.017	0.017	0.018	0.018	0.019
J01XB	Polymyxins	0.005	0.006	0.008	0.009	0.006	0.003	0.002	0.003	0.002	0.003
J01XD	Imidazole derivatives	0.027	0.027	0.025	0.026	0.030	0.027	0.029	0.030	0.030	0.032
J01XE	Nitrofuran derivatives	0.016	0.018	0.016	0.017	0.018	0.015	0.018	0.016	0.018	0.018
J01XX08	Linezolid	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
	other antibiotics	0.002	0.002	0.003	0.003	0.003	0.002	0.003	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**0.965**	**0.952**	**0.941**	**1.008**	**1.061**	**0.971**	**0.963**	**0.950**	**0.953**	**0.982**

Figure 3.2.2 Use of macrolides, aminoglycoside, fluoroquinolones and glycopeptides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2006-2015 (Source: SWAB).

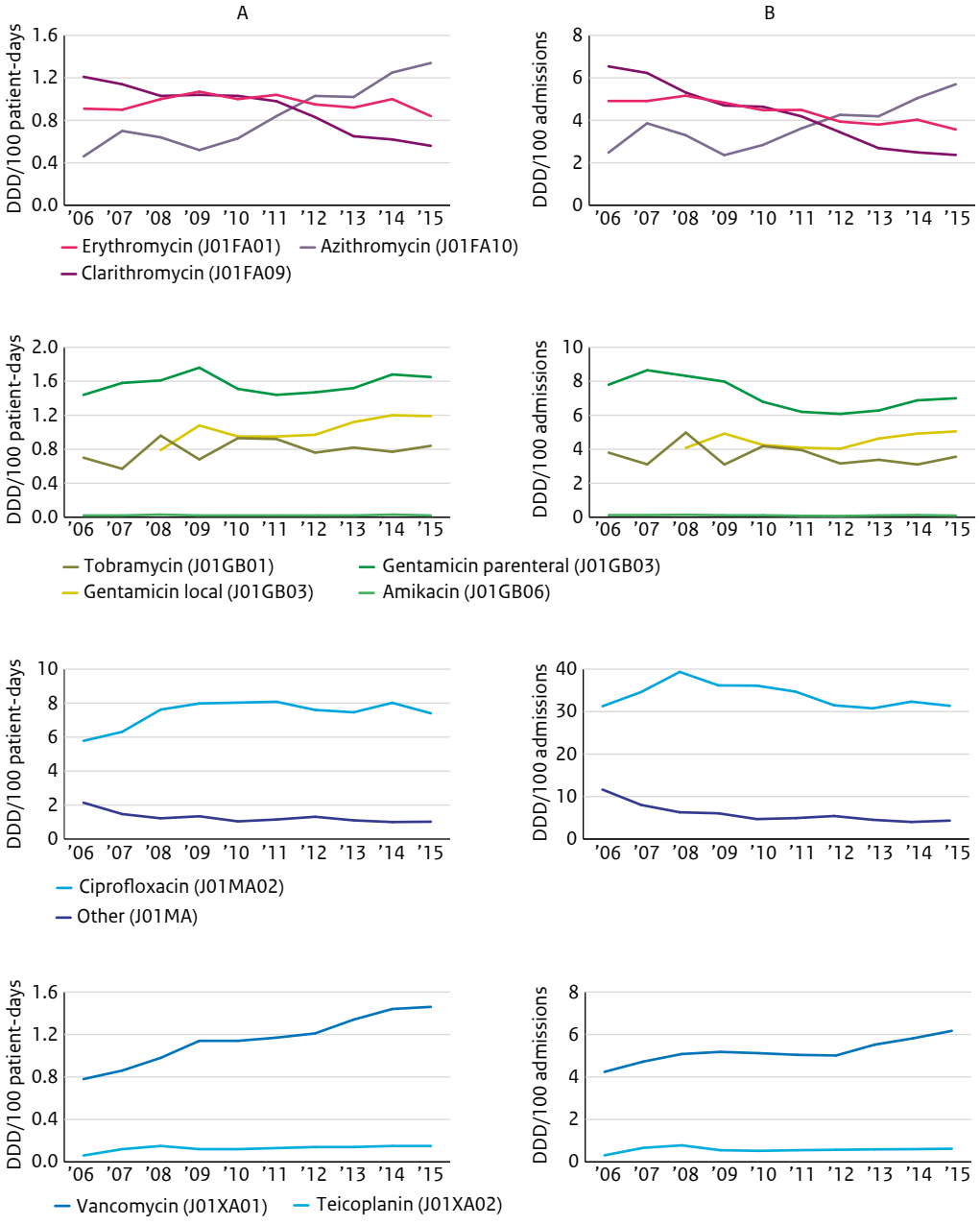


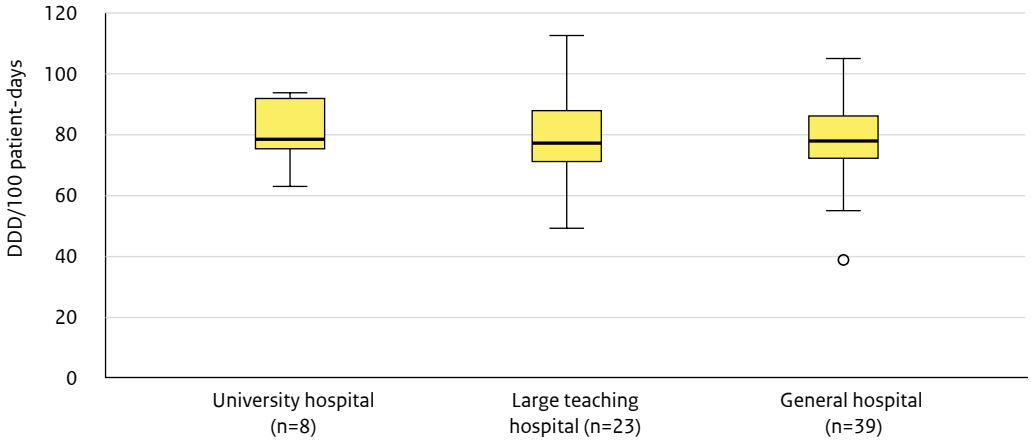
Table 3.2.3 Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2007-2015 (Source: SWAB).

ATC group *	Therapeutic group	2007	2008	2009	2010	2011	2012	2013	2014	2015
J02AA01	Antibiotics (amphotericin B)	4.44	1.12	1.35	1.65	1.77	2.43	3.01	3.46	4.17
J02AB02	Imidazole derivatives (ketoconazole)	0.12	0.11	0.08	0.15	0.09	0.10	0.06	0.24	0.34
J02AC	Triazole derivatives	5.18	6.36	6.72	6.31	5.83	6.25	6.29	7.15	7.55
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
J02	**Antimycotics for systemic use (total)**	**9.93**	**7.98**	**8.77**	**8.66**	**8.26**	**9.33**	**10.06**	**11.47**	**12.70**
J04AA	Aminosalicylic acid and derivatives	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
J04AC	Hydrazides (mainly isoniazide)	0.39	0.29	0.40	0.34	0.30	0.40	0.57	0.56	0.35
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.12
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
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.04	0.07
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
J04	**Antimycobacterials for systemic use (total)**	**2.74**	**2.33**	**2.35**	**2.58**	**2.62**	**2.57**	**2.88**	**2.87**	**2.76**
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
J05AD	Phosphonic acid derivatives (J05AD)	0.06	0.11	0.13	0.10	0.10	0.15	0.12	0.16	0.14
J05AE	Protease inhibitors (J05AE)	0.70	0.92	0.75	0.78	0.55	0.81	0.63	0.40	0.33
J05AF	Nucleoside reverse transcriptase inhibitors (J05AF)	0.83	0.74	0.64	0.67	0.63	0.69	0.54	0.59	0.71
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
J05AH	Neuraminidase inhibitors (J05AH)	0.02	0.05	n.a.#	0.21	0.42	0.19	0.49	0.16	0.30
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
J05AX	Other antivirals (J05AX)	0.00	0.06	0.06	0.15	0.17	0.24	0.29	0.22	0.33
J05	**Antivirals for systemic use (total)**	**3.86**	**4.65**	**4.59**	**4.91**	**4.89**	**5.41**	**5.47**	**5.37**	**5.75**

* *from the 2015 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

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

Figure 3.2.3 Total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals in 2015 (Source: SWAB).



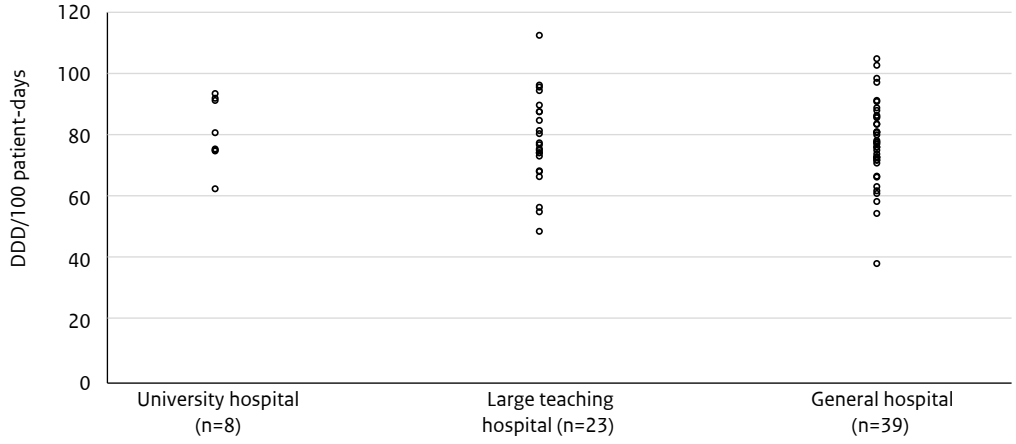


Figure 3.2.4 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2015 (Source: SWAB).

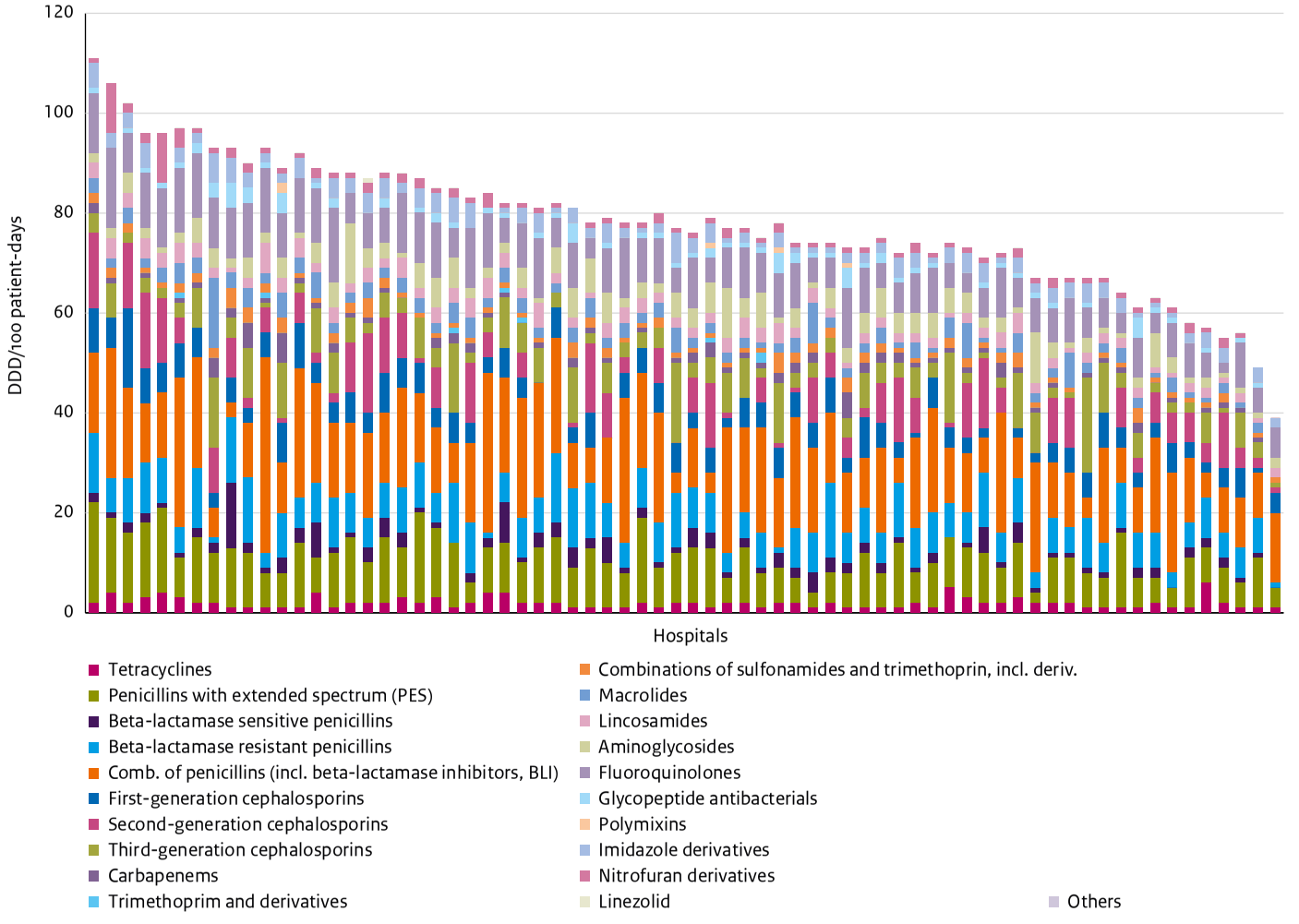


Figure 3.2.5 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2015 (Source: SWAB).

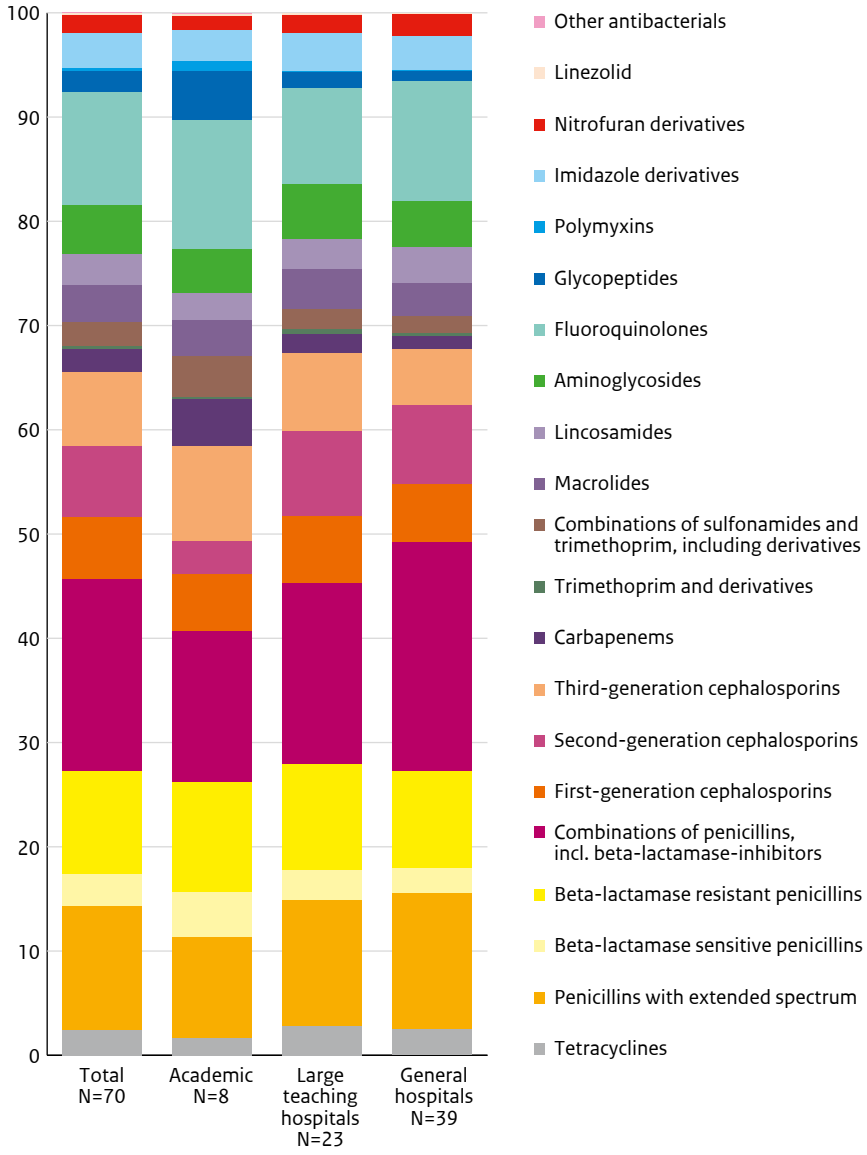


Figure 3.2.6 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days,2006-2015 (Source: SWAB).

Figure 3.2.6 (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, 2006-2015 (Source: SWAB).

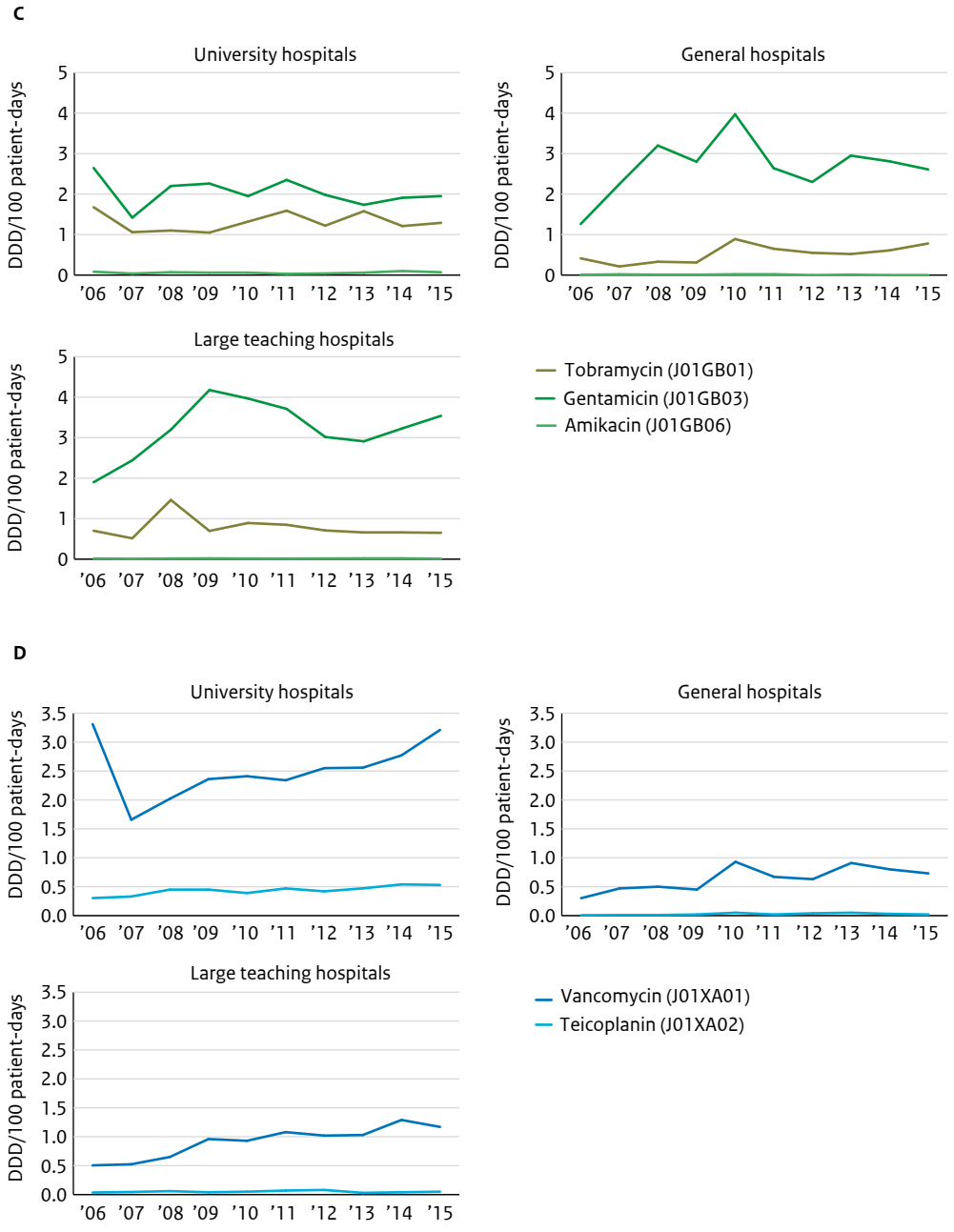


Figure 3.2.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2006-2015 (Source: SWAB).

E

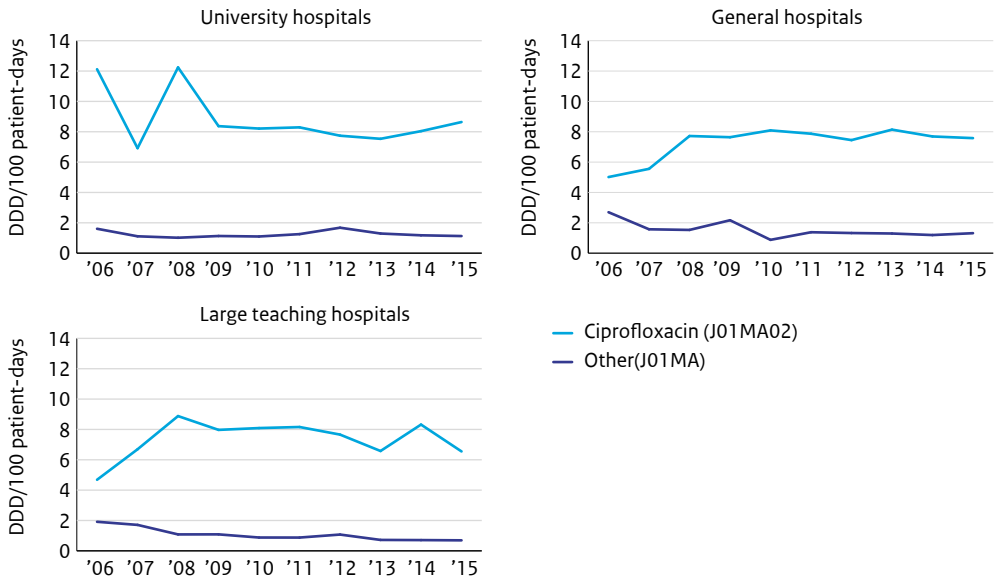


Figure 3.2.7 Use of 1st (A), 2nd (B) and 3th (C) generation cephalosporins in university, large teaching and general hospitals at ATC5 level in 2015 (source: SWAB).

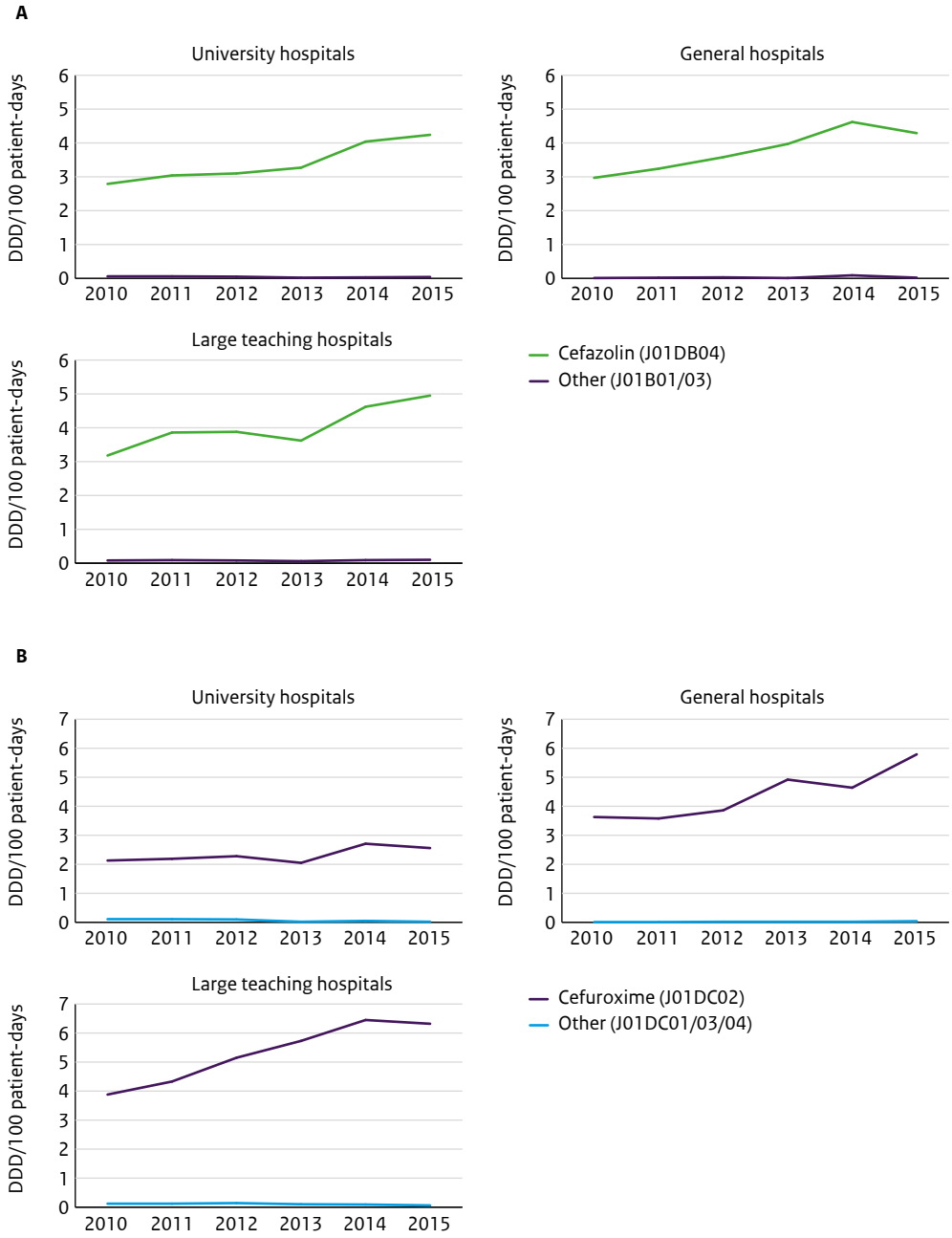


Figure 3.2.7 (continued) Use of 1st (A), 2nd (B) and 3th (C) generation cephalosporins in university, large teaching and general hospitals at ATC5 level in 2015 (source: SWAB).

C

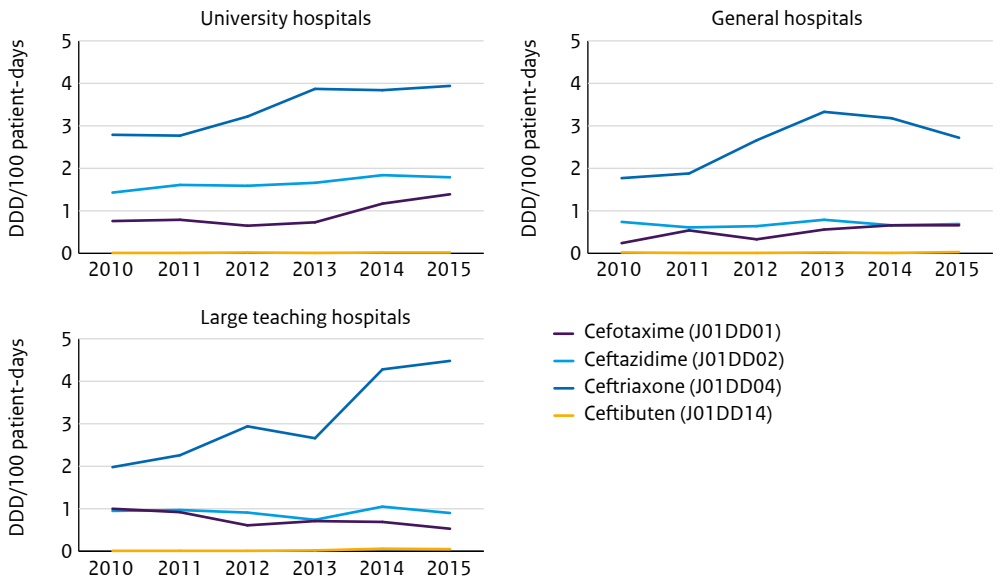
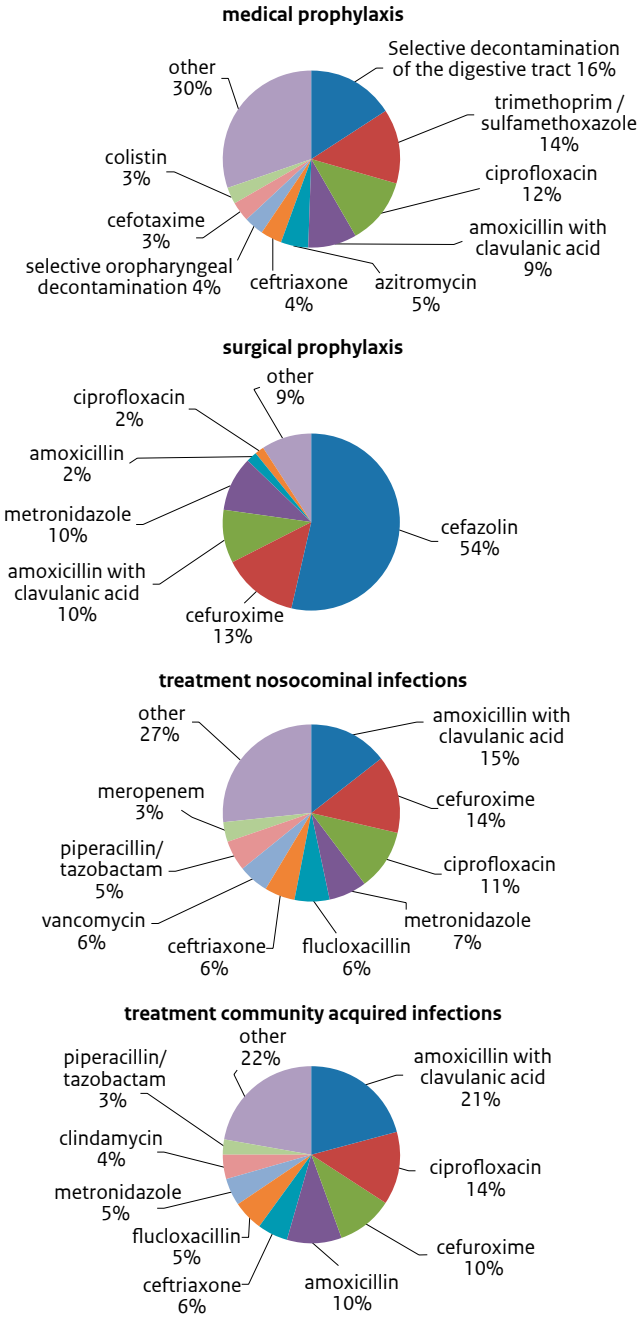


Figure 3.2.8 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2016 (source: PREZIES).



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/1000 residents/day was calculated, and their weighed mean was calculated.

In nursing homes of the SNIV network of RIVM, a prevalence study was performed according to the same method as described above under 3.2 (PREZIES), with the exception that systemic antibacterials (ATC-code J01) were included with a maximum of four concomitant substances per patient.

Results

The antibiotic use of 9862 residents of nursing homes was included in data analysis for 2015. The size of nursing homes varied from 15 to 1100 residents per home, with a mean of 411 residents. The mean antibiotic use increased by 8 DDD/1000 residents/day to 65.3 DDD/1000 residents/day. The use varied hugely with a minimum of 13 and a maximum of 130 DDD/1000 residents/day. As in 2014, combinations of penicillins (mainly amoxicillin with clavulanic acid), with 19.6 DDD/1000 residents/day, nitrofurantoin derivates (11.7 DDD/1000 residents/day) and fluoroquinolones (9.5 DDD/1000 residents/day) were most frequently used (Table 3.3.1).

Figure 3.3.1 depicts antibiotics used in the SNIV prevalence study in 74 nursing homes in 2016. A total of 7914 residents were participating, of which 486 patients on antibiotics, with a total of 515 prescriptions. Nitrofurantoin is used most frequently (21% of total patients on antibiotics) and mainly represents prophylactic use in revalidation and psychogeriatric care (Figure 3.3.1A and B). Amoxicillin with clavulanic acid comprises 15% of total patients on antibiotics and is used most for treatment of infections (Figure 3.3.1A).

Discussion

Compared with previous years, more or less the same pattern of usage is seen. The most frequently used antibiotic is amoxicillin with clavulanic acid (30 %), followed by nitrofurantoin (18%) and fluoroquinolones (15%). 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, 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.

Table 3.3.1 Distribution of the use of antibiotics (J01) in nursing homes, expressed as DDD/1000 residents/day, 2011-2015 (Source: SWAB).

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

* *From the 2015 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.3.1 A Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2016 (source: SNIV).

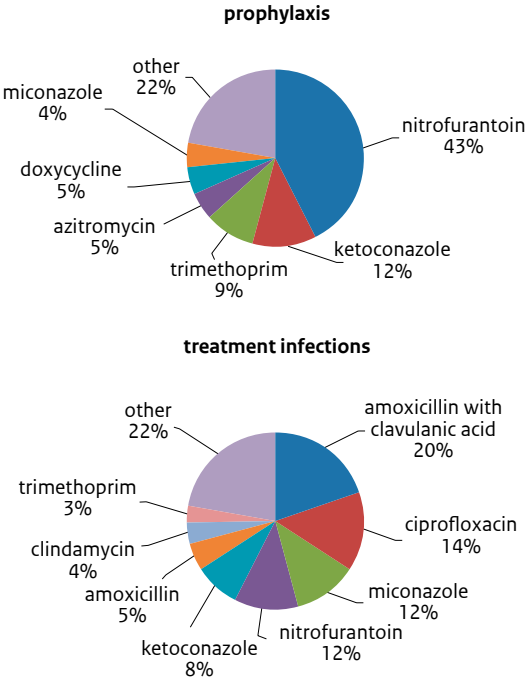
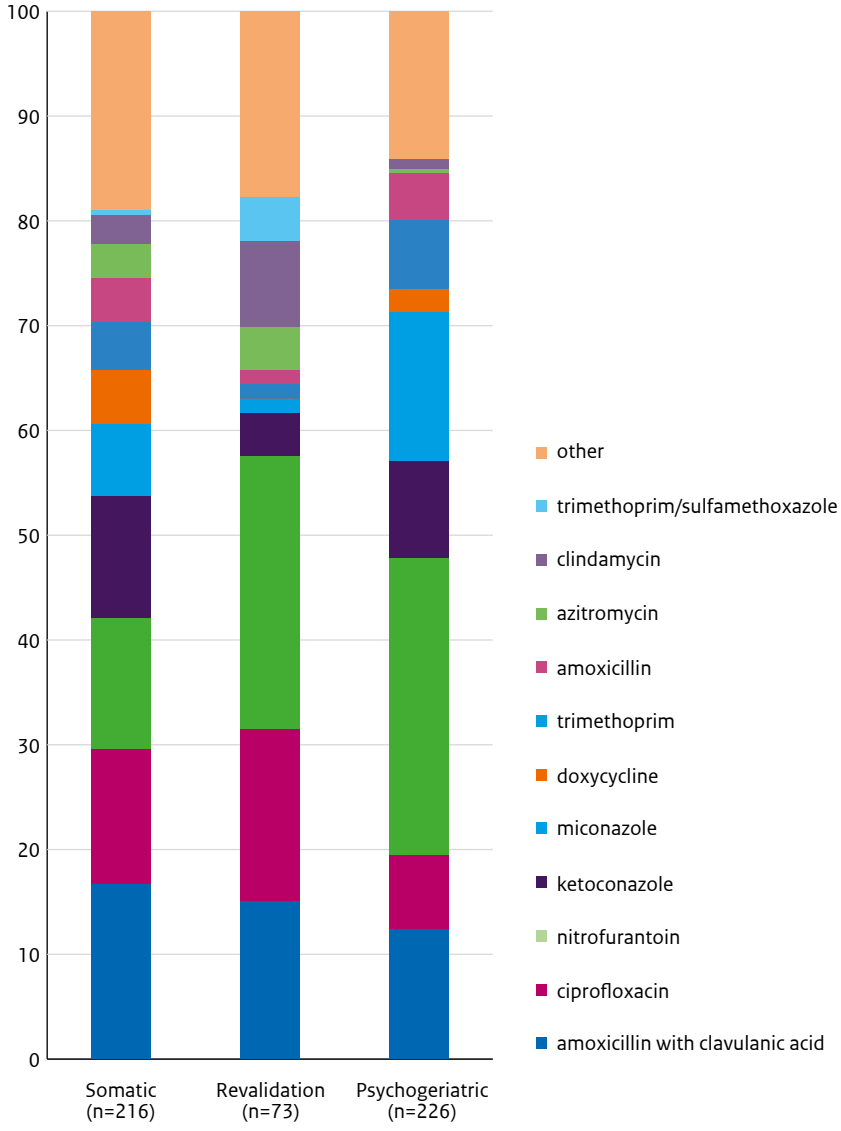


Figure 3.3.1 B Comparison of the distribution of antibiotic usage (J01) in nursing homes in somatic, revalidation and psychogeriatic care in 2016 (source: SNIV).



3.4 Comparison between sites of care

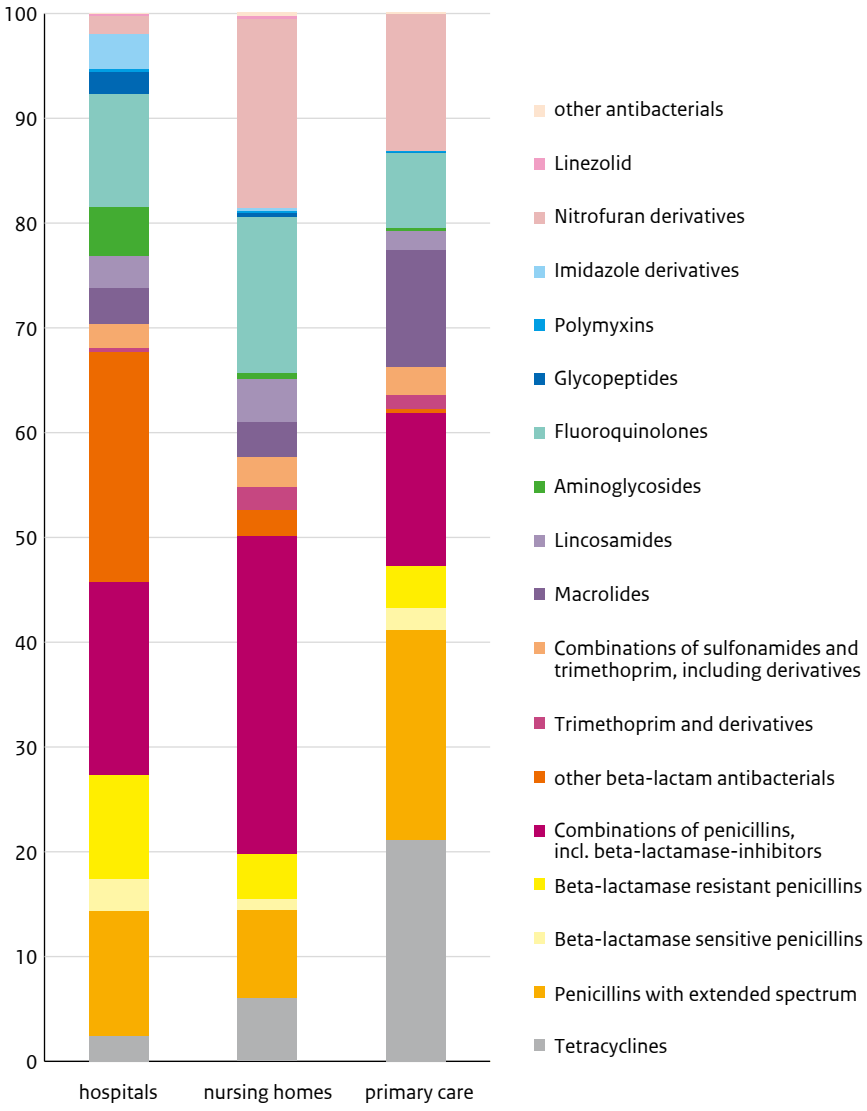
A comparison between the distribution of antibiotic usage expressed as percentage of total antibiotic use in hospitals, nursing homes and in primary care in 2015 is depicted in Figure 3.4.1. Combinations of penicillins, including beta-lactamase inhibitors, and nitrofurantoin derivates make up a larger part of total antibiotic use in nursing homes compared to primary care. Also notable is the relatively lower use of tetracyclines (6%) in nursing homes compared to primary care. In hospitals antibiotic use is more varied and, as expected, relatively more intravenous antibiotics, e.g. cephalosporins, and less oral antibiotics, such as nitrofurantoin derivates and tetracyclines, are used.

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.

Figure 3.4.1 Comparison of the distribution of antibiotic usage (J01) in primary care, hospital care and care in nursing homes in 2015.



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 Dutch medical laboratories, 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 2016, ISIS-AR received data from 41 laboratories of which 25 laboratories had complete data over the five most recent years (2012 to 2016). Three of these laboratories served university hospitals, 21 laboratories served non-university hospitals and general practitioners and one laboratory only served general practitioners and long-term care facilities. To avoid bias in time trends due to incomplete data we used data from these 25 laboratories only for most analyses in the current report. We calculated resistance percentages and linear time trends over the five most recent years (2012 to 2016) 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 (*Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) we used data from 29 laboratories for which at least complete data for the year 2016 were available, and that were known to use EUCAST testing guidelines (3 serving university hospitals, 24 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* isolates from

general practitioner's patients an extra analysis was conducted to calculate resistance to a selection of antibiotics in 2016 by NUTS3-region. For this analysis we used data from a separate set of 26 non-university laboratories for which at least complete data for the year 2016 were available.

Selection of isolates

Resistance levels and, if applicable, time trends were calculated as the percentage resistant isolates 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 and long-term care facilities (chapters 4.2 and 4.4) we selected urinary isolates for analysis of resistance in Enterobacteriaceae, and wound/pus for analysis of resistance in *Staphylococcus aureus*. For urology departments (chapter 4.3.5) we selected only urinary isolates. For the outpatient departments (chapter 4.3.1), inpatient departments (excl. intensive care units, chapter 4.3.2), and intensive care units (chapter 4.3.3), the selected isolates originated from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound/pus. Additionally, we conducted a separate analysis for blood isolates from inpatients (incl. patients from intensive care units, chapter 4.3.4). 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.
For the calculation of resistance levels and time trends, we selected the first isolate per species per patient per year per site to avoid bias due to multiple testing. 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 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 variance in breakpoint guidelines and expert rules used in the participating laboratories, these calculations were conducted using reinterpreted MIC values from automated susceptibility test systems or gradient tests according to EUCAST 2016 breakpoints. However, in 2016 BioMérieux introduced a new testpanel to test resistance of Gram-negative microorganisms in VITEK2. In this testpanel resistance to co-amoxiclav is tested according to the testing guidelines from EUCAST, 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 testing 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 microorganisms in 2016 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 the data because the maximum test value of >16 mg/L that can be measured by VITEK2 (BioMérieux), which is the automated system used by most laboratories, does not reach the resistance breakpoint of >32 mg/L. Therefore, in every chapter in the current report 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 staphylococci (CNS) including *Staphylococcus epidermidis*) at least 75% of the reported MICs were reinterpretable according to EUCAST 2016 clinical breakpoints. When reinterpretation could not be achieved, this was because of a lack of crude data or an MIC that was not compatible with 2016 breakpoints. For *Enterococcus faecium*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* less than 75% of MICs could be reinterpreted. Therefore, for these pathogens calculation of resistance percentages was based on the S/I/R interpretations of all isolates, as reported by laboratories known to have used EUCAST testing guidelines in 2016.
Because results of inducible clindamycin resistance tests were not available in ISIS-AR, two different values for clindamycin resistance in *S. aureus* and coagulase-negative staphylococci are presented. The first value was based on reinterpreted MIC-values, which do not show inducible resistance, and the second value on laboratory S/I/R interpretation in which 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 staphylococci was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin interpretation was available, for oxacillin/flucloxacillin.

In some tables, resistance levels are presented for a combination of agents to which 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, 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 + ciprofloxacin, and tobramycin + ceftazidime). For these combinations, resistance was defined as resistance to both agents.
For *S. aureus* and coagulase-negative staphylococci resistance to ciprofloxacin was calculated as class indicator for resistance against 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 urinary isolates from general practices, outpatient departments, urology departments, and long-term care facilities, multidrug resistance in Enterobacteriaceae was calculated, defined as resistance to the oral agents co-trimoxazole, co-amoxiclav and ciprofloxacin combined.

Calculation of time trends

In addition to resistance levels in 2016, we calculated time trends over the five most recent years (2012 to 2016), using logistic regression. Because adoption of new guidelines or changes in breakpoints can have a substantial effect on resistance levels, we only analysed trends for those species for which ≥75% of MICs each year were interpretable using EUCAST clinical breakpoints (i.e. *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus aureus* and coagulase-negative staphylococci including *Staphylococcus epidermidis*). We do not expect spurious time trends in flucloxacillin resistance in staphyloccocci, even though resistance percentages for this agent are based on laboratory S/I/R interpretation for cefoxitin or oxacillin/ flucloxacillin. In testing guidelines of CLSI and EUCAST there were no differences in breakpoints and in both guidelines breakpoints were not changed between 2012 and 2016, except for *S. saprophyticus* for which the diameter breakpoint in the EUCAST guideline was changed from 25 to 22 in 2013 and an MIC breakpoint was added in 2015. However, *S. saprophyticus* comprised only 0.2% (ICU), 0.4% (blood isolates) and 1.2% (inpatient excluding intensive care units) of our datasets. We do therefore not expect that use of different versions of the testing guidelines will influence the time trend. Almost the same holds for clindamycin including inducible resistance. However, for clindamycin MIC-values between 0.5 and 4 mg/L are considered intermediate by CLSI but resistant by EUCAST. We avoided causing spurious time trends because of laboratories adopting EUCAST testing guidelines instead of CLSI testing guidelines by changing the interpretation from intermediate to resistant if the MIC value was between 0.5 and 4 mg/L.

Two sided p-values <0.05 were considered statistically significant. If resistance in 2016 was below 10%, a change of ≥2.5% in the last 5 years was considered clinically relevant. If resistance in 2016 was above 10%, 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 percentage resistant isolates was between 0.5% and 30% in at least three years the resistance levels from 2012 to 2016 are shown in graphs.

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. For some laboratories that were connected to the ISIS-AR surveillance system data could not be included in the analyses in chapters 4.1 through 4.3 (see methods section for inclusion criteria). Therefore, connected laboratories are shown in separate colours, based on inclusion status. In figure 4.1.2.2 the percentage of residents for whom at least one isolate was included in the analyses in chapters 4.1 through 4.3 is shown by 4-digit postal code area. In figure 4.1.2.3 the same is presented for isolates from general practitioner's patients that were used to calculate regional resistance levels in chapter 4.2. In table 4.1.2.1 descriptive characteristics are compared between included and excluded laboratories from the ISIS-AR system. In table 4.1.2.2 more detailed descriptive characteristics from included laboratories only are listed by pathogen. Finally, the age distribution of patients included in the analyses is presented in figure 4.1.2.4 by type of care.

Figure 4.1.2.1 Geographical distribution of laboratories by connection status.

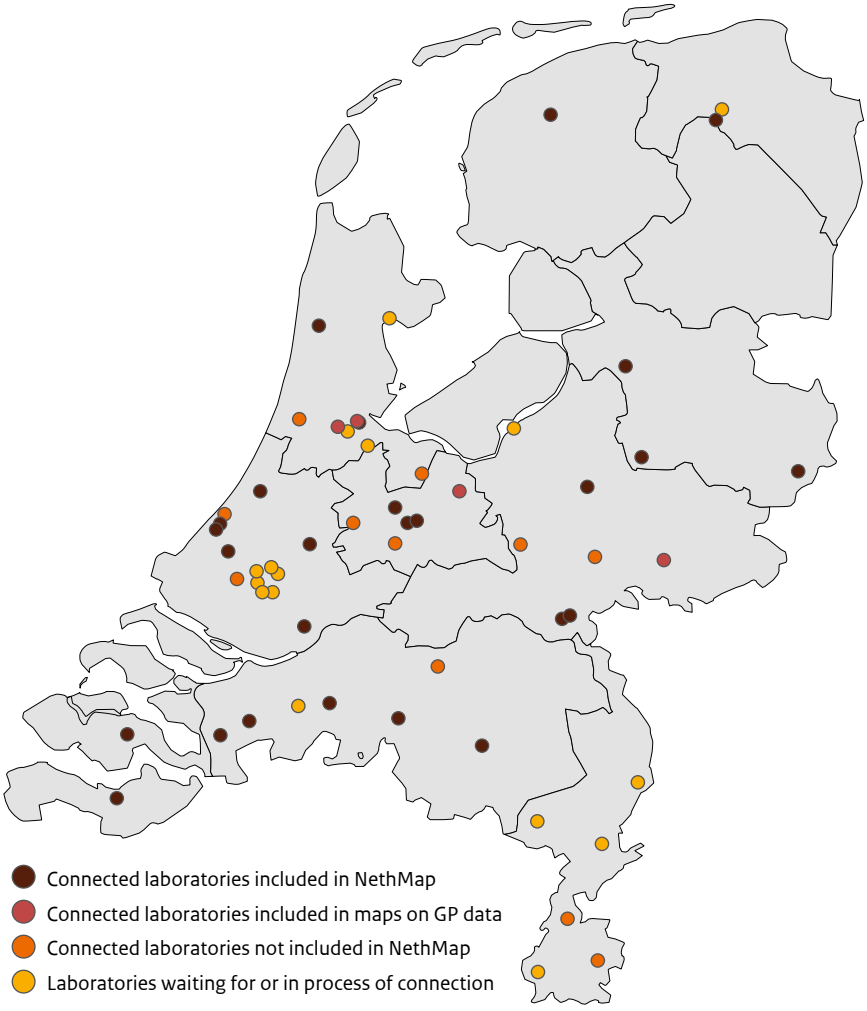


Figure 4.1.2.2 Percentage of residents for whom at least one isolate was included in the analyses in chapters 4.1 through 4.4, by 4-digit postal code area.

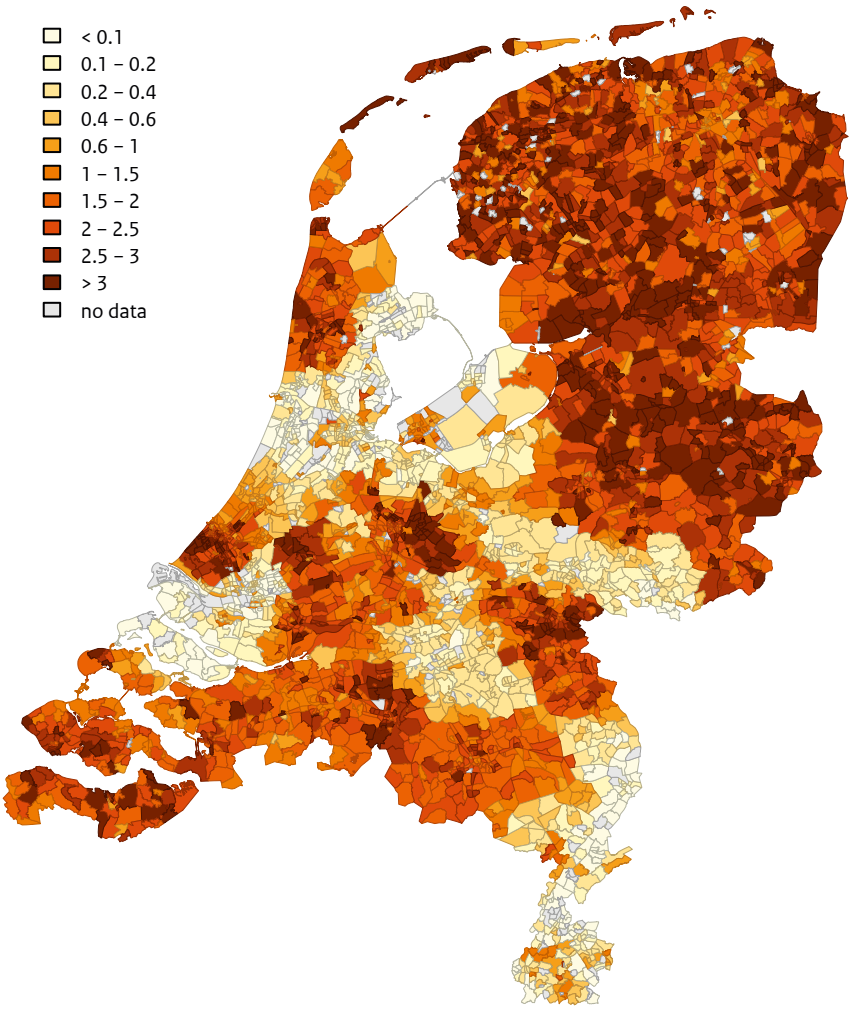


Figure 4.1.2.3 Percentage of residents for whom at least one isolate from a general practitioner's patient was included in the analyses as shown in figure 4.2.3 and 4.2.4 by 4-digit postal code area.

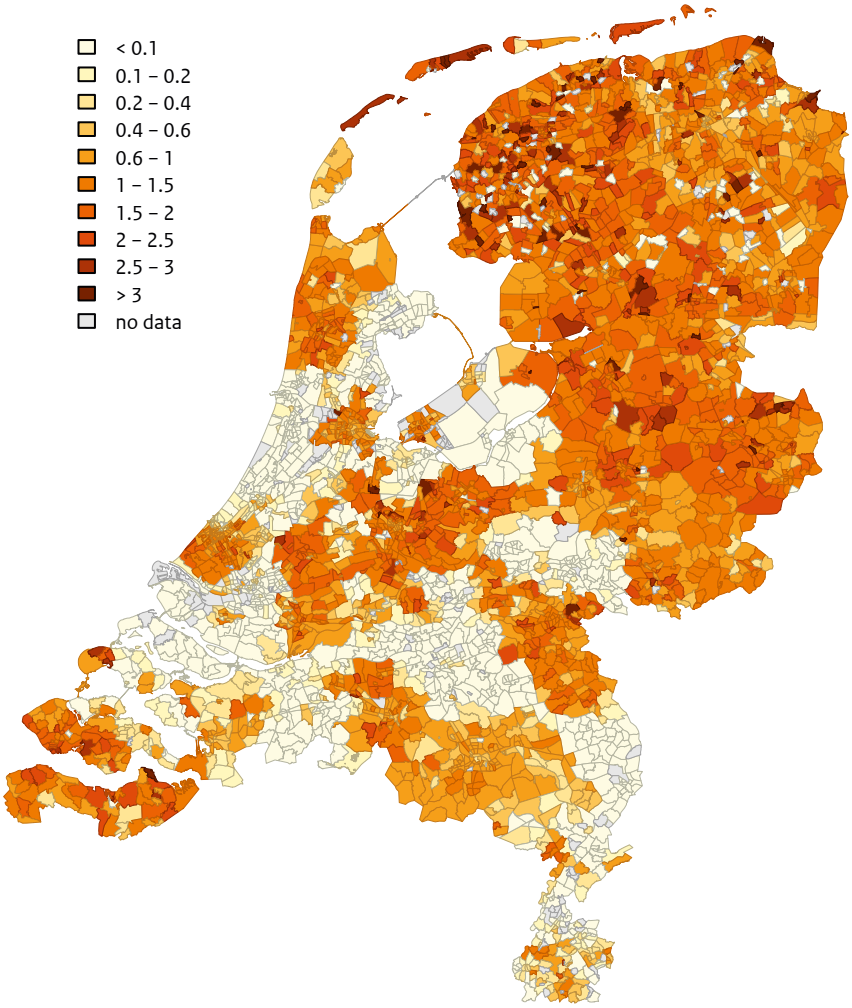


Table 4.1.2.1 Characteristics of isolates in 2016 from 29 laboratories for which data were included in the analyses in chapters 4.1 through 4.4 and 12 laboratories for which data were excluded.

	Included	Excluded
Total number of isolates	349050	64735
Mean number of isolates per laboratory	12036	7193
Pathogen		
E. coli	38	36
K. pneumoniae	6	6
E. cloacae	2	2
P. mirabilis	5	5
P. aeruginosa	5	4
Acinetobacter spp.	1	1
E. faecalis	6	6
E. faecium	1	1
S. aureus	12	12
CNS	5	6
S. pneumoniae	1	2
H. influenzae	2	3
M. catarrhalis	1	1
Other Enterobacteriaceae*	7	6
Other non-fermenters**	1	1
Other gram-positives	7	7
Sex of patient		
Male	39	40
Female	61	60
Type of care		
General practitioners	43	35
Outpatient departments	23	29
Inpatient departments (excl. Intensive Care Units)	27	28
Intensive Care Units	3	3
Long-term care facilities	4	4

Table 4.1.2.1 (continued) Characteristics of isolates in 2016 from 29 laboratories for which data were included in the analyses in chapters 4.1 through 4.4 and 12 laboratories for which data were excluded.

	Included	Excluded
Age category of patient (y)		
0-4	4	3
5-18	5	4
19-64	36	35
>65	54	58
Isolate source		
Blood	5	6
Lower respiratory tract	8	9
Urine	61	56
Wound/Pus	14	11
Other sterile	12	18
Type of hospital		
Not applicable (GP or LTCF) or missing data	47	40
General	19	25
Top clinical	27	36
University hospital	7	0

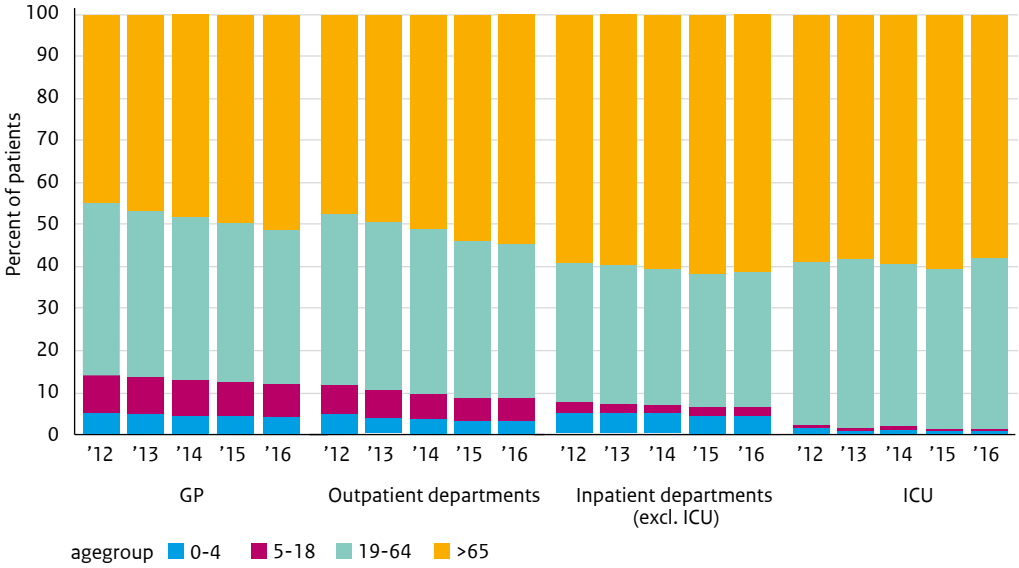
Values are percentages of the total number of isolates unless indicated otherwise
GP=general practitioners, LTCF=long-term care facilities
Only the first clinical isolate per patient was included
* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae)*
** *Pseudomonas spp. (non-aeruginosa), Stenotrophomonas spp.*

Table 4.1.2.2 Characteristics of 313640 isolates included in the analyses in chapters 4.1 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	138302	21620	7782	18068	18125	4242	21714	4456	44038	19328	4755	8748	2462
Sex of patient													
Male	28	34	54	41	53	49	51	52	53	50	54	53	51
Female	72	66	46	59	47	51	49	48	47	50	46	47	49
Type of care													
General practitioners	57	46	29	44	30	50	38	9	24	19	8	11	11
Outpatient departments	16	21	25	20	30	23	24	11	39	14	27	40	37
Inpatient departments (excl. Intensive Care Units)	21	26	37	23	31	21	31	59	31	56	55	41	45
Intensive Care Units	2	3	6	2	4	3	3	19	3	10	9	6	5
Long-term care facilities	4	5	3	10	5	2	4	2	3	1	0	1	2
Age category of patient (y)													
0-4	4	2	5	3	2	6	4	1	5	5	7	8	9
5-18	6	1	2	2	5	4	3	1	7	6	3	4	3
19-64	35	28	31	22	30	34	28	31	44	43	37	35	30
>65	55	68	62	73	62	56	65	68	44	47	53	54	59
Isolate source													
Blood	3	4	4	1	2	2	3	11	4	39	25	2	0
Lower respiratory tract	2	5	10	3	18	8	0	2	9	0	57	84	86
Urine	87	81	51	79	41	57	82	51	13	31	1	0	0
Wound/Pus	4	6	25	12	19	21	12	28	44	22	7	4	5
Other sterile	4	5	10	5	19	12	3	8	29	9	9	10	8
Type of hospital													
Not applicable (GP or LTCF) or missing data	61	51	32	55	35	53	42	12	27	20	9	13	14
General	15	18	24	18	21	15	21	26	26	26	34	30	30
Top clinical	19	25	33	22	33	24	30	47	37	39	47	47	46
University hospital	4	7	11	5	11	8	7	15	10	15	9	11	10

Values are percentages of the total number of isolates unless indicated otherwise.
GP=general practitioners, LTCF=long-term care facilities

Figure 4.1.2.4 Age distribution of patients, by year and type of care.



Key results

- Included laboratories were well distributed throughout the country, although the proportion of laboratories with complete data in Noord-Holland and Limburg was low (Figure 4.1.2.1).
- The distribution of included laboratories was reflected in the coverage data (Figure 4.1.2.2). The coverage was high in the north of the Netherlands. In the rest of the country coverage was more scattered. This pattern was also found in figure 4.1.2.3 displaying coverage of isolates from general practitioner's patients.
- Although the mean number of isolates per laboratory was lower in excluded laboratories (12036 in included laboratories versus 7193 in excluded laboratories), data were largely comparable between included and excluded laboratories (table 4.1.2.1). The main differences were a lower proportion of isolates from general practitioners (43% versus 35%) and from university hospitals (7% versus 0%) in excluded laboratories. This was 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 included in the analyses. However, because the ISIS-AR database contains large numbers of data, it is not expected that inclusion of this type of laboratories influenced the overall resistance percentages towards a deviation from the true mean of the Netherlands.
- Most pathogens were isolated from patients older than 65 years (44-73%, depending on the pathogen, table 4.1.2.2).
- Over the years the proportion of patients aged >65 years has increased (figure 4.1.2.4; 45% in 2012 to 51% in 2016 in general practices, 47-55% in outpatient departments, 59-61% in inpatient departments excluding intensive care units, and 86-88% in long-term care facilities), except in intensive care units (59-58%).
- Enterobacteriaceae were more often isolated from female patients (e.g. 72% of *E. coli* and 66% of *K. pneumoniae* in women), likely because women are more prone to urinary tract infections (table 4.1.2.2). For the other pathogens, the percentage of male and female patients was more comparable.
- The percentage of women was relatively large in general practice populations (~71%), whereas in intensive care units the percentage of men was relatively high (~61%). However, the distributions have remained stable over time (data not shown).
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *S. aureus* were most often isolated from patients from general practitioners and outpatient departments (combined 54-73%, depending on the pathogen, table 4.1.2.2), whereas the main part of *E. faecium* and coagulase-negative staphylococci was isolated from inpatients (78% and 66% respectively).
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecium*, and *E. faecalis* were mainly isolated from urine (41-87%, depending on the pathogen), whereas *S. aureus* was mainly isolated from wound or pus (44%), and *H. influenzae*, *S. pneumoniae*, *M. catarrhalis* from the respiratory tract (57-86%, Table 4.1.2.2).

4.2 Primary care

For the resistance analyses in patients from general practitioners (GP) on the pathogens *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* only urinary isolates were included. For *S. aureus*, only wound and pus isolates were included.
The distribution of pathogens in selected GP patients is presented in table 4.2.1 for pathogens isolated from urine samples and in table 4.2.2 for pathogens isolated from wound and pus samples. The resistance levels for the pathogens isolated from these samples in 2016 are presented in table 4.2.3 and table 4.2.4, respectively. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*) and figure 4.2.2 (*S. aureus*). For urinary isolates resistance levels and five-year trends are calculated for patients aged ≤12 years and patients aged >12 years separately in accordance with age categories used in the urinary tract infection guidelines of the Dutch College of General Practitioners (NHG). Finally, in figures 4.2.3 and 4.2.4, resistance levels in *E. coli* are shown by NUTS3-region (http://ec.europa.eu/eurostat/web/nuts) for a selection of antibiotics.

GPs usually send samples for culture and susceptibility testing in case of complicated urinary tract infection or antimicrobial therapy failure. As a result, the presented resistance levels are not representative for all patients with urinary tract infections or *S. aureus* wound and pus infections presenting at the GP. Therefore, these patients are further referred to as 'selected general practitioner's patients'.

Table 4.2.1 Distribution of isolated pathogens in clinical urinary isolates from selected general practitioner's patients, by age category, ISIS-AR 2016.

Pathogen	Age≤12 N (%)	Age>12 N (%)
E. coli	6506 (71)	57948 (57)
K. pneumoniae	144 (2)	7801 (8)
P. mirabilis	405 (4)	5967 (6)
P. aeruginosa	131 (1)	2539 (3)
S. aureus	127 (1)	2029 (2)
Other Enterobacteriaceae*	357 (4)	7088 (7)
Other non-fermenters**	164 (2)	1930 (2)
Enterococcus spp.	886 (10)	8401 (8)
Other Gram-positives***	506 (5)	7547 (7)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp., Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*
** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*
*** *Enterococcus spp., Streptococcus spp., coagulase-negative staphylococci*

Table 4.2.2 Distribution of isolated pathogens in clinical wound and pus isolates from selected general practitioner's patients, ISIS-AR 2016.

Pathogen	N (%)
S. aureus	2803 (50)
Enterobacteriaceae*	1049 (19)
Other non-fermenters**	741 (13)
Other Gram-positives***	973 (17)

* *Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp., Serratia spp., Morganella spp., Citrobacter spp., Salmonella spp., Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*
** *Acinetobacter spp., Pseudomonas spp., and Stenotrophomonas spp.*
*** *Enterococcus spp., Streptococcus spp., coagulase-negative staphylococci*

Table 4.2.3 Resistance levels (%) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioner's patients, by age category, ISIS-AR 2016.

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	age≤12	age>12	age≤12	age>12	age≤12	age>12	age≤12	age>12
median age	5	66	4	73	3	75	4	79
Antibiotic								
amoxicillin/ampicillin	35	39	-	-	20	21	-	-
co-amoxiclav* - non-uuti	17	20	11	10	6	5	-	-
cefuroxime	4	7	2	14	0	1	-	-
cefotaxime/ceftriaxone	2	3	1	5	0	1	-	-
ceftazidime	1	2	0	4	0	0	1	2
ciprofloxacin	3	9	0	4	2	7	1	6
norfloxacin	6	15	6	23	5	12	-	-
gentamicin	2	4	0	2	4	5	1	3
tobramycin	2	4	2	3	2	3	1	0
fosfomycin	1	1	22	32	9	17	-	-
trimethoprim	21	25	10	22	27	35	-	-
co-trimoxazole	20	23	8	11	22	28	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
HRMO**	2	5	1	5	1	2	-	-
multidrug resistance*** - non-uuti	1	3	0	2	0	0	-	-

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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*

*** *Multidrug resistance, defined as resistance to all of the following oral agents: co-trimoxazole, co-amoxiclav and ciprofloxacin.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioner's patients in ISIS-AR, by age category.

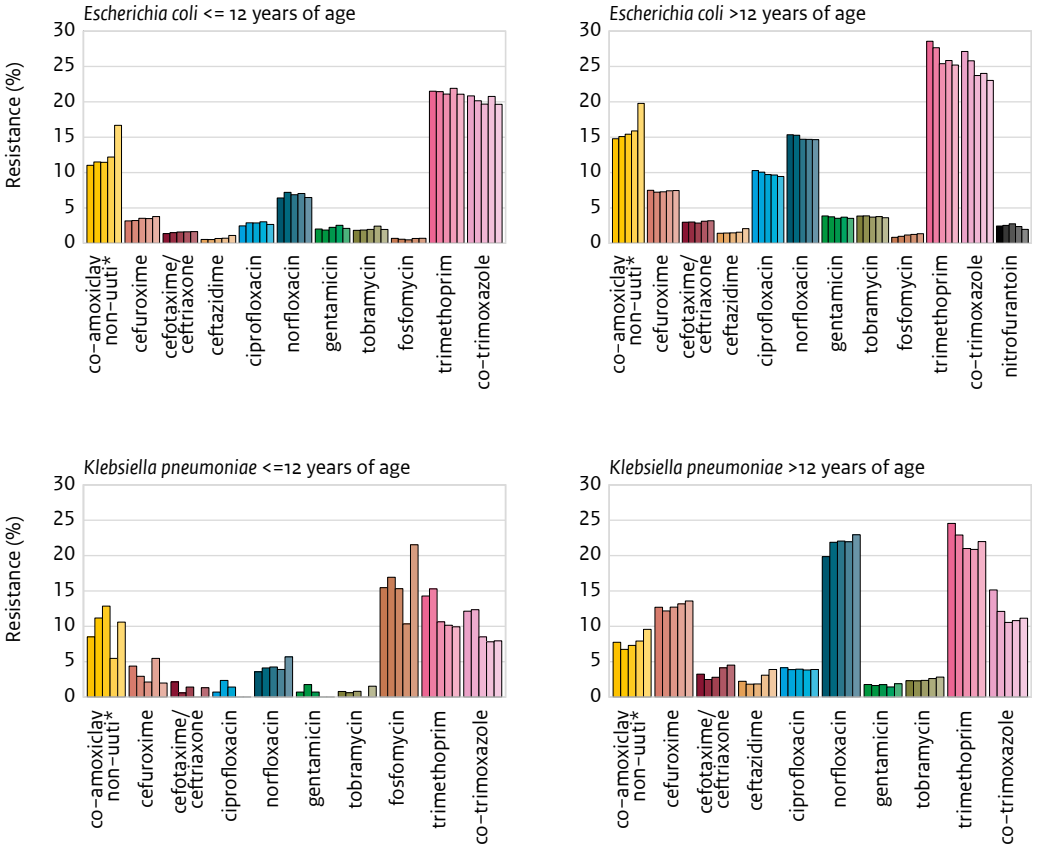
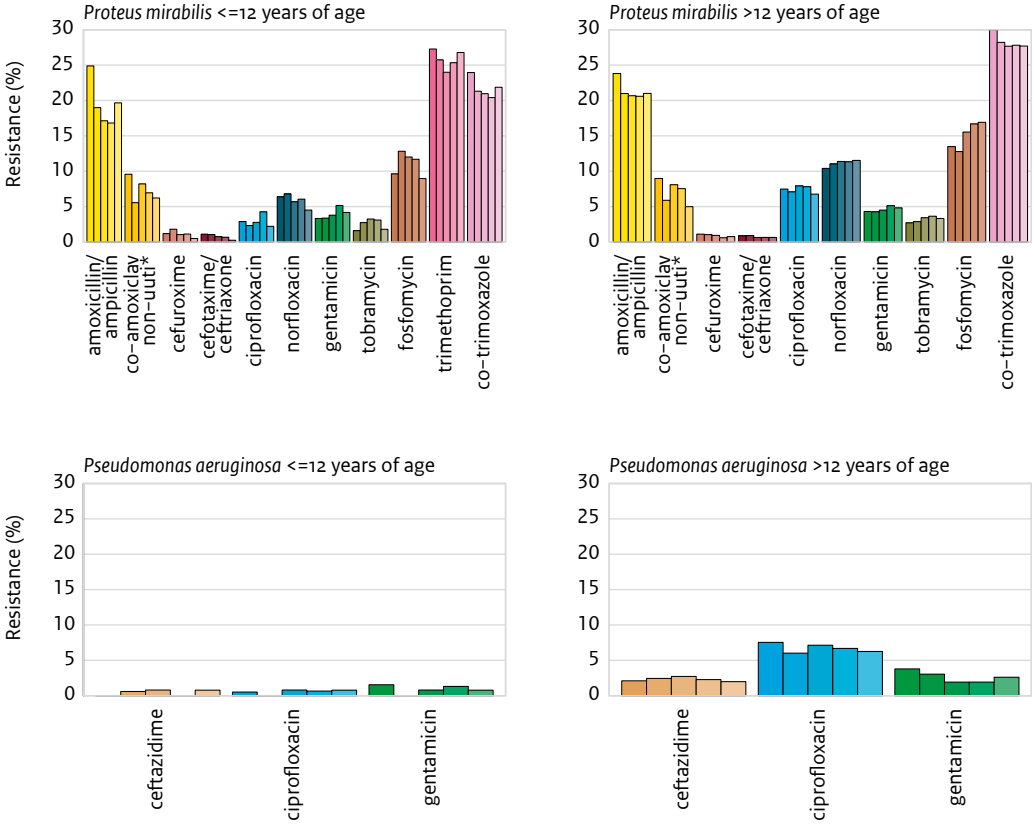


Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioner's patients in ISIS-AR, by age category.



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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.2.4 Resistance levels (%) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients, ISIS-AR 2016.

	S. aureus
Antibiotic	
flucloxacillin*	3
ciprofloxacin**	5
erythromycin	11
clindamycin	2
clindamycin including inducible resistance***	9
doxycycline/tetracycline	5
fusidic acid	16
co-trimoxazole	4

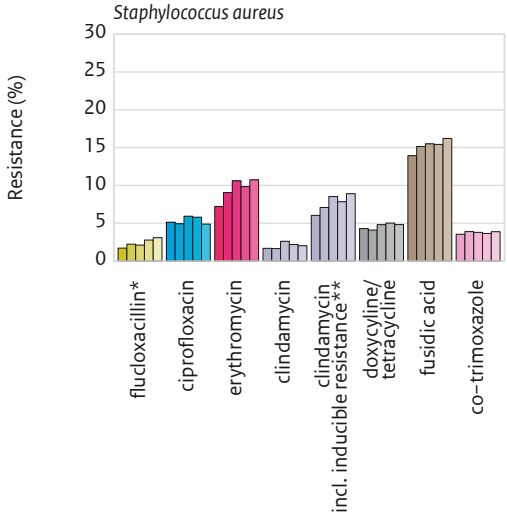
10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

* *Resistance against 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).*

** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

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

Figure 4.2.2 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients in ISIS-AR.



* *Resistance against 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)*

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

Figure 4.2.3 Resistance levels (%) for nitrofurantoin, fosfomycin, and cefotaxime/cefriaxone/ceftazidime among clinical urinary isolates of *E. coli* from selected general practitioner's patients in ISIS-AR in 2016, by NUTS3-region.

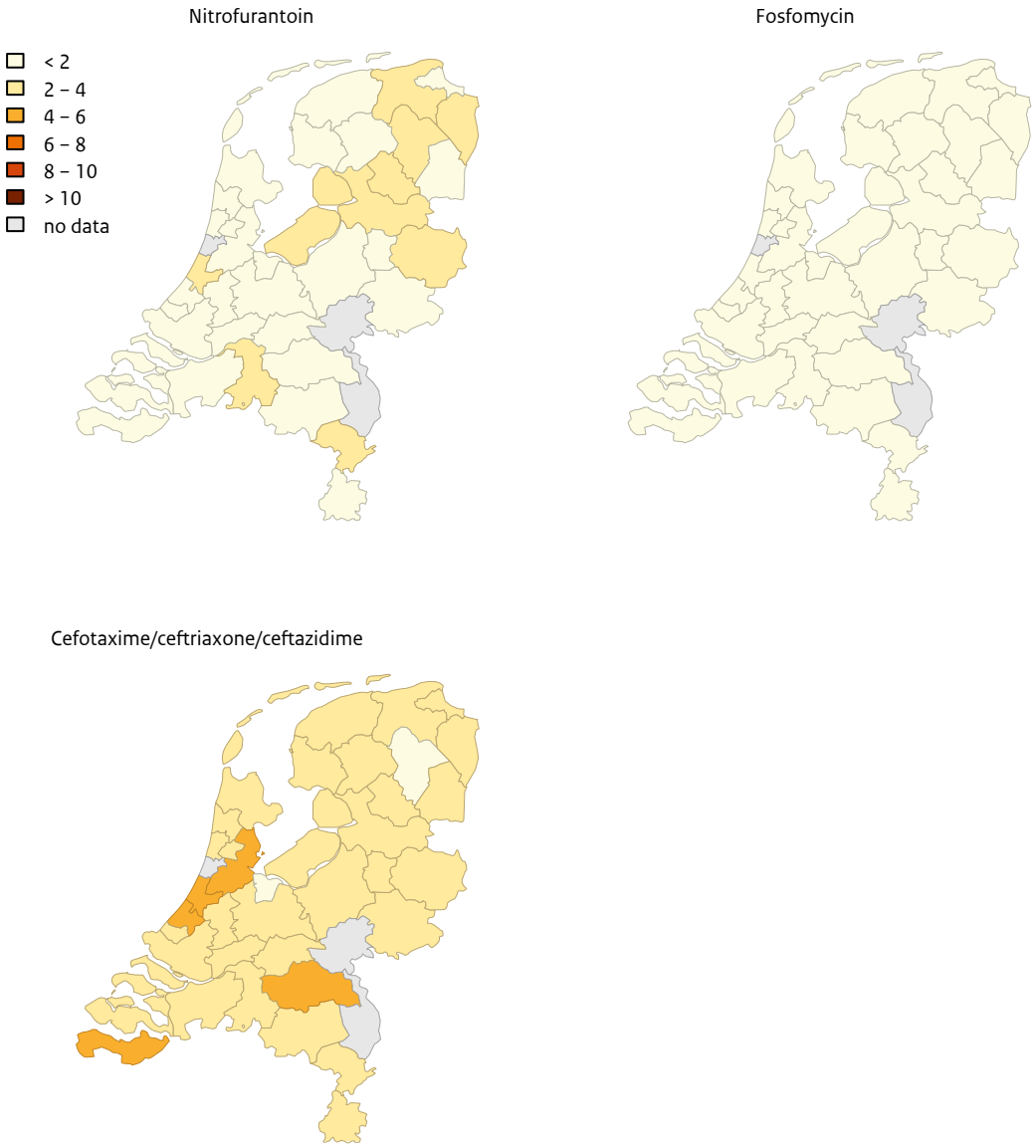
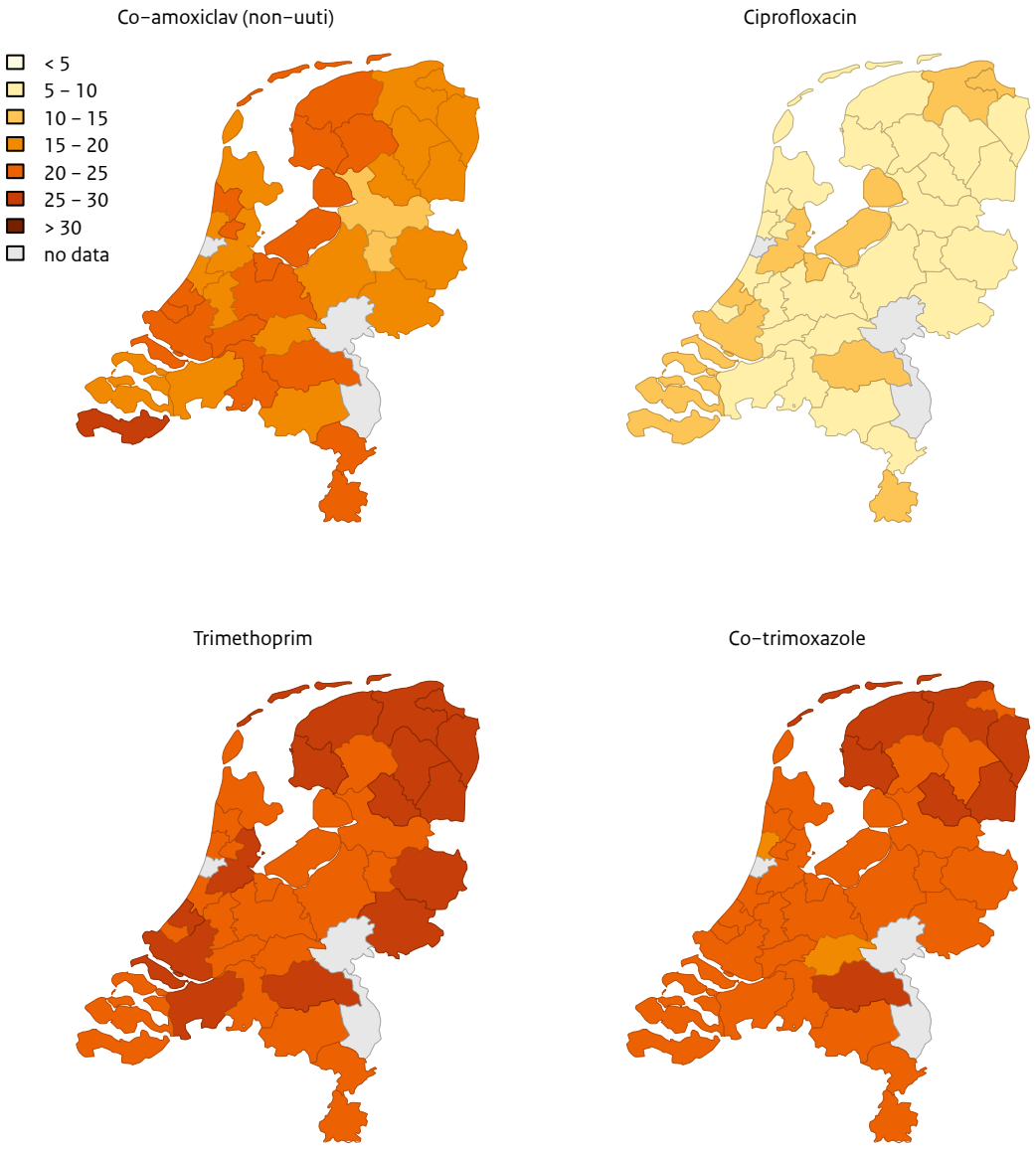


Figure 4.2.4 Resistance levels (%) for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole among clinical urinary isolates of *E. coli* from selected general practitioner's patients in ISIS-AR in 2016, by NUTS3-region.



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

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, cefotaxime/ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, and tobramycin were below 10%, except for cefuroxime in *K. pneumoniae* in patients aged >12 years (14%). Additionally, resistance levels below 10% were found for norfloxacin (≤6%) in *E. coli*, *K. pneumoniae*, and *P. mirabilis* from patients aged ≤12 years, fosfomycin (1%) and nitrofurantoin (≤2%) in *E. coli*, co-trimoxazole in *K. pneumoniae* (8%) from patients aged ≤12 years, and for fosfomycin (9%, patients aged ≤12 years only) and co-amoxiclav (≤6%) in *P. mirabilis*.
- With regard to patients aged ≤12 years resistance levels ≥20% were found in *E. coli* and *P. mirabilis* for amoxicillin/ampicillin (respectively 35% and 20%), trimethoprim (21% and 27%), and for co-trimoxazole (20% and 22%), and in *K. pneumoniae* for fosfomycin (22%). With regard patients aged >12 years resistance levels were ≥20% for amoxicillin/ampicillin in *E. coli* (39%) and *P. mirabilis* (21%), co-amoxiclav in *E. coli* (20%), norfloxacin (23%) and fosfomycin (32%) in *K. pneumoniae*, trimethoprim in all Enterobacteriaceae (≥22%), and co-trimoxazole in *E. coli* (23%) and *P. mirabilis* (28%).
- In *P. mirabilis*, there was a statistically significant and clinically relevant decrease in resistance to amoxicillin/ampicillin in patients aged ≤12 years (from 25% in 2012 to 20% in 2016). Also resistance of *P. mirabilis* to co-amoxiclav decreased significantly and to a clinically relevant extent in patients aged >12 years, from 9% in 2012 to 5% in 2016.
- The percentage of HRMO and multidrug resistance remained low in all Enterobacteriaceae (≤5%).
- Resistance levels for *E. coli* were comparable between all geographical regions for fosfomycin and nitrofurantoin (<2.5% between the region with the lowest resistance and the region with the highest resistance). For cefotaxime/ceftriaxone/ceftazidime, co-amoxiclav, ciprofloxacin, and trimethoprim resistance levels were comparable between most regions (interquartile range <2.5%), but not all. However, no clear pattern could be distinguished. Resistance levels for co-trimoxazole (average 23%) were somewhat higher in the northern regions of the Netherlands (25-29%).

P. aeruginosa

- Resistance below 10% was found for each of the selected agents (≤6%).

S. aureus

- Resistance to each of the selected agents was <10%, except for erythromycin (11%) and fusidic acid (16%)
- A significant and clinically relevant increase from 6% in 2012 to 9% in 2016 was found for clindamycin including inducible resistance.

4.3 Hospital departments

In the analyses for outpatient departments and inpatient departments (including intensive care units), the resistance levels were calculated based on antimicrobial susceptibility results in isolates from blood, cerebrospinal fluid, lower respiratory tract, urine and wound combined. Additionally, two separate analyses were conducted; 1) for blood isolates from patients admitted to inpatient hospital departments including ICU departments, presented in chapter 4.3.4, and 2) for urinary isolates from patients in urology departments (outpatient and inpatient departments), presented in chapter 4.3.5.

4.3.1 Outpatient departments

The distribution of pathogens isolated from clinical specimens (lower respiratory tract, urine, and wound) from patients attending outpatient departments is presented in table 4.3.1.1. The resistance levels for pathogens isolated from these patients in 2016 are presented in tables 4.3.1.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*) and 4.3.1.3 (*S. aureus*). Five-year trends in resistance are shown in figures 4.3.1.1 and 4.3.1.2 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 a good reflection of resistance in outpatient departments.

Table 4.3.1.1 Distribution of isolated pathogens in clinical specimens from patients attending outpatient departments, ISIS-AR 2016.

	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**
E. coli	443 (8)	16647 (45)	1326 (8)
K. pneumoniae	193 (4)	3155 (9)	302 (2)
P. mirabilis	140 (3)	1970 (5)	866 (5)
P. aeruginosa	1181 (22)	1379 (4)	1252 (7)
E. faecalis	2 (0)	4106 (11)	626 (4)
S. aureus	1242 (24)	1292 (4)	7614 (45)
Other Enterobacteriaceae*	672 (13)	3587 (10)	1802 (11)
Other non-fermenters**	494 (9)	645 (2)	526 (3)
Other Gram-positives***	917 (17)	3996 (11)	2783 (16)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp., Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Enterococcus spp., Streptococcus spp.*

Table 4.3.1.2 Resistance levels (%) among clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments, ISIS-AR 2016.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	46	-	24	-
co-amoxiclav* - non-uuti	23	11	7	-
piperacillin-tazobactam	5	6	0	5
cefuroxime	12	14	1	-
cefotaxime/ceftriaxone	5	7	1	-
ceftazidime	3	6	0	3
meropenem/imipenem	0	0	0	-
meropenem	-	-	-	1
imipenem	-	-	-	5
ciprofloxacin	16	6	9	7
norfloxacin	23	21	16	-
gentamicin	5	4	7	4
tobramycin	6	5	5	1
fosfomycin	-	29	-	-
trimethoprim	29	21	35	-
co-trimoxazole	27	15	29	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
gentamicin + amoxicillin/ampicillin	5	-	5	-
gentamicin + co-amoxiclav - non-uuti	3	2	1	-
gentamicin + cefuroxime	2	3	0	-
gentamicin + cefotaxime/ceftriaxone	1	2	0	-
gentamicin + ceftazidime	1	2	0	1

Table 4.3.1.2 (continued) Resistance levels (%) among clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments, ISIS-AR 2016.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Multidrug resistance				
HRMO**	8	9	4	2
multidrug resistance*** - non-uuti	5	3	1	-

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

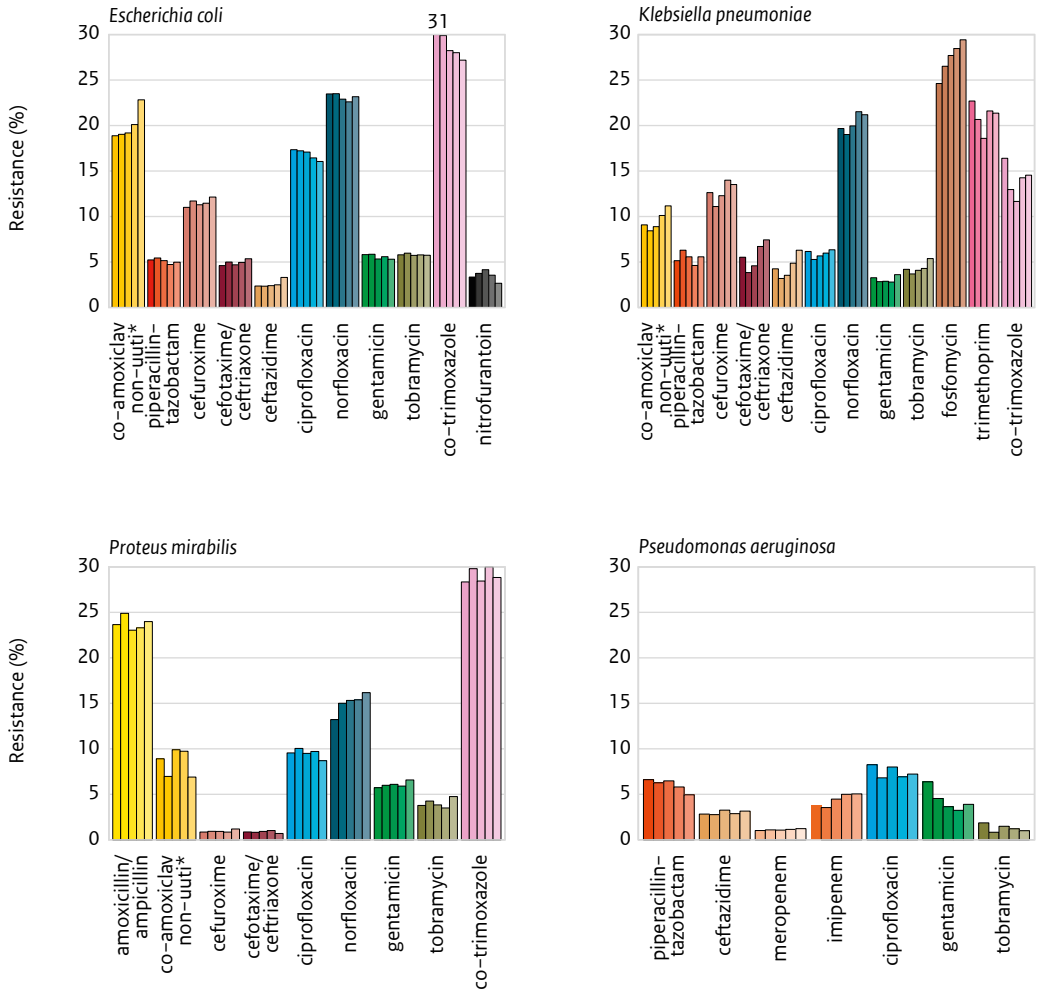
- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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 P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

*** *Multidrug resistance, defined as resistance to all of the following oral agents: co-trimoxazole, co-amoxiclav, and ciprofloxacin.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical 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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.3.1.3 Resistance levels (%) among clinical isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2016.

	S. aureus
Antibiotic	
flucloxacillin*	2
ciprofloxacin**	9
gentamicin	1
erythromycin	13
clindamycin	4
clindamycin including inducible resistance***	12
doxycycline/tetracycline	4
fusidic acid	9
linezolid	0
co-trimoxazole	3
rifampicin	0

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend

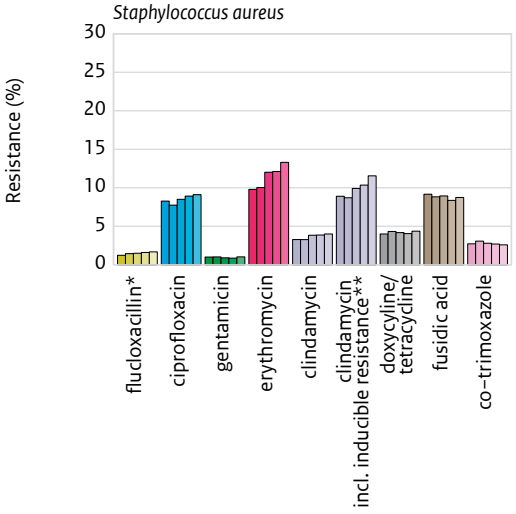
(For the definition of a clinically relevant trend see the methods section)

* *Resistance against 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).*

** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

*** *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 2012 to 2016) among clinical isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR.



* *Resistance against 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)*

** *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

- Resistance levels of 10% or lower were found for piperacillin/tazobactam (≤6%), cefotaxime/ceftriaxone (≤7%), ceftazidime (≤6%), meropenem/imipenem (0%), gentamicin (≤7%), and tobramycin (≤6%) in all Enterobacteriaceae in 2016. Resistance levels lower than 10% were also found for nitrofurantoin in *E. coli* (3%), ciprofloxacin in *K. pneumoniae* (6%) and *P. mirabilis* (9%), and cefuroxime (1%) and co-amoxiclav (7%) in *P. mirabilis*.
- Resistance of 20% or higher were found for amoxicillin/ampicillin in all Enterobacteriaceae (≥24%), norfloxacin in *E. coli* (23%) and *K. pneumoniae* (21%), co-trimoxazole in *E. coli* (27%) and *P. mirabilis* (29%), co-amoxiclav in *E. coli* (23%), and fosfomycin in *K. pneumoniae* (29%).
- Resistance to empiric therapy combinations was ≤5%.
- The percentage of HRMO was ≤9%, and multidrug resistance was ≤5% for all Enterobacteriaceae.
- In *K. pneumoniae*, a significant and clinically relevant increase was seen in resistance to cefotaxime/ceftriaxone, with the increase starting in 2013 (4% in 2013 to 7% in 2016). Additionally, the percentage of HRMO increased significantly and to a clinically relevant extent, with the increase starting in 2013 (5% in 2013 to 9% in 2016).

P. aeruginosa

- Resistance to each of the selected agents was ≤7%.

S. aureus

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

4.3.2 Inpatient hospital departments (excl. ICU)

The distribution of pathogens from clinical specimens (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 2016 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 staphylococci). 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 staphylococci).

In Dutch hospital departments, a sample is taken from the majority of infections for routine diagnostic purposes and susceptibility testing. Therefore, bias due to selective sampling of patients is expected to be limited.

Table 4.3.2.1 Distribution of isolated pathogens in clinical specimens from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2016.

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	N (%)	N (%)	N (%)	N (%)
E. coli	3590 (25)	1161 (14)	15829 (44)	3607 (16)
K. pneumoniae	641 (5)	529 (6)	2834 (8)	691 (3)
P. mirabilis	216 (2)	218 (3)	2404 (7)	824 (4)
E. cloacae	222 (2)	423 (5)	818 (2)	854 (4)
P. aeruginosa	308 (2)	1331 (16)	1794 (5)	1293 (6)
Acinetobacter spp.	72 (1)	98 (1)	250 (1)	254 (1)
E. faecalis	426 (3)	36 (0)	3972 (11)	1447 (7)
E. faecium	292 (2)	19 (0)	1195 (3)	849 (4)
S. aureus	1663 (12)	1630 (20)	1238 (3)	6055 (28)
CNS	4738 (34)	16 (0)	942 (3)	2494 (11)
Other Enterobacteriaceae*	598 (4)	1021 (12)	2597 (7)	1701 (8)
Other non-fermenters**	32 (0)	464 (6)	168 (0)	263 (1)
Other Gram-positives***	1309 (9)	1305 (16)	1557 (4)	1646 (7)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp. (non-cloacae), Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Enterococcus spp., Streptococcus spp.*

Table 4.3.2.2 Resistance levels (%) among clinical 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 2016.

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	45	-	-	22	-	-
co-amoxiclav* - non-uuti	24	12	-	7	-	-
piperacillin-tazobactam	5	5	-	1	7	-
cefuroxime	12	13	-	1	-	-
cefotaxime/ceftriaxone	6	8	-	1	-	-
ceftazidime	3	7	-	0	4	-
meropenem/imipenem	0	0	0	0	-	2
meropenem	-	-	-	-	1	-
imipenem	-	-	-	-	4	-
ciprofloxacin	12	6	3	8	6	4
gentamicin	5	4	2	5	3	4
tobramycin	5	5	3	4	1	3
trimethoprim	26	17	6	33	-	-
co-trimoxazole	25	13	5	26	-	2
nitrofurantoin	2	-	-	-	-	-
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	4	-	-	4	-	-
gentamicin + co-amoxiclav - non-uuti	3	3	-	1	-	-
gentamicin + piperacillin-tazobactam	1	1	-	0	1	-
gentamicin + cefuroxime	2	3	-	0	-	-
gentamicin + cefotaxime/ceftriaxone	1	3	-	0	-	-
gentamicin + ceftazidime	1	2	-	0	1	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	0	-
Multidrug resistance						
HRMO**	8	9	1	3	2	2

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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 2012 to 2016) among clinical 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.

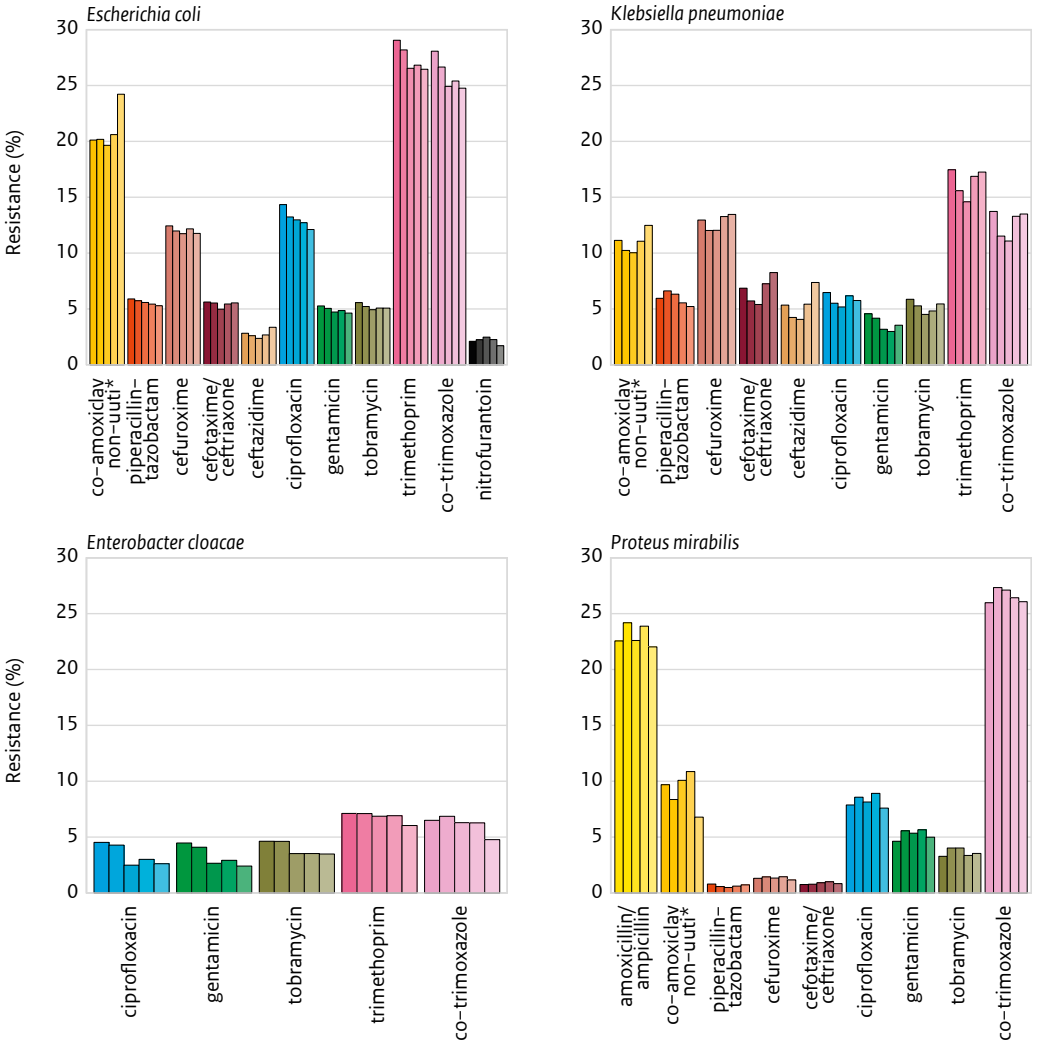
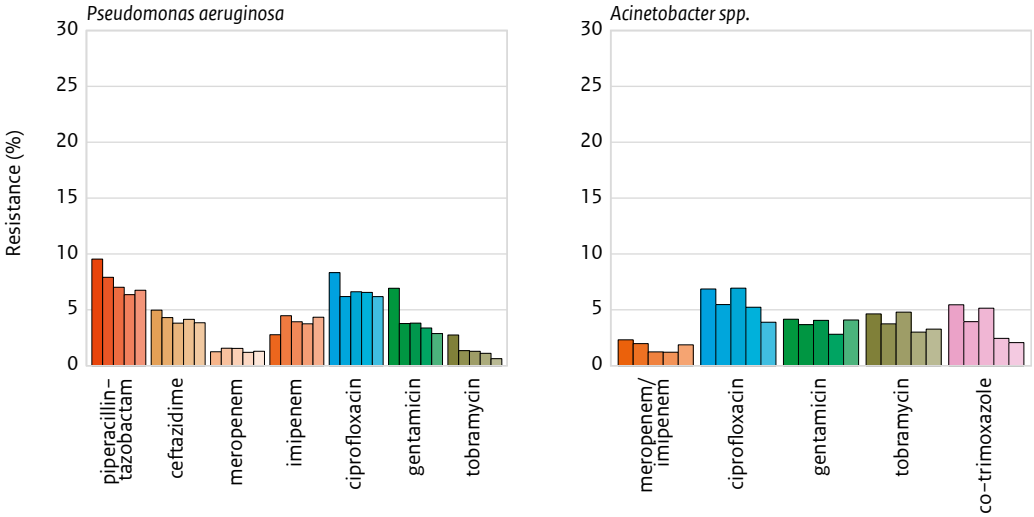


Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.



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

** 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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.3.2.3 Resistance levels (%) among clinical isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2016.

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

- = Resistance not calculated.
** Resistance based on isolates from urine only.*

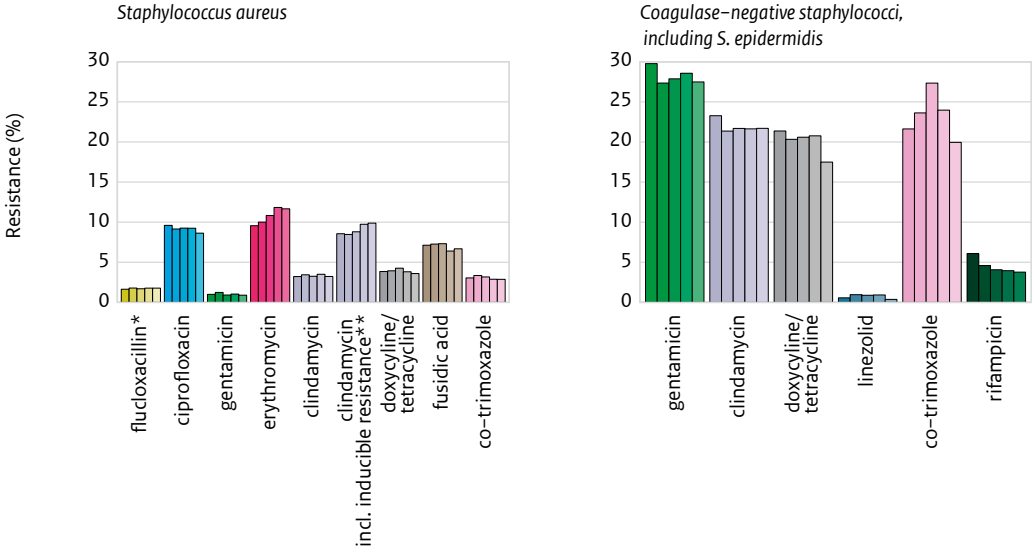
Table 4.3.2.4 Resistance levels (%) among clinical isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2016

	S. aureus	*CNS*
Antibiotic		
flucloxacillin*	2	44
ciprofloxacin**	9	32
gentamicin	1	27
erythromycin	12	45
clindamycin	3	22
clindamycin including inducible resistance***	10	31
doxycycline/tetracycline	4	17
fusidic acid	7	45
linezolid	0	0
co-trimoxazole	3	20
rifampicin	0	4

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

* *Resistance against 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).*
** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*
*** *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 2012 to 2016) among clinical isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR.



* *Resistance against 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)*

** *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 (≤5%), cefotaxime/ceftriaxone (≤8%), ceftazidime (≤7%), meropenem/imipenem (0%), ciprofloxacin (≤8%, except for *E. coli*; 12%), gentamicin (≤5%), and tobramycin (≤5%). Resistance levels ≤10% were also found for nitrofurantoin in *E. coli* (2%), trimethoprim (6%) and co-trimoxazole (5%) in *E. cloacae*, and co-amoxiclav (7%) and cefuroxime (1%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥22%), trimethoprim (≥26%), and co-trimoxazole (≥25%) in *E. coli* and *P. mirabilis*. Furthermore, co-amoxiclav resistance *E. coli* was 24%.
- For empiric therapy combinations, resistance was ≤4%.
- The percentage of HRMO was 9% (*K. pneumoniae*) or lower.

P. aeruginosa

- Resistance to each of the selected agents, empiric therapy combinations, and the percentage HRMO, was ≤7% in 2016.
- A significant and clinically relevant decrease in resistance was observed for piperacillin-tazobactam (from 10% in 2012 to 7% in 2016), and for gentamicin (from 7% to 3%).

Acinetobacter spp.

- Resistance to each of the selected agents, and the percentage HRMO, was ≤4% in 2016.
- A significant and clinically relevant decrease in resistance was observed for co-trimoxazole (from 5% in 2012 to 2% in 2016).

E. faecalis and E. faecium

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

S. aureus

- Resistance to each of the selected agents was ≤10%, except for erythromycin (12%).

Coagulase-negative staphylococci

- Apart from linezolid (0%), rifampicin (4%), and doxycycline/tetracycline (17%), resistance to each of the selected agents was ≥20%.
- Statistically significant and clinically relevant decreasing or increasing trends over the last five years were not observed in coagulase-negative staphylococci, however, co-trimoxazole resistance decreased from 27% to 20% in the last three years (2014-2016, $p<0.0001$).

4.3.3 Intensive Care Units

The distribution of pathogens from clinical specimens (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 2016 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 staphylococci). 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 staphylococci).

In intensive care units in the Netherlands, a sample is taken from almost all infections for routine diagnostic purposes and susceptibility testing. Bias due to selective sampling of patients is therefore unlikely.

Table 4.3.3.1 Distribution of isolated pathogens in clinical specimens from patients admitted to intensive care units, ISIS-AR 2016.

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	358 (14)	466 (14)	739 (42)	504 (19)
K. pneumoniae	71 (3)	216 (6)	135 (8)	105 (4)
P. mirabilis	21 (1)	97 (3)	96 (5)	77 (3)
E. cloacae	28 (1)	177 (5)	27 (2)	102 (4)
P. aeruginosa	52 (2)	330 (10)	123 (7)	165 (6)
Acinetobacter spp.	10 (0)	79 (2)	14 (1)	20 (1)
E. faecalis	88 (4)	51 (2)	205 (12)	231 (9)
E. faecium	197 (8)	96 (3)	151 (9)	357 (14)
S. aureus	198 (8)	771 (23)	51 (3)	295 (11)
CNS	1190 (48)	31 (1)	54 (3)	297 (11)
Other Enterobacteriaceae*	91 (4)	524 (16)	116 (7)	254 (10)
Other non-fermenters**	11 (0)	169 (5)	11 (1)	50 (2)
Other Gram-positives***	170 (7)	355 (11)	40 (2)	148 (6)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp. (non-cloacae), Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Enterococcus spp., Streptococcus spp.*

Table 4.3.3.2 Resistance levels (%) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units, ISIS-AR 2016.

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	49	-	-	27	-	-
co-amoxiclav* - non-uuti	29	15	-	8	-	-
piperacillin-tazobactam	6	6	-	1	13	-
cefuroxime	17	18	-	1	-	-
cefotaxime/ceftriaxone	8	12	-	1	-	-
ceftazidime	5	10	-	0	9	-
meropenem/imipenem	0	0	0	0	-	3
meropenem	-	-	-	-	4	-
imipenem	-	-	-	-	7	-
ciprofloxacin	12	7	4	7	6	3
gentamicin	4	5	4	6	5	5
tobramycin	4	7	6	4	3	4
co-trimoxazole	24	15	5	28	-	1
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	4	-	-	5	-	-
gentamicin + co-amoxiclav - non-uuti	3	4	-	1	-	-
gentamicin + piperacillin-tazobactam	0	2	-	0	3	-
gentamicin + cefuroxime	2	4	-	0	-	-
gentamicin + cefotaxime/ceftriaxone	2	4	-	0	-	-
gentamicin + ceftazidime	1	4	-	0	2	-
tobramycin + ceftazidime	-	-	-	-	2	-
tobramycin + ciprofloxacin	-	-	-	-	2	-
Multidrug resistance						
HRMO**	10	12	3	3	4	3

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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 4.3.3.1 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical 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.

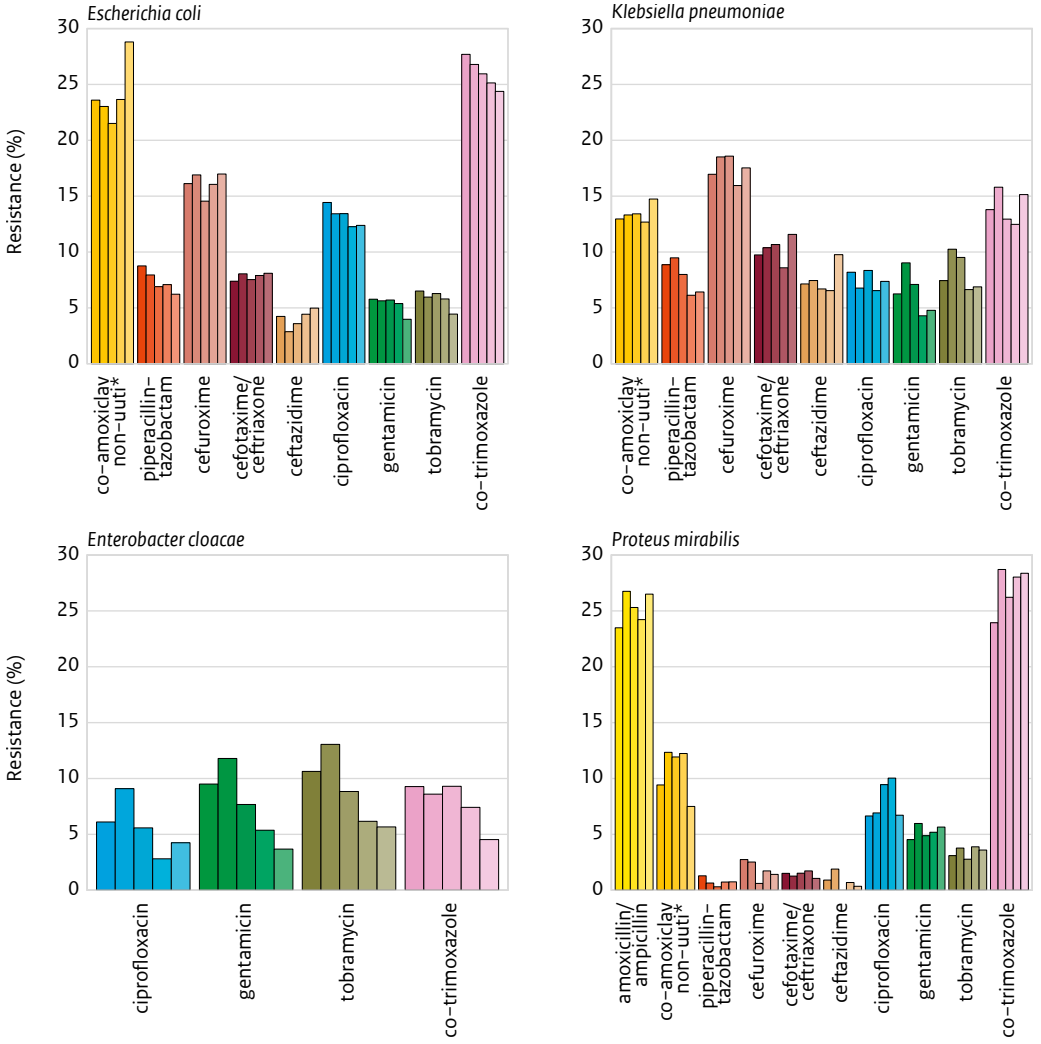
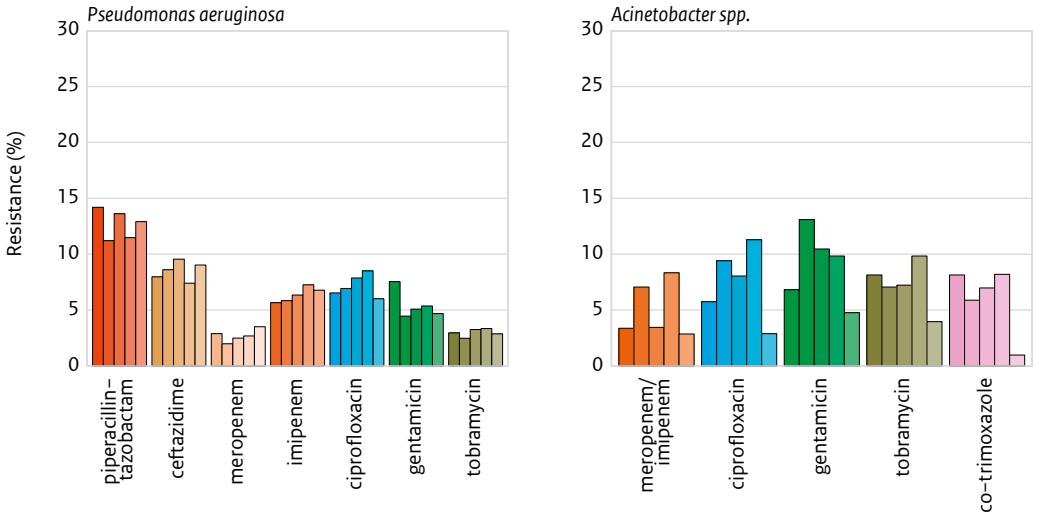


Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR.



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

** 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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.3.3.3 Resistance levels (%) among clinical isolates of *E. faecalis* and *E. faecium* from patients admitted to intensive care units, ISIS-AR 2016.

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	89
vancomycin	0	1

- = Resistance not calculated.

Table 4.3.3.4 Resistance levels (%) among clinical isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to intensive care units, ISIS-AR 2016.

	S. aureus	*CNS*
Antibiotic		
flucloxacillin*	2	70
ciprofloxacin**	5	57
gentamicin	1	51
erythromycin	11	66
clindamycin	2	43
clindamycin including inducible resistance***	9	56
doxycycline/tetracycline	3	21
linezolid	0	0
co-trimoxazole	3	39
rifampicin	0	9

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

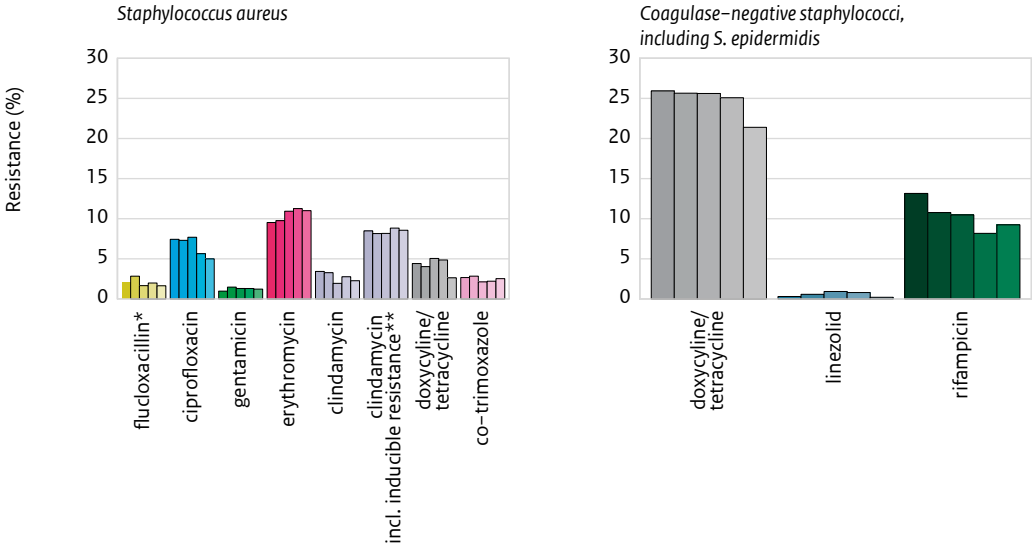
CNS=Coagulase-negative staphylococci, including S. epidermidis

* *Resistance against 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).*

** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

*** *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 2012 to 2016) among clinical isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to intensive care units in ISIS-AR.



* *Resistance against 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)*

** *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 (≤6%), ceftazidime (≤10%), meropenem/imipenem (0%), ciprofloxacin (≤7%, except for *E. coli*; 12%), gentamicin (≤6%), and tobramycin (≤7%). Resistance levels ≤10% were also found for cefotaxime/ceftriaxone in *K. pneumoniae* (8%) and *P. mirabilis* (1%), co-trimoxazole in *E. cloacae* (5%), and co-amoxiclav (8%) and cefuroxime (1%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥27%) and co-trimoxazole (≥24%) in *E. coli* and *P. mirabilis*, and for co-amoxiclav in *E. coli* (29%).
- Resistance to the empiric therapy combinations was ≤5%.
- The percentage HRMO was ≤10% for all Enterobacteriaceae except *K. pneumoniae* (12%).
- In *K. pneumoniae*, resistance to piperacillin-tazobactam decreased significantly and to a clinically relevant extent from 9% in 2012 to 6% in 2016. A similar trend was observed for gentamicin, with the decrease starting in 2013 (from 9% in 2013 to 5% in 2016). In *E. cloacae*, significant and clinically relevant decreases in resistance levels between 2012 and 2016 were found for ciprofloxacin (from 6% to 4%), gentamicin (from 10% to 4%), tobramycin (from 11% to 6%) and co-trimoxazole (from 9% to 5%). Furthermore, the percentage of HRMO decreased significantly and to a clinically relevant extent, with the decrease starting in 2013 (6% in 2013 to 3% in 2016).

P. aeruginosa

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

Acinetobacter spp.

- Resistance levels for each of the selected agents and the percentage HRMO were ≤5%.

E. faecalis and E. faecium

- Resistance to vancomycin was rare (0% in *E. faecalis*, 1% in *E. faecium*).
- In *E. faecium*, resistance to amoxicillin/ampicillin was 89%.

S. aureus

- Resistance to each of the selected agents was 10% or lower, except for erythromycin (11%).
- A statistically significant and clinically relevant decreasing trend was observed for ciprofloxacin (from 7% in 2012 to 5% in 2016).

Coagulase-negative staphylococci

- Apart from linezolid (0%) and rifampicin (9%), resistance to each of the selected agents was ≥20%.
- Resistance to flucloxacillin (from 74% in 2012 to 70% in 2016), ciprofloxacin (from 63% to 57%), and rifampicin (from 13% to 9%) decreased significantly and to a clinically relevant extent in the last five years.

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 2016 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 staphylococci). 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 staphylococci).

In most hospitals blood samples are taken for routine diagnostic purposes and susceptibility testing from all patients with a body temperature of >38.5 °C. Bias due to selective sampling of patients is therefore unlikely.

Table 4.3.4.1 Distribution of pathogens in clinical blood isolates from patients admitted to non-intensive care inpatient departments (non-ICU), and intensive care units (ICU) ISIS-AR 2016.

Pathogen	Non-ICU N (%)	ICU N (%)
E. coli	3571 (26)	317 (14)
K. pneumoniae	637 (5)	60 (3)
P. mirabilis	213 (2)	19 (1)
E. cloacae	217 (2)	26 (1)
P. aeruginosa	299 (2)	42 (2)
Acinetobacter spp.	68 (0)	10 (0)
E. faecalis	420 (3)	87 (4)
E. faecium	284 (2)	190 (8)
S. aureus	1638 (12)	158 (7)
CNS	4601 (33)	1151 (50)
Other Enterobacteriaceae*	595 (4)	83 (4)
Other non-fermenters**	29 (0)	11 (0)
Other Gram-positives***	1291 (9)	160 (7)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp., Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Enterococcus spp., Streptococcus spp.*

Table 4.3.4.2 Resistance levels (%) among clinical 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 2016.

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	45	-	-	26	-
co-amoxiclav* - non-uuti	24	11	-	8	-
piperacillin-tazobactam	5	5	-	1	4
cefuroxime	12	12	-	1	-
cefotaxime/ceftriaxone	6	8	-	0	-
ceftazidime	4	8	-	0	3
meropenem/imipenem	0	0	0	0	-
meropenem	-	-	-	-	2
imipenem	-	-	-	-	4
ciprofloxacin	13	6	3	8	4
gentamicin	5	3	2	4	2
tobramycin	6	5	2	3	1
co-trimoxazole	26	13	4	27	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	-	3	-
gentamicin + co-amoxiclav - non-uuti	3	3	-	0	-
gentamicin + piperacillin-tazobactam	1	1	-	0	1
gentamicin + cefuroxime	2	3	-	0	-
gentamicin + cefotaxime/ceftriaxone	2	3	-	0	-
gentamicin + ceftazidime	1	2	-	0	0
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	1
Multidrug resistance					
HRMO**	8	10	2	2	2

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical 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.

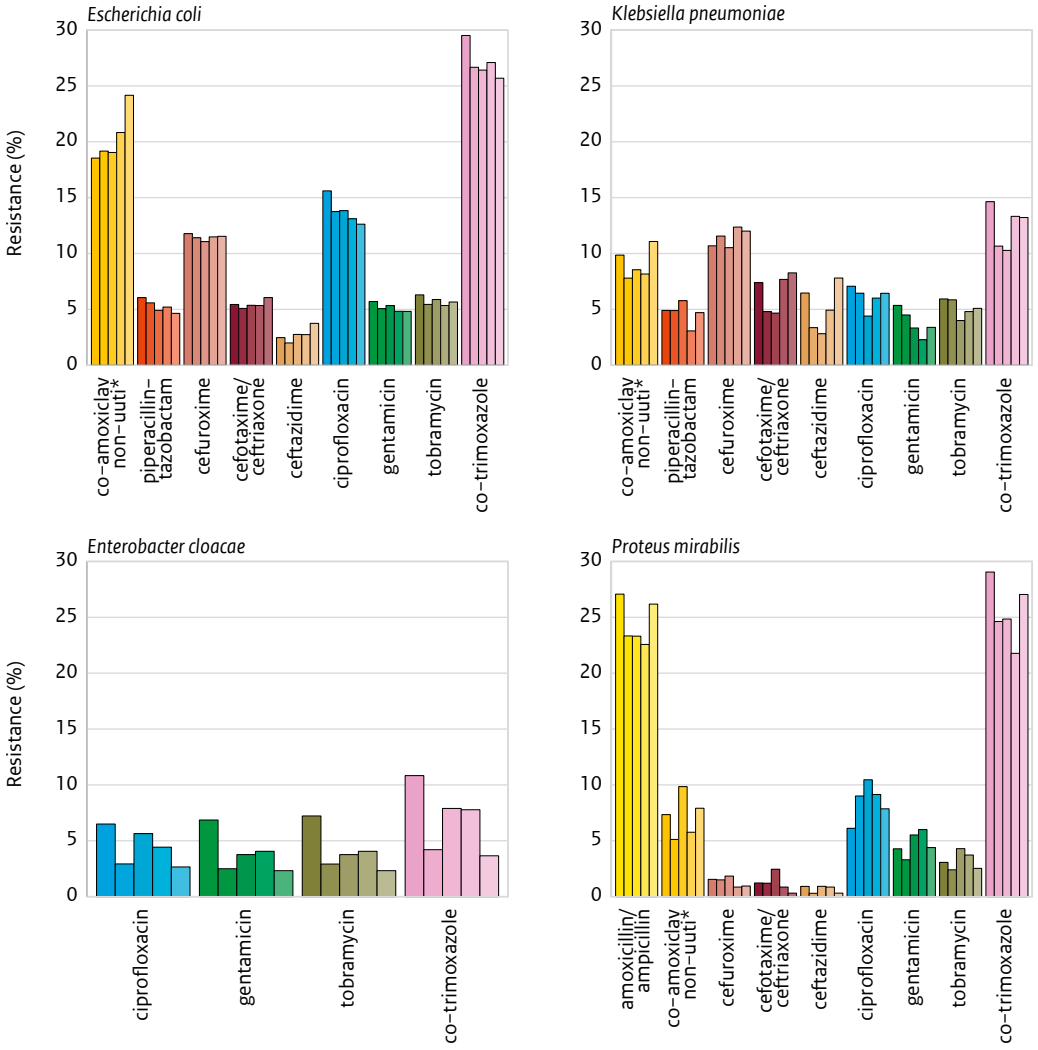
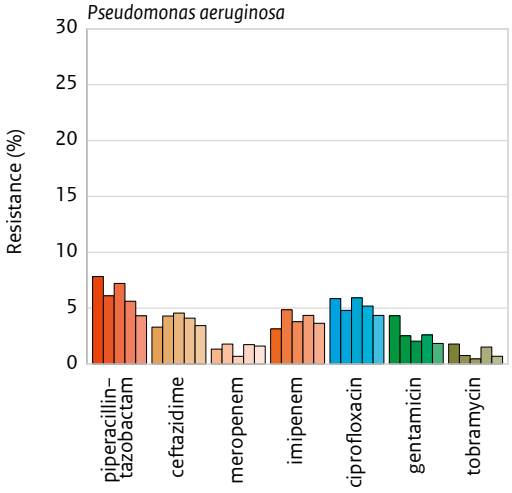


Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



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

** 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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.3.4.3 Resistance levels (%) among clinical blood isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2016.

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

- = Resistance not calculated.

Table 4.3.4.4 Resistance levels (%) among clinical blood isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2016.

	S. aureus	*CNS*
Antibiotic		
flucloxacillin*	1	49
ciprofloxacin**	7	33
gentamicin	1	30
erythromycin	10	49
clindamycin	2	23
clindamycin including inducible resistance***	8	33
doxycycline/tetracycline	3	20
linezolid	0	0
co-trimoxazole	2	20
rifampicin	0	3

10	Significant and clinically relevant increasing trend since 2012
10	Significant and clinically relevant decreasing trend since 2012
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see the methods section)

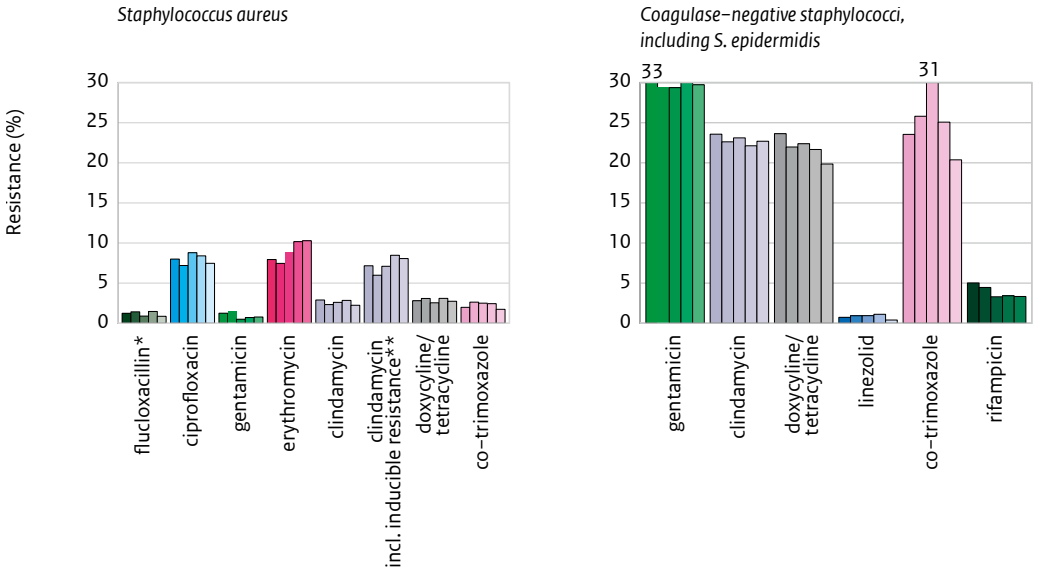
CNS=Coagulase-negative staphylococci, including S. epidermidis

* *Resistance against 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).*

** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

*** *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 2012 to 2016) among clinical blood isolates of *S. aureus* and coagulase-negative staphylococci from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



* *Resistance against 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).*

** *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 and P. aeruginosa

- The majority (86%) of inpatient blood isolates originated from non-ICU departments, and resistance was similar to resistance in these departments in all clinical specimens combined (chapter 4.3.2).
- In *E. coli*, resistance to co-amoxiclav increased significantly and to a clinically relevant extent, from 19% in 2012 to 24% in 2016. In *E. cloacae*, statistically significant and clinically relevant decreasing trends were observed between 2012 and 2016 for resistance to gentamicin, tobramycin (both from 7% to 2%), and co-trimoxazole (from 11% to 4%).

E. faecalis and E. faecium

- Resistance levels in blood were similar to those in all specimens.

S. aureus

- Resistance levels in blood were similar to those in all specimens.

Coagulase-negative staphylococci

- Resistance levels and time trends in blood were similar to those in all specimens from inpatient hospital departments (excl. ICU).

4.3.5 Urology services

The distribution of pathogens in urine 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 in these patients in 2016 are presented in tables 4.3.5.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *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 clinical urinary isolates from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2016.

Pathogen	OPD N (%)	IPD N (%)
E. coli	8136 (42)	1516 (34)
K. pneumoniae	1754 (9)	298 (7)
P. mirabilis	1009 (5)	259 (6)
P. aeruginosa	735 (4)	249 (6)
E. faecalis	2318 (12)	590 (13)
E. faecium	143 (1)	104 (2)
Other Enterobacteriaceae*	2206 (11)	656 (15)
Other non-fermenters**	422 (2)	143 (3)
Other Gram-positives***	2651 (14)	602 (14)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp., Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Enterococcus spp., Streptococcus spp.*

Table 4.3.5.2 Resistance levels (%) among clinical 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 2016.

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	46	51	-	-	23	27	-	-
co-amoxiclav* - non-uuti	22	27	11	15	6	7	-	-
piperacillin-tazobactam	5	6	5	8	0	2	3	5
cefuroxime	13	15	14	16	1	2	-	-
cefotaxime/ceftriaxone	6	8	7	12	1	1	-	-
ceftazidime	4	5	6	11	0	0	2	2
meropenem/imipenem	0	0	0	0	0	0	-	-
meropenem	-	-	-	-	-	-	1	1
imipenem	-	-	-	-	-	-	5	6
ciprofloxacin	19	23	6	9	10	10	8	10
gentamicin	6	7	3	5	7	6	4	3
tobramycin	6	8	5	8	4	2	0	0
fosfomycin	2	1	32	24	-	12	-	-
trimethoprim	31	33	24	22	37	35	-	-
co-trimoxazole	29	31	15	16	29	28	-	-
nitrofurantoin	4	3	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ampicillin	5	7	-	-	6	6	-	-
gentamicin + co-amoxiclav - non-uuti	3	4	2	5	2	2	-	-
gentamicin + piperacillin-tazobactam	-	1	-	2	-	0	1	0
gentamicin + cefuroxime	2	3	2	4	0	0	-	-
gentamicin + cefotaxime/ceftriaxone	1	2	2	4	0	0	-	-
gentamicin + ceftazidime	1	2	2	4	0	0	0	0
tobramycin + ceftazidime	-	-	-	-	-	-	0	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	0	0
Multidrug resistance								
HRMO**	9	12	8	13	4	4	1	1
multidrug resistance*** - non-uuti	5	7	3	6	1	1	-	-

10 Significant and clinically relevant increasing trend since 2012
10 Significant and clinically relevant decreasing trend since 2012
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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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 P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

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

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical 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.

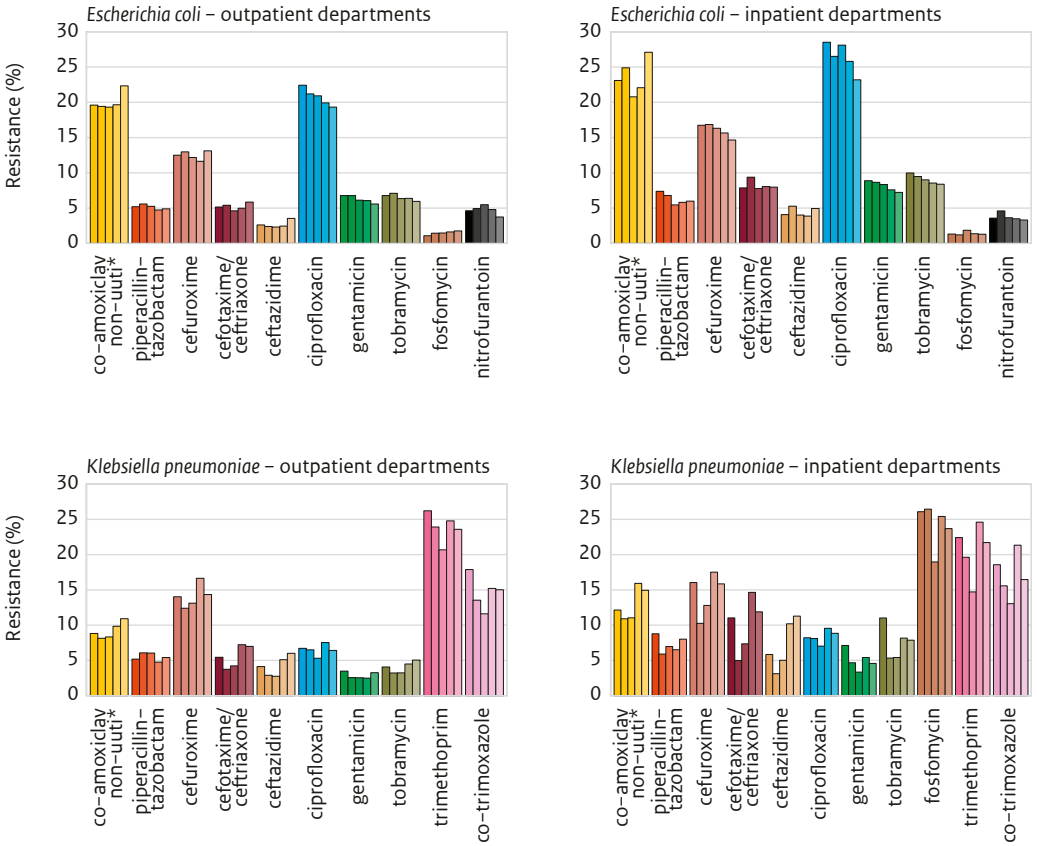
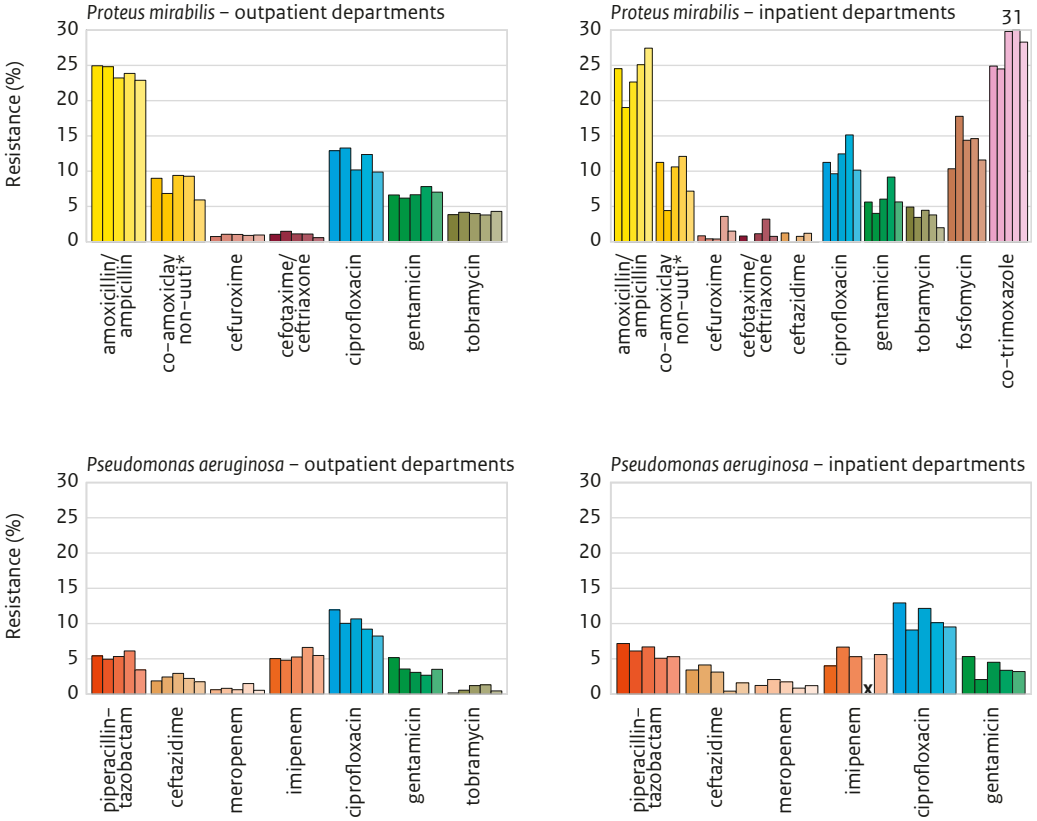


Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2012 to 2016) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.



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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

Table 4.3.5.3 Resistance levels (%) among clinical 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 2016.

	E. faecalis		*E. faecium*	
	OPD	**IPD**	**OPD**	**IPD**
Antibiotic				
amoxicillin/ampicillin	-	-	80	89
vancomycin	0	0	0	0
nitrofurantoin	1	1	-	-

Key results

Enterobacteriaceae

- Resistance levels of 10% or lower in all Enterobacteriaceae were found for piperacillin-tazobactam (≤8%), cefotaxime/ceftriaxone and ceftazidime (≤7%, except in *K. pneumoniae* isolates from IPD patients; 12% and 11% respectively), meropenem/imipenem (0%), ciprofloxacin (≤10%, except in *E. coli*; 19% in OPD and 23% in IPD), gentamicin (≤7%), and tobramycin (≤8%). In addition, levels of 10% or lower were found for fosfomycin and nitrofurantoin in *E. coli* (≤4%), and for co-amoxiclav, cefuroxime, and tobramycin (≤7%) in *P. mirabilis*.
- Resistance of 20% or higher were found for trimethoprim (≥22%) in all Enterobacteriaceae, for co-amoxiclav in *E. coli* (≥22%), for ciprofloxacin in *E. coli* (IPD only; 23%), for fosfomycin in *K. pneumoniae* (≥24%), and for amoxicillin/ampicillin and co-trimoxazole in *E. coli* and *P. mirabilis* (≥23%).
- In *E. coli*, there was a statistically significant and clinically relevant decrease in resistance to trimethoprim in isolates from IPD patients (from 39% in 2012 to 33% in 2016). Resistance levels for co-trimoxazole decreased significantly and to a clinically relevant extent in *E. coli* isolates from both patient groups (from 34% to 29% in OPD and from 37% to 31% in IPD). In *K. pneumoniae*, there was a significant and clinically relevant increase in resistance to cefotaxime/ceftriaxone in patients from OPD (from 5% to 7%) and in resistance to ceftazidime both in OPD (from 4% to 6%) and IPD (from 6% to 11%). Finally, although not significant over five years, over the last four years an increase in resistance was found for amoxicillin/ampicillin in *P. mirabilis* in isolates from IPD patients (from 19% in 2013 to 27% in 2016).
- Resistance to empiric therapy combinations was ≤7%.
- In *K. pneumoniae*, the percentage of HRMO increased to a significant and clinically relevant extent in OPD, from 6% in 2012 to 8% in 2016.

P. aeruginosa

- Resistance to each of the selected agents was ≤10%.
- In IPD, resistance to ceftazidime decreased significantly and to a clinically relevant extent, especially in the first four years (from 3% in 2012 to 0% in 2015). In OPD, resistance to ciprofloxacin decreased significantly and to a clinically relevant extent from 12% to 8%.
- Resistance to empiric therapy combinations and the percentage HRMO remained low (≤1%).

E. faecalis and E. faecium

- Resistance to vancomycin (0%) and nitrofurantoin (1%, presented for *E. faecalis* only) were both rare.
- In *E. faecium*, resistance to amoxicillin/ampicillin was ≥80%.

4.3.6 Respiratory pathogens

For respiratory pathogens, resistance levels were calculated for general practitioner's patients and hospital patients (outpatient and inpatient, incl. intensive care units) separately. In table 4.3.6.1 the distribution of respiratory pathogens isolated from clinical lower and upper respiratory tract specimens from GP patients is presented. The resistance levels for pathogens isolated from GP patients are displayed in table 4.3.6.2. The distribution of pathogens and the resistance levels for pathogens isolated from hospital patients are presented in table 4.3.6.3 and table 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 Dutch hospitals, 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 respiratory pathogens in clinical specimens from general practitioner's patients, ISIS-AR 2016.

Pathogen	Lower respiratory tract N (%)	Upper respiratory tract N (%)
S. pneumoniae	157 (17)	25 (37)
H. influenzae	604 (64)	34 (51)
M. catarrhalis	188 (20)	8 (12)

Table 4.3.6.2 Resistance levels (%) among clinical isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioner's patients, ISIS-AR 2016.

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)	0	-	-
(benzyl)penicillin (I+R)	2	-	-
amoxicillin/ampicillin	-	22	-
co-amoxiclav	-	11	1
erythromycin	16	-	3
doxycycline/tetracycline	15	1	1
co-trimoxazole	7	20	9

- = Resistance not calculated.

Table 4.3.6.3 Distribution of isolated respiratory pathogens in clinical specimens from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2016.

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)
S. pneumoniae	1185 (89)	2605 (23)
H. influenzae	134 (10)	6728 (60)
M. catarrhalis	12 (1)	1942 (17)

Table 4.3.6.4 Resistance levels (%) among clinical 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 2016.

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)	0	-	-
(benzyl)penicillin (I+R)	4	-	-
amoxicillin/ampicillin	-	23	-
co-amoxiclav	-	9	2
erythromycin	10	-	3
doxycycline/tetracycline	9	1	1
co-trimoxazole	7	19	9

- = Resistance not calculated.

Key results

S. pneumoniae

- In both GP patients and hospital patients resistance (0% in both patient groups) and nonsusceptibility (2% in GP patients and 4% in hospital patients) to (benzyl)penicillin was ≤10%. Furthermore, resistance levels of 10% or lower were found for co-trimoxazole (7% in both patient groups), and doxycycline/tetracycline in hospital patients (9%).

H. influenzae

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

M. catarrhalis

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

4.4 Long-term care facilities

For the resistance analyses on *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* in residents of long-term care facilities (LTCFs), only urinary isolates were included. For *S. aureus* from LTCF residents, only wound and pus isolates were included. The distribution of pathogens from LTCF residents is presented in table 4.4.1 for pathogens isolated from urine samples and in table 4.4.2 for pathogens isolated from wound and pus samples. The resistance levels for the pathogens isolated from these samples in 2016 are presented in table 4.4.3 and table 4.4.4, respectively.

LTCFs usually send samples for culture and susceptibility testing in case of complicated urinary tract infection or antimicrobial therapy failure. As a result, the presented resistance levels are not representative for all residents with urinary tract infections or *S. aureus* (wound and pus) infections in LTCFs. Therefore, these residents 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 clinical urine isolates from selected residents of long-term care facilities, ISIS-AR 2016.

Pathogen	N (%)
E. coli	4207 (44)
K. pneumoniae	854 (9)
P. mirabilis	1415 (15)
P. aeruginosa	543 (6)
S. aureus	409 (4)
Other Enterobacteriaceae*	684 (7)
Other non-fermenters**	80 (1)
Enterococcus spp.	945 (10)
Other Gram-positives***	317 (3)

* *Klebsiella spp. (non-pneumoniae), Serratia spp., Morganella spp., Citrobacter spp., Enterobacter spp., Salmonella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas spp.*

*** *Streptococcus spp.*

Table 4.4.2 Distribution of isolated pathogens in clinical wound and pus isolates from selected residents of long-term care facilities, ISIS-AR 2016.

Pathogen	N (%)
S. aureus	385 (41)
Enterobacteriaceae*	297 (32)
Other non-fermenters**	129 (14)
Other Gram-positives***	123 (13)

* *Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp., Serratia spp., Morganella spp., Citrobacter spp., Salmonella spp., Providencia spp., Hafnia spp., Pantoea spp., Shigella spp., Yersinia spp.*

** *Acinetobacter spp., Pseudomonas spp., and Stenotrophomonas spp.*

Table 4.4.3 Resistance levels (%) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected residents of long-term care facilities, ISIS-AR 2016.

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	50	-	23	-
co-amoxiclav* - non-uuti	29	11	7	-
piperacillin-tazobactam	7	6	0	5
cefuroxime	14	16	1	-
cefotaxime/ceftriaxone	6	7	1	-
ceftazidime	4	6	0	3
meropenem/imipenem	0	0	0	-
meropenem	-	-	-	0
imipenem	-	-	-	6
ciprofloxacin	20	7	11	6
norfloxacin	27	24	21	-
gentamicin	5	3	5	3
tobramycin	6	5	4	1
fosfomycin	2	32	16	-
trimethoprim	29	20	39	-
co-trimoxazole	26	13	29	-
nitrofurantoin	4	-	-	-
Multidrug resistance				
HRMO**	10	8	3	1
multidrug resistance*** - non-uuti	5	3	1	-

- = Resistance not calculated

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

* *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 in 2016 to higher levels than before (see methods section for more detailed information).*

** *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 P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

*** *Multidrug resistance, defined as resistance to all of the following oral agents: co-trimoxazole, co-amoxiclav and ciprofloxacin*

Table 4.4.4 Resistance levels (%) among clinical wound and pus isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2016.

	S. aureus
Antibiotic	
flucloxacillin*	1
ciprofloxacin**	25
erythromycin	14
clindamycin	5
clindamycin including inducible resistance***	13
doxycycline/tetracycline	3
fusidic acid	11
co-trimoxazole	3

* *Resistance against 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).*

** *Resistance against ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

*** *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 (≤7%), ceftazidime (≤6%), meropenem/imipenem (0%), gentamicin (≤5%), and tobramycin (≤5%) were ≤10%. In addition, resistance of *P. mirabilis* to co-amoxiclav (7%) and cefuroxime (1%), of *K. pneumoniae* to ciprofloxacin (7%) and of *E. coli* to fosfomycin (2%) and nitrofurantoin (4%) were ≤10%.
- For all Enterobacteriaceae, resistance levels of 20% or higher were found for amoxicillin/ampicillin (≥23%), norfloxacin (≥21%) and trimethoprim (≥20%). Additionally, resistance levels for co-amoxiclav (29%) and ciprofloxacin (20%) in *E. coli*, fosfomycin (32%) in *K. pneumoniae* and for co-trimoxazole in *E. coli* (26%) and *P. mirabilis* (29%) were ≥20%.
- The percentage of HRMO and multidrug resistance was ≤10% in all Enterobacteriaceae.

P. aeruginosa

- Resistance levels for each of the selected agents were ≤6%.

S. aureus

- Resistance to flucloxacillin (1%), clindamycin (5%), doxycycline/tetracycline (3%) and co-trimoxazole (3%) were below 10%.
- Resistance for ciprofloxacin was 25%.

4.5 Highly resistant microorganisms

4.5.1 Carbapenem-resistant Enterobacteriaceae

Introduction

Carbapenem-resistant Enterobacteriaceae (CRE), particularly *Klebsiella pneumoniae* and *Escherichia coli*, are a growing worldwide public health threat. 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.[4] Below, the prevalence of CRE in the Netherlands is described based on information from ISIS-AR data and in addition information on molecular typing of carbapenemase-producing Enterobacteriaceae (CPE) is presented from the Type-Ned database.

Prevalence of CRE in the Netherlands

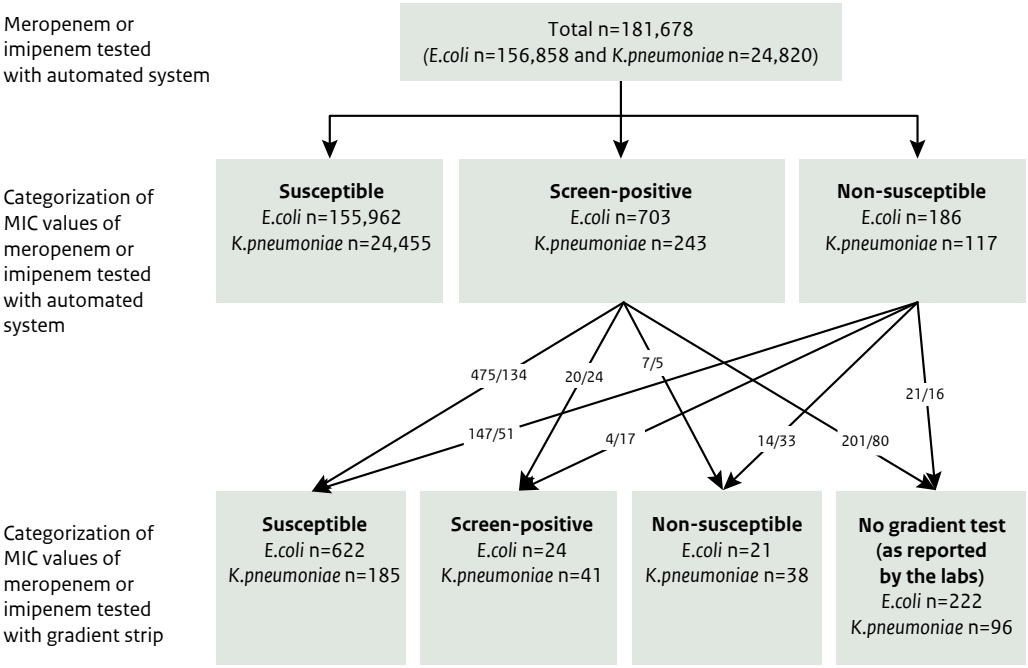
Methods

The ISIS-AR database (year 2016) was searched for *E. coli* and *K. pneumoniae* isolates that, based on susceptibility testing by automated system, were either i) non-susceptible to meropenem and/or imipenem based on EUCAST clinical breakpoints (MIC >2 mg/L) or ii) screen-positive for meropenem (MIC >0.25 mg/L) and/or imipenem (MIC >1 mg/L) as defined by the NVMM (NVMM Guideline Laboratory detection of highly resistant microorganisms, version 2.0, 2012). Both screening and clinical isolates were included. Only one isolate per patient, i.e. the most resistant and most completely tested isolate, was included. Data are based on isolates from 38 laboratories.

Results

Results of sequential testing of carbapenem susceptibility and genotypic/phenotypic testing of carbapenemase production, as prescribed by the NVMM, are presented in figure 4.5.1.1. Of a total number of 181,678 isolates (156,858 *E. coli* and 24,820 *K. pneumoniae*), an elevated meropenem and/or imipenem MIC on automated testing was found in 0.7% of isolates. Confirmation of these elevated carbapenem MIC values using gradient testing was performed in 74.5% of eligible isolates. Confirmatory testing in eligible isolates using a gradient strip method confirmed elevated carbapenem MIC values in 5% of *E. coli* and 22% of *K. pneumoniae*. This means that the overall yield of further testing was low: in the remaining 95% of *E. coli* and 78% of *K. pneumoniae* isolates, gradient strip testing showed MIC values below the screening breakpoint. Even in isolates non-susceptible using automated testing, 79% of *E. coli* and 44% of *K. pneumoniae* had MIC values below the screening breakpoint using gradient strip testing. In total, 21 carbapenem resistant *E. coli* isolates and 38 carbapenem resistant *K. pneumoniae* isolates were found. The overall proportion of confirmed non-susceptible *E. coli* and *K. pneumoniae* was 0.01% and 0.15% respectively.

Figure 4.5.1.1 Results of sequential testing of carbapenem susceptibility and genotypic/phenotypic testing of carbapenemase production, according to NVMM Guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012), in 38 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

Discussion

An elevated carbapenem MIC was found in 0.7% of isolates. This is the same percentage as in 2013/2014 and 2015. Confirmatory testing of elevated carbapenem MIC values is increasing: in 2013/2014, 59.8% of eligible isolates underwent gradient testing, compared to 65.8% in 2015 and 74.5% in 2016.
The yield of confirmatory testing remains low: in only 5% of *E. coli* isolates in 2016 an elevated carbapenem MIC could be confirmed by gradient testing, compared to 8% in 2013/2014 and 2015.
For *K. pneumoniae*, an elevated carbapenem MIC was found using confirmatory testing in 22% of isolates in 2016 compared to 32% in 2013/2014 and 41% in 2015.
The overall proportion of confirmed non-susceptible *E. coli* and *K. pneumoniae* is stable over the past 4 years and was 0.01% and 0.15% respectively in 2016 compared to 0.01% and 0.16% in 2013/2014 and 0.01% and 0.21% in 2015.

Conclusion

- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing remains stable over the past 4 years. Confirmatory testing of elevated MIC values with a gradient strip method has increased from 59.8% to 74.5%. The overall proportion of confirmed non-susceptible *E. coli* and *K. pneumoniae* is low (0.01% and 0.15% respectively).

Molecular epidemiology

Methods

For the enhanced CPE surveillance, Dutch MMLs submit isolates with a MIC for meropenem >0.25mg/L and/or MIC for imipenem >1mg/L using the Type-Ned system for molecular typing, 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-coding genes by PCR (carba-PCR). From August 2016 on, next-generation sequencing (NGS) has been added to the enhanced CPE surveillance for all isolates that were CIM positive.
The data described in this chapter are based on the first unique CIM positive species-gene combination per person per year. Samples from non-human origin and isolates without a person ID were excluded from further analysis.

Results

A total of 403 Enterobacteriaceae isolates obtained in 2016 were submitted to the RIVM by 52 Dutch MMLs, of which 384 isolates from 348 persons met the inclusion criteria. The CIM test showed that 165 unique carbapenemase-producing Enterobacteriaceae isolates were obtained from 151 persons submitted by 43 MMLs (mean age 62 years and 52% male).
In 142 of the 151 persons, a single carbapenemase-producing species was found, whereas multiple unique carbapenemase-producing species (23 isolates) were isolated from 9 persons (Table 4.5.1.1).

The most frequently identified genes were *bla*$_{OXA-48}$, *bla*$_{NDM}$ and *bla*$_{KPC}$. In five persons both *bla*$_{OXA-48}$ and *bla*$_{NDM}$ genes were detected in the same isolate. Seven out of the eight carbapenemase-producing isolates that did not yield a PCR product in the carba-PCR were *Enterobacter* species. The highest number of unique species-gene combinations found in a single patient was five. NGS analysis was performed for 76 isolates originating from 67 persons (Table 4.5.1.2).

Table 4.5.1.1 Carbapenemase encoding genes in Enterobacteriaceae isolates submitted in 2016 as detected by PCR, based on first isolate per patient per year.

Single carbapenemase-producing isolate per person	No gene	*bla*$_{OXA-48}$	*bla*$_{NDM}$	*bla*$_{OXA-48}$ and *bla*$_{NDM}$	*bla*$_{KPC}$	*bla*$_{VIM}$	*bla*$_{OXA-23}$	Num. of persons
Klebsiella pneumoniae	1	34	20	3	10	1		69
Escherichia coli		24	15	1				40
Enterobacter spp.	7	5	1	1		2		16
Other species		6	7		1	1	2	17
Total	**8**	**69**	**43**	**5**	**11**	**4**	**2**	**142**

Multiple different carbapenemase-producing isolates per person	No gene	*bla*$_{OXA-48}$	*bla*$_{NDM}$	*bla*$_{OXA-48}$ and *bla*$_{NDM}$	*bla*$_{KPC}$	*bla*$_{VIM}$	*bla*$_{OXA-23}$	Num. of persons
2 isolates *pp* belonging to different species carrying the same gene		8	2					5
2 isolates *pp* belonging to different species with each isolate carrying a different gene		1	1					1
3 isolates *pp* belonging to 2 different species carrying 3 different genes		1	1			1		1
3 isolates *pp* belonging to 3 different species carrying 2 different genes		1	2					1
5 isolates *pp* belonging to 5 different species carrying 3 different genes		3			1	1		1
Total	**0**	**14**	**6**	**0**	**1**	**2**	**0**	**9**

pp: per person

Table 4.5.1.2 Carbapenemase encoding alleles in Enterobacteriaceae isolates submitted from August 2016 on as detected by next-generation sequencing, based on first isolate per patient per year.

	Single carbapenemase-producing isolate per person	Multiple different carbapenemase-producing isolates per person
bla$_{OXA-48}$	30	11
bla$_{NDM-1}$	7	2
bla$_{OXA-48}$ and *bla*$_{NDM-1}$	3	
bla$_{NDM-5}$	5	
bla$_{NDM-7}$	1	
bla$_{KPC-2}$	1	1
bla$_{KPC-3}$	4	
bla$_{OXA-23}$	1	
bla$_{OXA-181}$	4	
bla$_{OXA-232}$	2	
bla$_{OXA-244}$	1	
bla$_{OXA-181}$ and *bla*$_{NDM-5}$	1	
bla$_{VIM-1}$	1	1
Num. of persons	61	6
Num. of isolates	61	15

Additional epidemiological data (from questionnaires in the OSIRIS and Type-Ned system) was available for 67 (44%) of the patients with a confirmed CPE isolate. Of those, 37 (55%) had a history of admission to a foreign hospital longer than 24 hours within the previous two months, and three patients (4%) had been in contact with a hospital abroad in a different way. Two (3%) patients 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. Overall, no risk factor was identified in 15 patients (22%).

Discussion

In 2016, more Enterobacteriaceae isolates were submitted to the RIVM than in 2015, and as a result more CIM positive isolates were detected. The proportion of persons carrying a carbapenemase-producing strain remained stable (151/348; 43%).

Conclusion

- The most frequently identified carbapenemase encoding genes in Enterobacteriaceae were *bla*$_{OXA-48}$, *bla*$_{NDM}$ and *bla*$_{KPC}$.

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. 2014 Dec 29. doi: 10.1111/joim.12342. [Epub ahead of print]

[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

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. The highest percentage was found in intensive care units, amounting to 0.8% of isolates.
VRE outbreaks were reported through the Signaling Consultation 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, 59 hospital outbreaks with VRE have been reported in the Netherlands: 9 in 2012, 10 in 2013, 14 in 2014, 16 in 2015, and 10 in 2016.
As of May 2012 the UMC Utrecht has offered molecular diagnostics on clinical VRE-isolates. Since then, 44 hospitals and laboratories have submitted 818 VRE to the UMC Utrecht (status of March 10th 2017), of which 815 were identified as *E. faecium*, 2 *E. faecalis* and 1 *E. gallinarum*. These represented 459 *E. faecium* isolates carrying the *vanA* gene cluster, 348 the *vanB* gene cluster, five isolates carried both the *vanA* and the *vanB* gene cluster and three isolates carried the *vanD* gene cluster. Of the 815 *E. faecium* VRE, 705 were typed by Multi Locus Sequence Typing (MLST). This revealed a total of 45 different Sequence Types, suggesting that at least 45 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, 18 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 (29 hospitals), ST203 (25 hospitals), ST18 (15 hospitals), ST80 (15 hospitals).

Table 4.5.2.1 Percentage of VRE in the Netherlands in 2016, based on ISIS-AR data.

Type of department	Tested isolates, N	VRE, N (%)*
GP	385	1 (0.3)
Outpatient departments	436	1 (0.2)
Inpatient departments excluding Intensive Care Units	2359	13 (0.6)
Intensive Care Units	666	5 (0.8)

* *VRE is defined as resistant to amoxicillin/ampicillin and vancomycin.*
Numbers based on a selection of 29 laboratories
The first Enterococcus faecium isolate per patient was selected
Based on interpretation of the laboratories

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 2016. Numbers are based on data from 29 laboratories that continuously reported to the ISIS-AR database during the whole year in 2016. 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. Nevertheless, the RIVM occasionally receives consecutive isolates from the same person. It is assumed that the enhanced MRSA surveillance includes more than 85% of all persons found to be MRSA-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) only (i.e. Staphylococcal protein A *(spa)*-typing is not performed anymore). MLVA is a typing technique based on the composition of eight genomic loci containing tandem repeats and is based on accurate band sizing using an automated DNA sequencer. Currently, the MRSA population can be divided into 28 MLVA-complexes (MCs). 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 2016 only, 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* appearing to be susceptible for methicillin, and isolates without a person ID were also excluded from further analysis.

Results

Prevalence

The proportion of *S. aureus* positive for MRSA in clinical isolates (including blood samples) based on ISIS-AR was 1.8% (518/28,840), ranging from 1.6% in outpatient and hospital departments to 2.5% in general practices (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 unbiased, the MRSA prevalence was 1.0% (24/2,386).

Table 4.5.3.1 Percentage of MRSA in the Netherlands in 2016, based on ISIS-AR data.

Type of department	Tested isolates, N	MRSA, N (%)*
GP	6,291	155 (2.5)
Outpatient departments	11,397	182 (1.6)
Inpatient departments excluding Intensive Care Units	10,061	164 (1.6)
Intensive Care Units	1,091	17 (1.6)
Total	**28,840**	**518 (1.8)**

* *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 for flucloxacillin/oxacillin.*

Numbers based on a selection of 29 laboratories
The first S. aureus isolate per patient was selected
Based on re-interpretation according to EUCAST 2016

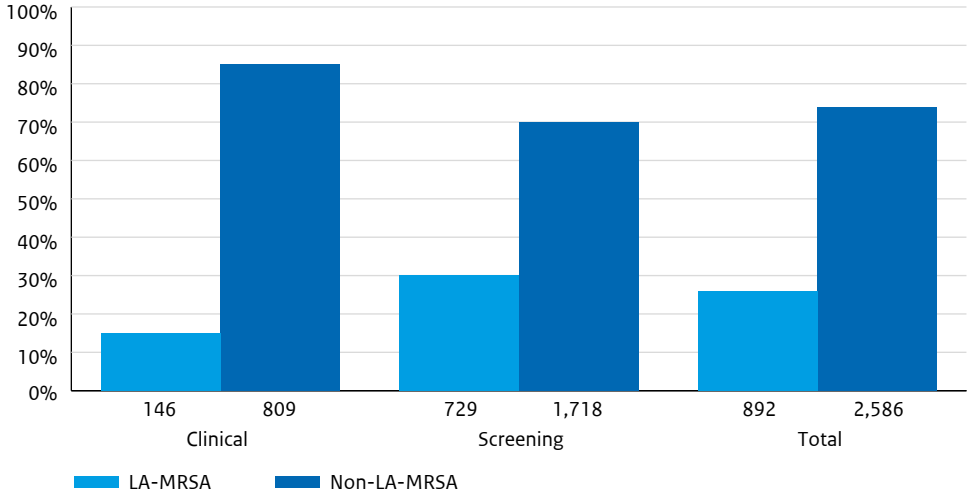
Molecular results and epidemiology

A total of 4,395 isolates obtained in 2016 were genotyped as part of the enhanced MRSA surveillance, of which 3,768 obtained from 3,478 persons (mean age 47 years and 53% male) submitted by 58 MMLs fulfilled the inclusion criteria (human origin, *S. aureus* resistant for methicillin, and isolates with a known person ID).

Based on culture methods and origin of the samples, 70% (2,447/3,478) of the isolates were identified as screening samples (mainly swabs of nose, throat and perineum) (Figure 4.5.3.1). A total of 955 samples (27%) were identified as clinical sample (material originating from blood, cerebrospinal fluid, sputum, pus, urine or wound), with the majority being wound material or pus (651/955; 68%) and only 26 blood samples (3%). For the remaining 76 samples (2%), the origin of the sample was unknown.

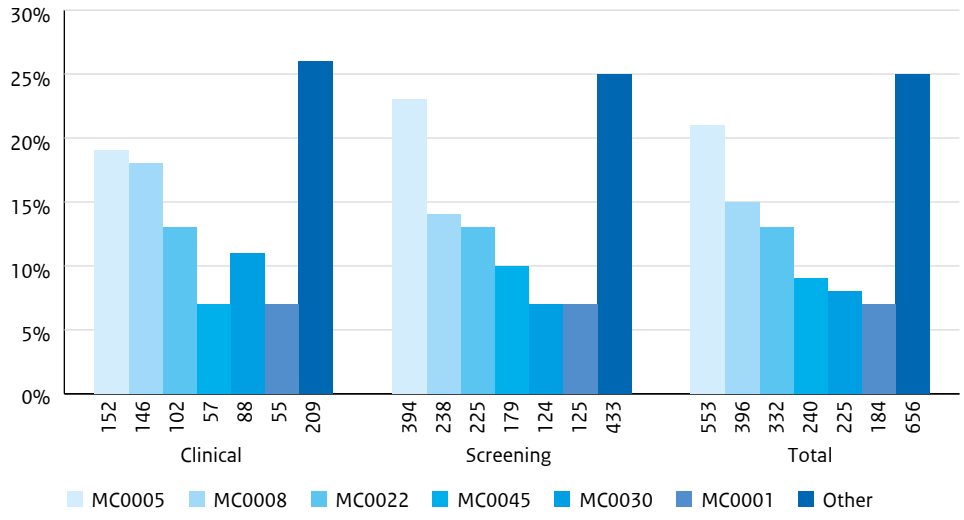
The most common MLVA-complex in the Dutch enhanced MRSA surveillance in 2016 was MC0398, representing livestock-associated MRSA (LA-MRSA), which was detected in 892/3,478 (26%) of the isolates. Of the LA-MRSA isolates, 16% were clinical isolates, 82% were obtained for screening purposes, and for 2% it was unknown (Figure 4.5.3.1). The number and proportion of clinical isolates was higher among the non-LA-MRSA (809/2,586; 31%). Among the clinical isolates, MC0005, MC0008 and MC0022 were the most prevalent non-LA-MRSA MLVA complexes (Figure 4.5.3.2).

Figure 4.5.3.1 Distribution of clinical and screening samples for LA-MRSA and non-LA-MRSA among MRSA isolates received in the Dutch enhanced MRSA surveillance in 2016 (N=3,478).



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 76 isolates for which it was unknown whether it was a clinical or screening sample (17 LA-MRSA and 59 non-LA-MRSA)

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 2016 (N=2,586).



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 59 isolates for which it was unknown whether it was a clinical or screening sample (7 MC0005, 12 MC0008, 5 MC0022, 4 MC0045, 13 MC0030, 4 MC0001, and 14 Other)

Risk groups

A risk factor questionnaire is requested to be completed as part of the enhanced surveillance. Questionnaires were available for 2,161 of the 3,478 genotyped isolates (62%) from unique persons: 2,006 (93%) were patients and 155 (7%) were employees.
Completed data on risk categories based on the WIP guidelines[1] were available for 2,121 (61%) of the persons/isolates. Hospitalization abroad during the previous two months was recorded for 112 persons (5%). Work-related exposure to livestock animals was reported for 330 persons (16%), almost all of them (93%) were positive for LA-MRSA (MLVA-complex MC0398). A total of 210 persons (10%) were already known to be MRSA-positive. For 38% (810/2,121), no risk factors that meet any of the WIP risk categories for MRSA carriage had been detected.
Of the clinical isolates, completed data on risk categories were available for 615/955 (64%). The large majority was not suspected of MRSA carriage (513/615; 83%), 9% had a high risk for carriage, and 5% was already known to be MRSA positive. Twelve (2%) had been hospitalized abroad in the previous two months and fifteen (2%) had work-related exposure to livestock animals.

Discussion

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. 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 38% of the isolates. Therefore, some misclassification of screening and clinical isolates may have occurred.

Conclusions

- The proportion of *S. aureus* that was MRSA positive in unbiased blood-culture isolates was 1%. The prevalence in biased samples ranged from 1.6% in hospital departments to 2.5% in general practices.
- LA-MRSA is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance.
- Non-LA-MRSA subtypes are more often found in clinical isolates than observed for LA-MRSA.
- Most of the persons positive for MRSA do not seem to have a risk factor as defined in the WIP risk categories.

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.[1] However, the organism may also acquire additional resistance either by chromosomal mutations or by horizontal gene transfer. The intrinsic resistance is caused by a concerted action of efflux pumps and low permeability of the outer membrane. *P. aeruginosa* may become multidrug-resistant (MDR) due to the simultaneous acquisition of several resistance genes that are clustered in integrons or plasmids through horizontal gene transfer. The emergence of these MDR *P. aeruginosa* is a problem of global concern. On 27 February 2017 the World Health Organization classified carbapenem-resistant *P. aeruginosa* as 'priority 1: critical' on a global priority list of the 12 most important antibiotic-resistant pathogens. Currently, there are reports of hospital outbreaks of MDR *P. aeruginosa* from countries around the world, including the Netherlands. More recently, *P. aeruginosa* with metallo-β-lactamases, such as Verona integron-encoded metallo-β-lactamase (VIM) and imipenemase (IMP), are encountered. Outbreaks, especially caused by these carbapenemase producing *P. aeruginosa* may be large and persistent, despite infection control measures and management. 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. Most other carbapenemase encoding genes in *P. aeruginosa* and other Gram-negatives are carried by plasmids, adding to the risk of transfer of these resistance genes.

Methods

Since 2010 the RIVM performs surveillance of carbapenemase producing Enterobacteriaceae (CPE). Although this surveillance is aimed at collecting Enterobacteriaceae, more than half of the submitted isolates are non-fermenters. However, these data cannot be used to infer prevalence or accurate distribution of carbapenemase producing non-fermenters in the Netherlands. From August 2016 on, medical microbiology laboratories (MMLs) were asked to submit their carbapenem-resistant Enterobacteriaceae and *P. aeruginosa* to the RIVM for characterization as well. Submission was performed via the Type-Ned CPE-CPPA system. 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 that produce carbapenemase and/or carry a carbapenemase coding gene are subjected to next-generation sequencing (NGS) to assess the whole genome sequence.

Results

A search was performed in the ISIS-AR database on multidrug resistant *P. aeruginosa* isolates (Table 4.5.4.1). Multidrug resistance (MDR) *P. aeruginosa* is defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin-tazobactam. The analysis revealed that 1.1% of the *P. aeruginosa* isolates was MDR and that this was highest (3.9%) for isolates obtained from patients at intensive care units (ICUs). Of all MDR *P. aeruginosa* isolates 58.4% was resistant to carbapenems. This fraction was highest in isolates from ICUs (19/20; 95%) and lowest for isolates obtained from patients attending the GP (2/14; 15%).

In 2016 the RIVM received 421 *P. aeruginosa* isolates (one isolate per person per year) and 28 (6.7%) produced carbapenemase (Table 4.5.4.2). PCR revealed that the majority of these carbapenemase-producing isolates (17/28; 60.7%) carried a bla_{VIM} gene, one carried a bla_{IMP} and one a bla_{NDM} gene. Nine of the carbapenemase producing isolates (32.1%) did not yield a PCR product. All 28 carbapenemase producing isolates were subjected to NGS. This revealed that all 17 VIM-positive isolates carried the bla_{VIM-2} allele. NGS also revealed the presence of bla_{IMP-26} and bla_{NDM-1} in the other two isolates. One of the nine PCR-negative carbapenemase producing isolates carried the bla_{GES-5} gene, a gene that is not detected by our multiplex PCR. However, no known carbapenemase coding gene could be identified in the remaining eight isolates.

The range of the MICs for meropenem among the submitted *P. aeruginosa* during the 2013-2016 surveillance period varied (Figure 4.5.4.1). Approximately 71% (121/170) of all carbapenemase producing isolates had MICs for meropenem above the clinical breakpoint of 8 mg/L. However, several isolates with lower MICs also produced carbapenemases and can therefore easily be missed in routine practice. Of the isolates that did not produce carbapenemase 36% (377/1055) also had MICs for meropenem above the clinical breakpoint. In total only 121 of the 499 (24%) of the isolates with MICs above the clinical breakpoint produced carbapenemase.

Discussion

In 2016 only a minority of the *P. aeruginosa* in the Netherlands appeared to be MDR. However, more than half of these HRMOs were resistant to carbapenems. Characterization of the carbapenem resistant *P. aeruginosa* isolates submitted to the RIVM revealed that 36% did not produce carbapenemase and apparently were resistant to carbapenems due to other mechanisms. Remarkably, several *P. aeruginosa* isolates with MICs below the clinical breakpoint also produced carbapenemases. This supports the decision to confirm carbapenemase activity for isolates with MICs above 2 mg/L for meropenem. The majority of the carbapenemase-producing isolates (57.7%) carried a bla_{VIM-2} gene.

Conclusions

- Only 1.1% of *P. aeruginosa* isolates are MDR, but 58% of the MDR are also carbapenem resistant
- Of the isolates with MICs for meropenem above the clinical breakpoint only 24% produced carbapenemase
- Only 71% of the carbapenemase producing *P. aeruginosa* had MICs for meropenem above the clinical breakpoint
- In 2016 nearly 61% of carbapenemase producing *P. aeruginosa* carried the bla_{VIM-2} gene

References

[1] Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. 2013 Feb;19(2):141-60.

[2] van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One. 2015 Mar 23;10(3):e0123690.

Table 4.5.4.1 Multidrug resistant *P. aeruginosa* in the Netherlands in 2016, based on ISIS-AR data.

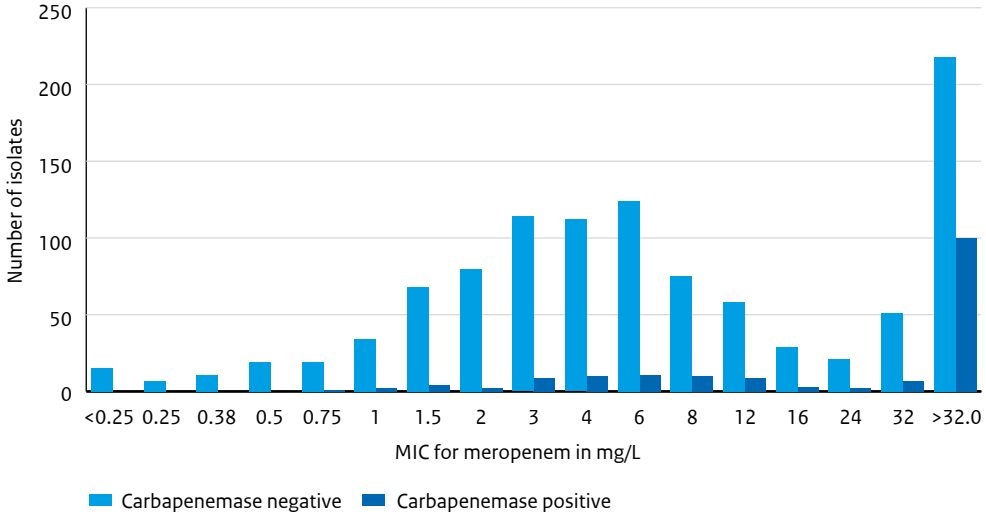
Type of department	Num. of isolates	MDR *P. aeruginosa* (%)	Carbapenem resistant MDR *P. aeruginosa* (%)
General practitioner	3,747	14 (0.4)	2 (14)
Outpatient departments	3,889	42 (1.1)	23 (55)
Inpatient departments, excl. ICUs	4,655	61 (1.3)	36 (59)
Intensive Care Units	512	20 (3.9)	19 (95)
Total	**12,803**	**137 (1.1)**	**80 (58.4)**

Numbers are based on a selection of 29 laboratories. The first P. aeruginosa isolate per patient was selected. Based on re-interpretation according to EUCAST 2016.

Table 4.5.4.2 Distribution of carbapenemase coding genes in carbapenemase producing *P. aeruginosa*. Only the first submitted isolate per person per year is included.

			Year			
Carbapenemase (CIM)	**Carba gene (PCR)**	**Carba allele (NGS, 2016 only)**	**2013**	**2014**	**2015**	**2016**
Negative	Negative	Not done	126	229	307	393
Positive	VIM	$bla_{\text{VIM-2}}$	33	58	31	17
	IMP	$bla_{\text{IMP-26}}$	5	7	2	1
	NDM	$bla_{\text{NDM-1}}$		1		1
	Negative	$bla_{\text{GES-5}}$				1
		No gene identified		1	4	8
Total positive			**38**	**67**	**37**	**28**
All isolates			**164**	**296**	**344**	**421**

Figure 4.5.4.1 Distribution of the MICs for meropenem among submitted *P. aeruginosa* isolates. Only the first submitted isolate per person per year has been included.



4.5.5 Extended spectrum beta-lactamases

Introduction

Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) have become a concern over the years in various countries. In the Netherlands, several nation-wide studies have been performed over the years. The first study was performed in 1997 and comprised 767 isolates, of which 18 were screening positive and, 13 (1.7%) showed beta-lactamase activity.[1] In 2006 in a large study comprising 1713 isolates , close to 6% of isolates were phenotypically positive for ESBL and 3.9% were confirmed ESBLs.[2] Since then, a number of individual studies have been performed, the % confirmed ESBLs ranging between 5-10%. Over the last years, the prevalence of ESBLs in the Netherlands was also estimated each year using the ISIS-AR database. We here present data from ISIS-AR.

Methods

Data were extracted from the ISIS-AR database. The presence of ESBL was estimated based on MIC-testvalues for cefotaxime and/or ceftriaxone and/or ceftazidime, interpreted using EUCAST 2016 breakpoint criteria.

Results and discussion

In table 4.5.5.1 the estimated percentage of ESBL carrying Enterobacteriaceae in 2016 is shown. Overall, compared to the data of 2015, the prevalence appears to have slightly increased, although the selection of laboratories for data analysis in the previous year was not completely similar to 2016. There is a clear increase correlated with the complexity of care.

One of the factors that are hypothetized to be important in acquiring ESBLs, is livestock. An extensive study was carried out in the South of the Netherlands to determine whether living in the proximity of livestock poses a risk to ESBL carriage.[3] Overall, carriage was 4.5% and living in close proximity to livestock animals and farms did not seem to be a risk factor for carriage of ESBL/pAmpC-Enterobacteriaceae. Of note, one of the major risk factors of ESBL carriage was travel to Africa, Asia or Latin America in the previous 12 months. The latter concurs with findings reported in NethMap last year.

References

[1] Stobberingh EE, Arends J, Hoogkamp-Korstanje JA, Goessens WH, Visser MR, Buiting AG, Debets-Ossenkopp YJ, van Ketel RJ, van Ogtrop ML, Sabbe LJ, Voorn GP, Winter HL, van Zeijl JH. Occurrence of extended-spectrum betalactamases (ESBL) in Dutch hospitals. Infection 1999 27(6):348

[2] Mouton J, Voss A, Arends J, Bernards S and the ONE study group. Prevalence of ESBL in the Netherlands: the ONE study. Int J Antimicrob Agents 2007 29:S91-S92

[3] Wielders CC, van Hoek AH, Hengeveld PD, Veenman C, Dierikx CM, Zomer TP, Smit LA, van der Hoek W, Heederik DJ, de Greeff SC, Maassen CB, 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

Table 4.5.5.1 Percentage of ESBL in the Netherlands in 2016 and 2015 as a reference, based on ISIS-AR data.

Type of department	Tested isolates 2016, N	ESBL 2016, N (%)*	ESBL 2015, (%)*
GP	101,330	3169 (3.1)	2.8
Outpatient departments	30,536	1490 (4.9)	4.3
Inpatient departments excluding Intensive Care Units	37,772	2190 (5.8)	5.5
Intensive Care Units	3,019	257 (8.5)	7.8

* *ESBL is estimated by resistance to cefotaxime and/or ceftriaxone and/or ceftazidime*
Numbers are based on isolates from Enterobacteriaceae from a selection of 29 laboratories.
The first isolate per organism per patient was selected Based on re-interpretation according to EUCAST, 2016

4.5.6 Signaling Consultation of Hospital acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR)

In 2012, the Signaling Consultation of Hospital acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded. The purpose of the SO-ZI/AMR is to prevent or mitigate large-scale outbreaks in hospitals 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 and 0 as contained. An outbreak 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.

Table 4.5.6.1 provides an overview of the fifty-one outbreaks reported in 2016. These were reported by 34 healthcare institutions (7 nursing homes, 25 hospitals and two other institutions). None of the outbreaks was considered uncontrollable or a direct threat to public health. Most outbreaks (n=41) ended in 2016; nine outbreaks were still ongoing but completed in 2017, two outbreaks 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.

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and Norovirus where most often reported, whereas in 2015 more outbreaks were related to multi-drug-resistant *Pseudomonas aeruginosa*. Outbreaks with CPE were also reported and caused by different microorganisms, namely *Klebsiella pneumoniae* (2), *Acinetobacter* spp. (1) and *Enterobacter cloacae* (1). Other bacteria or viruses causing outbreaks were reported sporadically.

Only four outbreaks included more than 10 patients. Six outbreaks were classified as phase 2 (n=3 MRSA and n=3 VRE) while no phase 3 outbreak occurred. In contrast, in 2015 three outbreaks were classified as phase 3 (2 VRE outbreaks and one MRSA outbreak).

The median (range) interval it took to report an outbreak to the SO-ZI/AMR, from the moment that the first patient was identified, was 14 days. Four outbreaks were only reported more than three months after identification of the first patient. Of these, one was reported after more than 7 months. 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 requested advice and help to control the outbreaks. One of these outbreaks was due to a carbapenemase-producing *Klebsiella pneumoniae* and the other MRSA. Both outbreaks occurred in nursing homes.

Conclusions

- On average four outbreaks a month are reported to the SO-ZI/AMR.
- All outbreaks were classified as phase 1 or phase 2.
- No MDR *Pseudomonas* outbreaks were reported, however more CPE outbreaks than the previous year.
- Most outbreaks are reported to SO-ZI/AMR within a month after detection
- MRSA and VRE remain the predominant outbreak microorganisms.
- Most outbreaks are controlled quickly (<2 months).
- The median number of patients involved in an outbreak was 5.

Table 4.5.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2016.

	2016 n=51 n (%)
Microorganism (resistance mechanism)*	
Staphylococcus aureus (MRSA)	20 (39)
Enterococcus faecium (VRE)	10 (20)
Klebsiella pneumoniae (CPE)	2 (4)
Klebsiella pneumoniae (ESBL)	1 (2)
Acinetobacter (CPE)	1 (2)
Enterobacter cloacae (CPE)	1 (2)
Escherichia coli (ESBL)	1 (2)
Escherichia coli (AG and FQ)	1 (2)
Acinetobacter	1 (2)
Serratia marcescens	2 (4)
Serratia spp.	1 (2)
Clostridium difficile	1 (2)
Staphylococcus aureus	1 (2)
Norovirus	6 (12)
Enterovirus	1 (2)
Rotavirus	1 (2)
Reason of reporting	
(threatened) closure	40 (78)
ongoing transmission	3 (6)
combination of both	4 (8)
HRMO outbreak (not in a hospital)	3 (6)
unknown	1(2)
Highest level phase	
phase 1	45 (88)
phase 2	6 (12)
phase 3	0 (0)
phase 4	0 (0)
phase 5	0 (0)
Median number of patients: (range)	5 (1-41)
Median duration outbreak in days from reporting date until end of the outbreak: (range)	55 (7-131)
Duration in days between detection of the first patient and day of reporting to the SO-ZI/AMR: (range)	14 (2-418)
Request for help	2 (4)

* 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 Centre for Bacterial Meningitis. Here we report the data of 2016. For N. *meningitidis* the interpretation of the phenotypic susceptibility testing is not 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* gene of all isolates was sequenced.

Methods

From 2009-2016, a total of 323 strains from cerebrospinal fluid (CSF) or CSF and blood and 527 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 and the 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. Serotyping was also performed for all strains.

Results

Penicillin resistance and ceftriaxone resistance were not found in 2016 (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 12% (17/140). In 2016, the 17 moderately susceptible strains from blood and/or CSF belonged to different serogroups: 10 belonged to serogroup B, 2 to serogroup C, 4 to serogroup W and one to serogroup Y. In 2016, 98.6% of the isolates were sensitive to rifampicin; only 2 isolates (one B and one W) were resistant.

Alterations in the *penA* gene, associated with non- susceptibility to penicillin according to the *Neisseria* typing database (https://pubmlst.org/neisseria/), were detected in 14 (10%) of the 140 isolates. Of these 14 isolates, three were phenotypically susceptible and ten were moderately susceptible by Etest (table 4.6.1.3). One isolate was *penA* resistant but phenotypically susceptible.

penA genotyping yielded less strains (10%) non-susceptible to penicillin than Etest with EUCAST criteria does (12%) and both methods do not agree completely.

Discussion and conclusions

Penicillin resistance is still sporadic and was not found in the last three years (two strains in 2013, one strain from CSF and one from blood, zero in 2014, 2015 and 2016). Likewise, the proportion of moderately susceptible strains did not alter over the last three years (around 12%) and resistance to ceftriaxone was not found. The percentage of strains moderately susceptible to penicillin (MIC 0.06-0.25 mg/L), stable around 1-5% until 2009 and thereafter sharply increasing to 33% for blood isolates and 39% for CSF isolates in 2012, has reached stability again. Rifampicin resistance was found in two strains, the last occasion was one strain in 2013 and may need attention the coming years. Alterations in *penA* associated with non-susceptibility to penicillin are present in 10% of all isolates. One or more of the following reasons may be involved: 1) other factors than *penA* gene alterations also confer non-susceptibility to penicillin; 2) a considerable number of minor interpretation errors occur 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, 2009-2016.

	Penicillin*								
	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**

* *MIC values in mg/L*

Table 4.6.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2016.

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2009	77	**88.5**	10	**11.5**	0	**0.0**	0	**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**

* *MIC values in mg/L*

Table 4.6.1.3 Alterations in the *penA* gene and penicillin susceptibility in *N. meningitidis*.

Alterations *penA* gene*	Number of strains with penicillin MIC (mg/L):			
	MIC ≤ 0.06 sensitive	0.064 < MIC ≤ 0.25	0.25 < MIC≤1.0	MIC >1.0
Yes	4	10	0	0
No	119	7	0	0
Total	123	17	0	0

* *Alterations in penA associated with non-susceptibility to penicillin*

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 one of the most common STIs and a major public health concern globally. It can result in severe reproductive complications and can increase the transmission of HIV. Gonorrhoea is the second most common bacterial sexually transmitted infection in the Netherlands. Third generation cephalosporins, such as ceftriaxone and cefixime, are the current first-line treatment for gonorrhoea in most countries. In the Netherlands, cefotaxime 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.

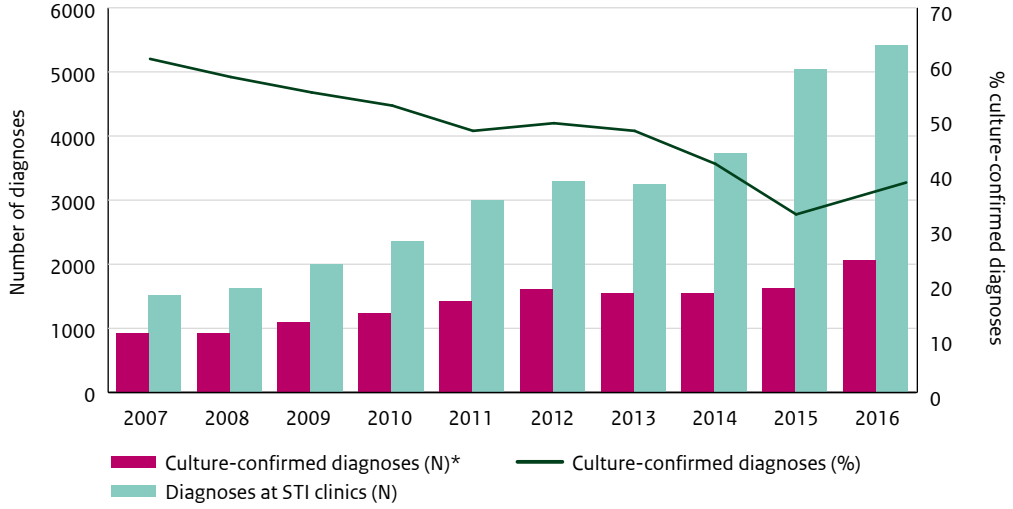
Methods

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. The participating STI centres represent 77% of the total population of STI centre attendees. Diagnosis of gonorrhoea was made by culture or PCR on patients' materials. Susceptibility testing for in total 14,204 isolates was performed by Etest 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 and in 2015, penicillin and tetracycline were removed from the panel. Resistance levels were calculated using the EUCAST breakpoints for resistance.[1]

Results

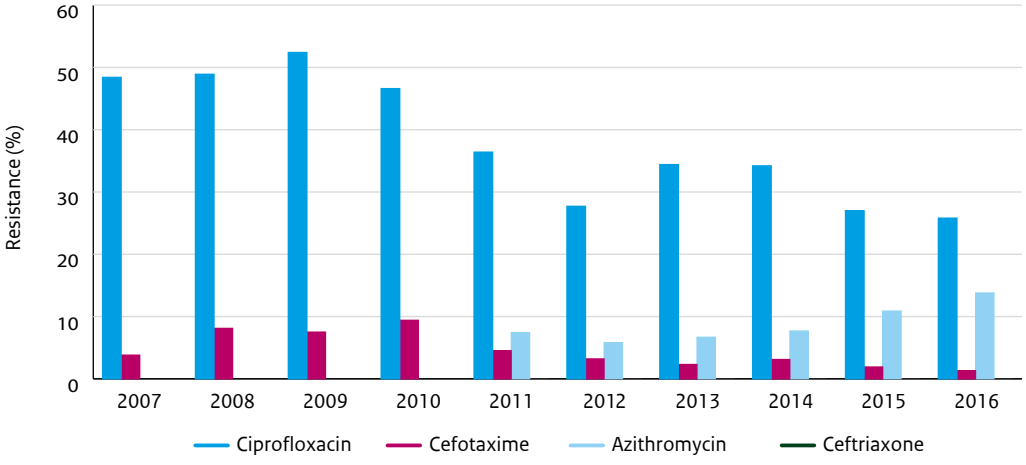
- Since 2007, the number of gonorrhoea diagnoses at STI clinics participating in GRAS has increased to 5,425 in 2016. The number of positive cultures has increased to 2,070 (38%) and the downward trend of culture confirmed diagnoses reversed (Figure 4.6.2.1)
- In 2016, the highest gonococcal resistance levels were reported for ciprofloxacin; however, the resistance level for ciprofloxacin has decreased from 52% in 2009 to 26% in 2016. No resistance was found for ceftriaxone. Resistance levels for cefotaxime have decreased since 2010 to 1% in 2016 and the gonococcal resistance level for azithromycin has increased since 2012 to 14% in 2016 (Figure 4.6.2.2).
- The MIC distribution of ceftriaxone is highly skewed to the right and shows a unimodal shape (Figure 4.6.2.3a), with the biggest proportion of isolates having a MIC smaller than or equal to 0.016mg/L. The MIC distribution of azithromycin shows a more normal distribution (Figure 4.6.2.3b); the proportion of isolates with a MIC of 1mg/L or MIC of 2mg/L and higher has been increasing since 2014.

Figure 4.6.2.1 Number of gonorrhoea diagnoses at STI clinics and number and percentage of culture-confirmed diagnoses in the Netherlands, 2007-2016*.



* *Failed cultures are excluded*
In 2015, some STI clinics had problems reporting resistance levels of cultured strains, leading to missing culture results.

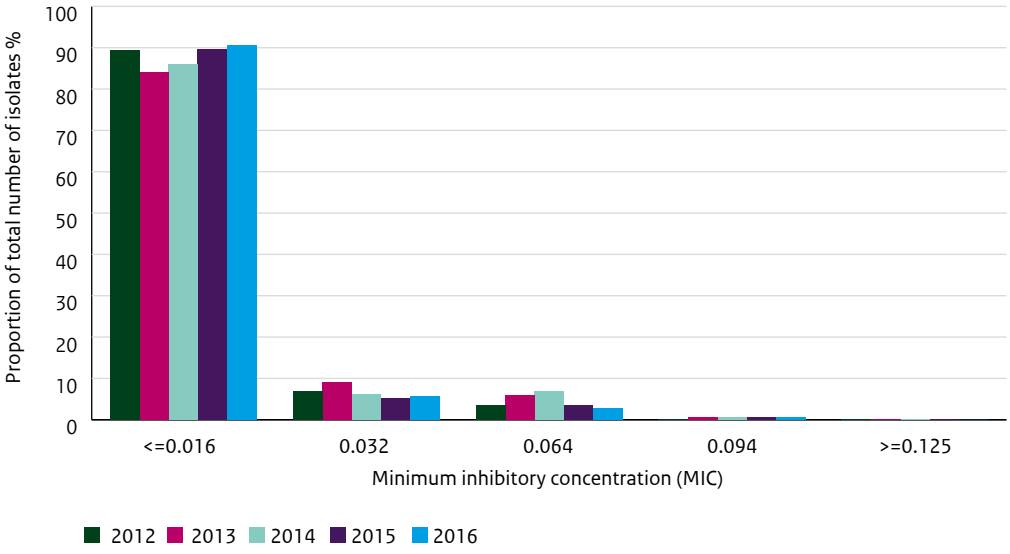
Figure 4.6.2.2 Trends in antibiotic resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands (n=14,204), 2007-2016*.



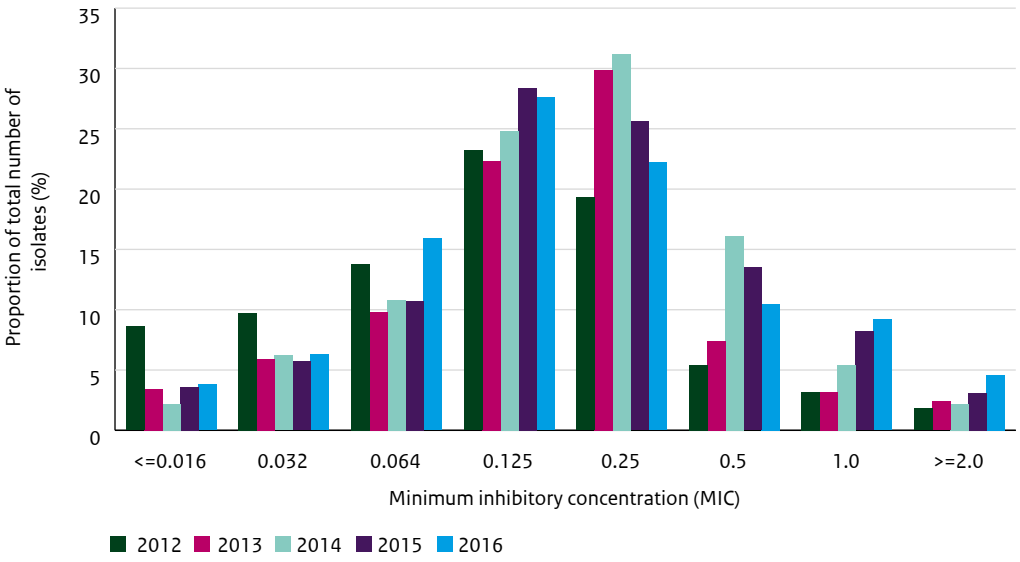
* *Ceftriaxone and azithromycin were added to the panel in 2011 and testing for penicillin and tetracycline became optional. Testing for spectinomycin became optional in 2014. Penicillin and tetracycline were removed from the panel in 2015.*
No resistance was found for ceftriaxone.

Figure 4.6.2.3 MIC (minimum inhibitory concentration) distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2012-2016.

A. MIC distribution for ceftriaxone



B. MIC distribution for azithromycin



Discussion

In less than half (38%) of all gonorrhoea diagnoses at STI clinics participating in GRAS resistance levels were measured by culture. This can partially be explained by negative cultures, making measurement of resistance levels impossible. In addition, the STI register data show that gonorrhoea diagnoses are often only confirmed by PCR, not by culture. The increase in cultures in 2016 compared to 2015 can potentially be explained by a change in reporting; since 2016, it is possible to report the results of more than one culture per patient to GRAS.

In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500 mg). Thus far, no resistance to ceftriaxone has been found yet, although a few isolates have reached the limit MIC value of 0.125mg/L in the last years. Other countries have been recommending combination therapy with azithromycin. The percentage of isolates resistant to azithromycin has been increasing from 6% in 2012 to 14% in 2016. As such, future challenges will probably include the increasing resistance to azithromycin.

Conclusions

- Increase in gonorrhoea diagnoses at STI clinics and a continuing low relative number of confirmations of diagnoses by culture (38% in 2016).
- No resistance to ceftriaxone, the current first-line treatment.
- Continuing increase of resistance to azithromycin from 6% in 2012 to 14% in 2016.

References

[1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. Available via: http://www.eucast.org.

4.6.3 *Mycobacterium tuberculosis*

Introduction

Of all infectious diseases, tuberculosis (TB) has the highest mortality worldwide. Although the incidence is slowly decreasing, 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 are currently found 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).

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 is performed at the RIVM and the results are used both for direct therapy guidance in patients and surveillance. The RIVM participates in the resistance proficiency study of the WHO for WHO supra-national laboratories to monitor the quality of the resistance testing.

Around 35 laboratories in the Netherlands send all *M. tuberculosis* isolates to the RIVM for epidemiological typing to support the investigations on transmission of TB by Municipal Health Services.

Methods

The current gold standard in drug antimicrobial susceptibility testing (AST) 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 incubator automatically monitors the growth of the bacteria.

Since 2011, not all drug susceptibility testing for first line drugs is performed at the RIVM; about 25% of these tests is performed at regional or peripheral laboratories. When resistance is observed 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 AST has been performed externally have been collected for the year 2016 and this confirms that this recommendation is followed.

Results

The presented data on 2016 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 2016, the RIVM received 572 *M. tuberculosis* complex isolates for epidemiological typing, of which 388 were subjected to AST with first line drugs at the RIVM.

Figure 4.6.3.1 Trends in antibiotic resistance for *M. tuberculosis* 2002-2016.

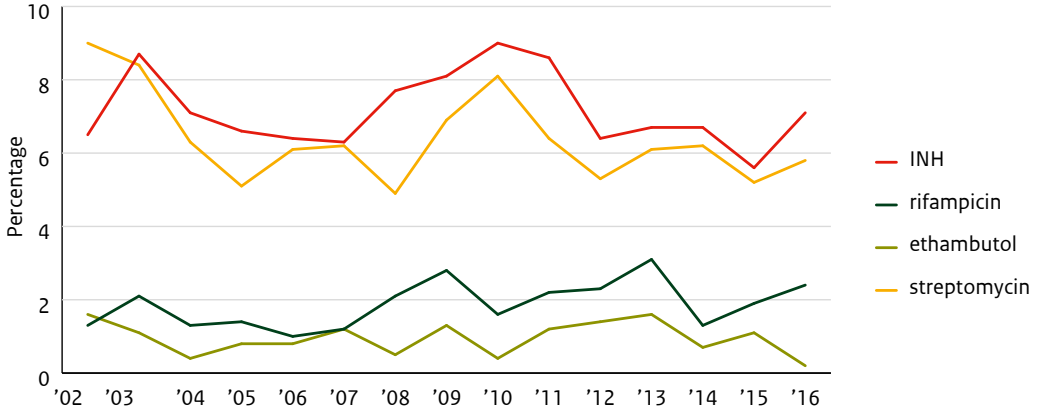
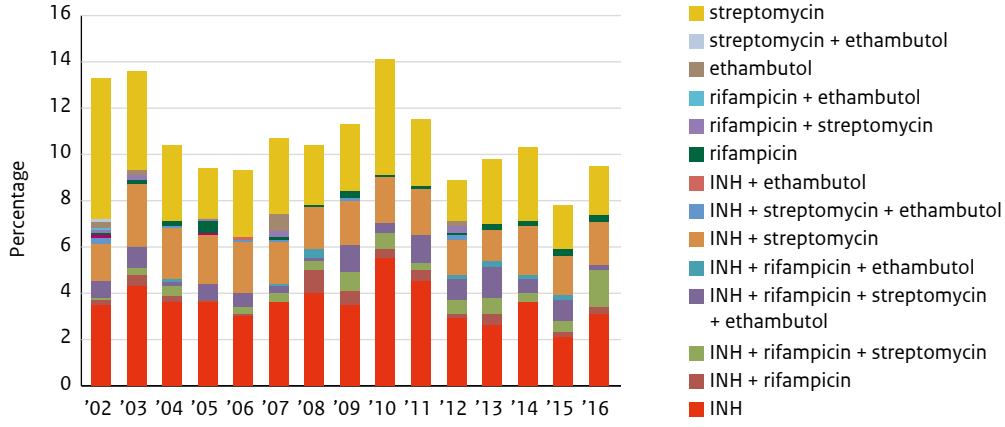


Figure 4.6.3.2 Trends in combined antibiotic resistance for *M. tuberculosis* 2002-2016.



The notification of TB cases again increased in 2016 by 3% to 889, and it is expected that the total number of culture positive cases will also be larger than in 2015. Since 2010, the total number of *M. tuberculosis* strains isolated in the Netherlands (60-70% of the notified cases are culture positive) gradually decreased from 784 in 2010, to 534 in 2014. In 2015, the number of *M. tuberculosis* isolates increased to 578 due to the increased influx of asylum seekers and immigrants.

Figure 4.6.3.1 shows the resistance rates for 2002-2016. In 2016 there was a clear increase in INH resistance to 7.1%. Until 2010, the rate of INH resistance increased to 9.0%, but since 2011 it decreased over the years to 5.4% in 2015. Rifampicin resistance decreased from 3.1% in 2013 to 1.3% in 2014. In 2015 and 2016 the rifampicin resistance increased slightly from to 1.9% to 2.4%.

Figure 4.6.3.2 shows trends for combinations of resistance. The rate of ethambutol resistance in combination with MDR decreased from 1.1% in 2015 to 0.2% in 2016. However, this may be due to a change in the approach. It is internationally recognized that AST is less reliable for ethambutol and streptomycin. Therefore, until 2015 the observations on ethambutol resistance in the MGIT were verified in the 7H10 agar dilution method, with slightly different results. In line with the international trend, currently the results in the standard MGIT approach are reported.

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. In 2016, 2.1 % of the isolates were reported as MDR-TB. XDR-TB was not diagnosed in 2016.

In recent years mono-resistance to rifampicin was incidentally found; in 2016 in 2 cases.

Discussion

Worldwide, resistance is an important aspect of TB control. Because there is a slight increase in the notification of TB in the Netherlands in the last two years, due to a higher influx of asylum seekers and immigrants from high prevalence areas, it remains important to continue the surveillance on resistance.

In 2016, 9.6% percent of the 572 isolates tested in the Netherlands revealed some form of resistance. Although the number of multidrug resistant isolates remained low and amounted to 12 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 of *Mycobacterium tuberculosis* isolates. It is being investigated whether the detection of mutations in 23 resistance genes is a reliable predictor of resistance.

Conclusions

- Resistance to the antibiotics to treat tuberculosis remained stable over the last 4 years.
- MDR-TB increased slightly from 1.8% in 2015 to 2.1% in 2016.
- Tuberculosis notification increased by 6% in 2015 and 3% in 2016.

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 Complexby Use of a High-Throughput, Reproducible, AbsoluteConcentration Method. Bert van Klingeren, Mirjam Dessens-Kroon, Tridia van der Laan, Kristin Kremer, and Dick van Soolingen*; 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 TM[1], Kohl TA[2], Omar SV[3], Hedge J[4], Del Ojo Elias C[4], Bradley P[5], Iqbal Z[5], Feuerriegel S[6], Niehaus KE[7], Wilson DJ[4], Clifton DA[7], Kapatai G[8], Ip CL[5], Bowden R[5], Drobniewski FA[9], Allix-Béguec C[10], Gaudin C[10], Parkhill J[11], Diel R[12], Supply P[13], Crook DW[14], Smith EG15, 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.
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] Findings for the influenza seasons 2005/2006 through 2009/2010 are presented in NethMap 2016.[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, 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.

Results

Table 4.6.4.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season and figure 4.6.4.1 shows the prescriptions for oseltamivir, zanamivir and amantadine since 2010. In the 2016/2017 season, for results obtained so far, no viruses with reduced inhibition for oseltamivir and zanamivir were found, and all viruses tested for M2B susceptibility had the M2 S31N amino acid substitution associated with resistance.

Oseltamivir and zanamivir prescriptions increased during the 2016/2017 influenza season, similar to levels seen during influenza epidemics since the 2010/2011 influenza season.
Amantadine prescriptions during the 2016/2017 season decreased 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.[2,3] 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.[2,3] 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.[2-4] 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 7 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.

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] Hurt AC, Besselaar TG, Daniels RS, Ermetal B, Fry A, Gubareva L, Huang W, Lackenby A, Lee RT, Lo J, Maurer-Stroh S, Nguyen HT, Pereyaslov D, Rebelo-de-Andrade H, Siqueira MM, Takashita E, Tashiro M, Tilmanis D, Wang D, Zhang W, Meijer A. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014-2015. Antiviral Res. 2016 Aug;132:178-85.

[3] Takashita E, Meijer A, Lackenby A, Gubareva L, Rebelo-de-Andrade H, Besselaar T, Fry A, Gregory V, Leang SK, Huang W, Lo J, Pereyaslov D, Siqueira MM, Wang D, Mak GC, Zhang W, Daniels RS, Hurt AC, Tashiro M. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013-2014. Antiviral Res. 2015 May;117:27-38.

[4] Meijer A, Lackenby A, Hungnes O, Lina B, van-der-Werf S, Schweiger B, Opp M, Paget J, van-de-Kassteele J, Hay A, Zambon M; European Influenza Surveillance Scheme. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007-08 season. Emerg Infect Dis. 2009 Apr;15(4):552-60.

Table 4.6.4.1 (Highly) reduced inhibition of influenza viruses by NAIs and M2Bs in the Netherlands, 2010/2011 - 2016/2017[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[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[9]	0/785	29/29 (100%)	0/6	1/1 (100%)	0/4

[1] Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; M2B = M2 ion channel blocker.

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

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

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

[5] One virus with highly reduced inhibition by oseltamivir due to the H275/Y amino acid substitution. No patient characteristics or anti 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 anti 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] Preliminary data, status by 6 March 2017.

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.

4.6.5 The antibiotic susceptibility profile of anaerobic bacteria

Introduction

The antibiotic susceptibility profile for amoxicillin, amoxicillin-clavulanic acid (only gram-negative anaerobic bacteria), clindamycin, metronidazole and meropenem (only *Bacteroides* spp. and *Prevotella* spp.) of different genera of anaerobic bacteria isolated at the University Medical Center Groningen (UMCG) was assessed.

Methods

Anaerobic strains isolated from human clinical specimens at the UMCG were identified using Matrix Assisted Laser Desorption Time-of-flight Mass Spectrometry (MALDI-TOF MS). The MIC of the strains for the different antibiotics was determined using Etest following manufacturer's instructions. Resistance was determined using breakpoints advised by EUCAST (2016).

Results

Results of susceptibility testing is displayed in Table 4.6.5.1.

Gram-negative anaerobic bacteria

Amoxicillin resistance was observed among all tested genera, except for the *Veillonella* spp. The highest resistance rate was among *Bacteroides* spp. and *Parabacteroides* spp. Compared with the previous year (for a full overview of susceptibility profiles over the years 2011-2015, see NethMap 2016 section 4.5.5) the resistance in the *Parabacteroides* spp. and *Prevotella* spp. increased, from 54.5% to 82.4% and from 40.7% to 52.4%, respectively. Among the *Porphyromonas* spp. the resistance decreased from 22.2% to 5.6%.

The resistance rates for amoxicillin-clavulanic acid and clindamycin remained similar as in the previous year, except for a decrease in resistance for amoxicillin clavulanic acid among the *Parabacteroides* spp., from 16.7% to 5.9%. An increase in resistance to clindamycin was observed, from none to 58.8%.

In the previous years we reported metronidazole resistance among *Prevotella bivia* strains and a *Bacteroides fragilis* strain. This year metronidazole resistance was encountered in a *Bacteroides fragilis* strain and a *Prevotella bivia* strain. All other strains showed no metronidazole resistance.

Meropenem resistance was only encountered among *Bacteroides* spp. All *Prevotella* strains were susceptible.

Gram-positive anaerobic bacteria

Resistance for amoxicillin was only observed among the clostridia. The resistance increased from 7.3% to 14.3%. Clindamycin resistance was observed for GPAC, clostridia, *Actinomyces* spp. and *Propionibacterium* spp. in similar rates as previous year.

Conclusions

- The susceptibility profile of *Parabacteroides* spp. is different compared with previous year.
- Clinicians should be aware of metronidazole resistant strains and possible multidrug resistant strains.

Table 4.6.5.1 Susceptibility profiles of anaerobes in 2016.

	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. (n=138-141)	32	>256	94.2	0.5	2	0	1.5	>256	17.7	0.38	0.75	0.7	0.125	0.75	1.4
Parabacteroides spp. (n=17)	>256	>256	82.4	3	4	5.9	6	12	58.8	0.38	1	0			
Fusobacterium spp. (n=30)	0.023	0.094	3.3	0.023	0.19	3.3	0.032	0.094	0	0.016	0.125	0			
Prevotella spp. (n=80-82)	3	128	52.4	0.125	1	0	0.016	>256	13.4	0.19	0.75	1.2	0.047	0.094	0
Porphyromonas spp. (n=18)	0.016	0.125	5.6	0.016	0.125	0	<0.016	>256	16.7	0.047	0.38	0			
Bilophila spp. (n=6-7)	48	128	100	1	1.5	0	0.5	0.75	0	0.125	0.125	0			
Veillonella spp. (n=10)	0.25	0.75	0	0.5	1	0	0.125	0.25	0	1	2	0			
Gram-positive anaerobes															
GPAC (n=135-136)	0.047	0.25	0				0.19	>256	16.9	0.19	0.75	0			
Clostridium spp. (n=28-33)	0.25	16	14.3				3	8	27.6	0.38	1	0			
Atopobium spp. (n=11)	0.125	0.5	0				0.75	2	0	0.25	0.38	0			
Eggerthella lenta (n=6-7)	1	1.5	0				0.125	0.25	0	0.19	0.25	0			
Actinomyces spp. (n=124-125)	0.125	0.38	0				0.125	1	6.5						
Propionibacterium spp. (n=157-159)	0.064	0.25	0				0.032	0.125	3.8						

4.6.6 *Clostridium difficile*

Introduction

The Dutch *C. difficile* Reference Laboratory operates since the recognition of PCR ribotype 027 outbreaks in the Netherlands in 2005. In 2009, the national *C. difficile* Infection (CDI) sentinel surveilllance program was initiated. This program is currently implemented in twenty-three acute care hospitals. *C. difficile* isolates of all included patients with CDI are sent to the reference laboratory for further characterisation. Resistance of *C. difficile* isolates to the most commonly prescribed antibiotics for treatment of CDI (metronidazole, vancomycin and fidaxomicin) is very rare.[1,2] Since most laboratories do not test *C. difficile* isolates routinely, the national reference laboratory considers this as a task to monitor development of resistance.

Methods

Antibiotic resistance was determined for 40 randomly selected *C. difficile* isolates, collected between January 2016 and January 2017. MICs were determined using the agar dilution method according to CLSI guidelines in Brucella Blood Agar (Becton and Dickinson, France), supplemented with haemin and vitamin KI.[2,3] *Bacteroides fragilis* ATCC 25285, *C. difficile* ATCC 700057 and *Clostridium glycolicum* were used as quality controls. Plates were incubated in an anaerobic cabinet and after 48 h plates were read. All isolates were tested in duplicate. The MIC50 and MIC90 were defined as the antibiotic concentrations at which 50% and 90%, respectively, of the tested strains were susceptible.

Results

Of 40 at random selected *C. difficile* isolates, 21 belonged to clade 1 (PCR ribotypes 001, 003, 012, 014, 020, 037, 076 and 245), 11 belonged to clade 2 (types 016, 027 and 265), and 8 belonged to clade 5 (Types 078 and 126). Table 4.6.6.1 summarizes the overall results, whereas table 4.6.6.2 depicts the results per individual clade. No resistance to metronidazole or fidaxomicin was found.

Table 4.6.6.1 MIC50, MIC90 and range (mg/L) of 40 *C. difficile* surveillance isolates.

	MIC50	MIC90	Range
Fidaxomicin	<0.06	<0.06	≤0.06-1
Vancomycin	0.25	0.5	0.06-2
Metronidazole	0.125	0.25	0.125-0.25

Table 4.6.6.2 MIC90 and range (mg/L) of 40 *C. difficile* isolates belonging to the most frequently found clades.

	MIC50	MIC90	Range
Clade 1 (n=21)			
Fidaxomicin	<0.06	<0.06	<0.06-0.25
Vancomycin	0.125	0.5	0.06-1
Metronidazole	0.125	0.25	0.125-0.25
Clade 2 (n=11)			
Fidaxomicin	<0.06	<0.06	<0.06-0.25
Vancomycin	0.25	1	0.06-2
Metronidazole	0.125	0.25	0.125-0.25
Clade 5 (n=8)			
Fidaxomicin	<0.06	<0.06	<0.06
Vancomycin	0.5	0.5	0.06-0.5
Metronidazole	0.125	0.125	0.125

Discussion

The EUCAST Clinical Breakpoint Recommendations do not provide any recommendation for clinical breakpoints and epidemiological cut-off (ECOFF) values of fidaxomicin, because it was concluded that the available data showed major variations in MIC distribution between studies.[4] Based on wild-type distributions and MIC data on fidaxomicin and *C. difficile* presented , an ECOFF value of 1.0 mg/L seems appropriate.[2] So far, the only clinical isolate with reduced susceptibility to fidaxomicin was obtained from a patient with recurrence of diarrhoea 6 days after cure with fidaxomicin.[5] The isolate at day 1 and the end of treatment had an MIC of 0.06 mg/L, but the isolate from the recurrent episode had reduced susceptibility with an MIC of 16 mg/L, which is still less than gut-level concentrations of the drug.

Conclusions

- Resistance to metronidazole and fidaxomicin was not found in 40 tested isolates collected in the sentinel surveillance of CDI in 2016.

References

[1] Crobach MJT, van Dorp SM, Harmanus C, Sanders IMG, Kuijper, EJ, Notermans DW, de Greeff SC et al. Tenth Annual Report of the National Reference Laboratory for Clostridium difficile and results of the sentinel surveillance May 2015 - May 2016. Availalbe at http://www.rivm.nl/Onderwerpen/C/Clostridium_difficile

[2] Debast SB, Bauer MP, Sanders IM, Wilcox MH, Kuijper EJ; ECDIS Study Group. Antimicrobial activity of LFF571 and three treatment agents against Clostridium difficile isolates collected for a pan-European survey in 2008: clinical and therapeutic implications. J Antimicrob Chemother. 2013; 68: 1305-11.

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

[4] 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/.

[5] Goldstein EJ, Babakhani F, Citron DM (2012). Antimicrobial activities of fidaxomicin. Clin Infect Dis 55 (Suppl 2): S143.

4.6.7 *Aspergillus fumigatus*

Introduction

In the Netherlands *Aspergillus fumigatus* is by far the most frequently recovered species from patients with aspergillus diseases. Triazoles are the main class of antifungals that are used for the prevention and treatment of aspergillus diseases.

The clinical use of triazoles is threatened by the emergence of triazole resistance, commonly due to mutations in the *Cyp51A*-gene, the target of triazole drugs.[1,2] Case series indicate that triazole resistance is associated with treatment failure, and a high mortality rate was observed in patients with acute invasive disease.[1,2,4] We here report the prevalence of azole resistance in *A. fumigatus* in the Netherlands.

Methods

In five University Medical Centers all *A. fumigatus* isolates isolated from clinical patients were screened for the presence of resistance using a four-wells agar plate (VIPcheck™). Three agars were supplemented with the medical triazoles itraconazole, voriconazole and posaconazole, while the fourth-well served as growth control. Isolates that grew on the triazole-containing agar have a high probability of resistance and were sent to the Center of Expertise in Mycology Radboudumc/CWZ for phenotypic and genotypic characterization. MICs were determined using the EUCAST microbroth dilution reference method and the presence of resistance mutations in the *Cyp51A*-gene were investigated using PCR and sequencing. The EUCAST clinical breakpoints were used to classify the isolates as susceptible and resistant. A patient was positive if any of the isolates were confirmed resistant.

Results

In 2016 *A. fumigatus* isolates from 784 patients were screened for the presence of triazole resistance (Table 4.6.7.1). Overall 101 patients (12.9%) harbored a triazole-resistant isolate. The frequency of resistance in individual centers ranged between 9.5% (Radboudumc, Nijmegen) and 20.5% (LUMC, Leiden). Compared with previous years, the frequency of triazole resistance had increased (Table 4.6.7.1). Between 86% (voriconazole) and 99% (isavuconazole) of *A. fumigatus* isolates were classified as resistant (Table 4.6.7.2). Environmental resistance mutations, i.e. TR34/L98H and TR46/Y121F/T289A, were most frequently present accounting for 83% of resistance mutations. The proportion of TR46/Y121F/T289A continued to decrease with a frequency of 9% in 2016. Recent environmental resistance mutations such as those with three TR46 repeats ($TR46^3$) or four repeats ($TR46^4$) were not encountered. In only two isolates other *Cyp51A*-mutations were found, with one, G54W, known to confer triazole resistance. In 20 isolates (15%) sequencing of the *Cyp51A*-gene showed a wildtype sequence, indicating another resistance mechanism. The MIC distributions according to the underlying resistance mutations for the four medical triazoles are shown in Figure 4.6.7.1.

Discussion

Surveillance of clinical *A. fumigatus* isolates indicated that the overall resistance frequency continues to increase, with 12.9% of patients showing resistance. As in previous years the majority of triazole-resistant isolates harbored mutations that are associated with environmental resistance selection. The distribution of environmental triazole resistance mutations appears to be variable, with TR34/L98H now being highly dominant and fewer patients harboring TR46-resistance mutations. The environmental factors that determine the distribution of resistance mutations remain unclear, but as new mutations may continue to emerge surveillance of clinical and environmental *A. fumigatus* isolates is important.[5] Direct detection of triazole resistance mutations in clinical specimens through PCR has shown to improve our ability to diagnose triazole-resistant aspergillosis even in culture-negative patients.[6] However, this tool relies on a close association between resistance genotype and phenotype and on resistance mutations that have been characterized.

In the 2016 flu epidemic a high number of invasive aspergillosis cases were seen in Dutch ICUs; of 144 patients admitted to the ICU of the eight University Intensive Care Units, 23 (16%) were found to have invasive aspergillosis. This represents a new risk group for invasive aspergillosis, with uncharacteristic risk profiles and clinical presentation.[7] The triazole resistance frequency in this group was 29% of those with a positive *A. fumigatus* culture. Increasingly centers are moving away from triazole monotherapy as initial therapy in critically ill patients suspected of invasive aspergillosis.

Conclusions

- The triazole resistance frequency in *A. fumigatus* continues to increase with 12.9% of unselected patients with positive culture harboring a resistant isolate.
- The high triazole resistance frequency in unselected patient isolates and in specific patient groups questions the use of voriconazole monotherapy as first-line treatment for acute invasive aspergillosis, especially in critically-ill patients.
- The triazole resistance mutations are dominated by those associated with environmental resistance selection, as they were found in 83% of triazole-resistant isolates.

References

1 Verweij PE, Chowdhary A, Melchers WJG, Meis JF. Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles? Clin Infect Dis 2016;62:362-8.

2 Meis JF, Chowdhary A, Rhodes JL, Fisher MC, Verweij PE. Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*. Philos Trans R Soc Lond B Biol Sci. 2016; 371(1709).

3 Chowdhary A, Sharma C, Hagen F, Meis JF. Exploring azole antifungal drug resistance in *Aspergillus fumigatus* with special reference to resistance mechanisms. Future Microbiol. 2014;9:697-711.

4 Fuhren J, Voskuil WS, Boel CH, Haas PJ, Hagen F, Meis JF, Kusters JG. High prevalence of azole resistance in *Aspergillus fumigatus* isolates from high-risk patients. J Antimicrob Chemother. 2015;70:2894-8

5 Verweij PE, Zhang J, Debets AJM, Meis JF, van de Veerdonk FL, Schoustra SE, Zwaan BJ, Melchers WJG. In-host adaptation and acquired triazole resistance in *Aspergillus fumigatus*: a dilemma for clinical management. Lancet Infect The emergence of high-level pan-triazole resistant phenotypes suggest the accumulation of new resistance Dis 2016;16:e251-60.

[6] Chong GM, van der Beek MT, von dem Borne PA, Boelens J, Steel E, Kampinga GA, Span LF, Lagrou K, Maertens JA, Dingemans GJ, Gaajetaan GR, van Tegelen DW, Cornelissen JJ, Vonk AG, Rijnders BJ. PCR-based detection of *Aspergillus fumigatus Cyp51A* mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay® in 201 patients with haematological disease suspected for invasive aspergillosis. J Antimicrob Chemother. 2016;71:3528-3535.

[7] van de Veerdonk FL, Kolwijck E, Lestrade PPA, Hodiamont CJ, Rijnders BJA, van Paassen J, Haas PJ, Oliveira dos Santos C, Kampinga G, Bergmans DCJJ, van Dijk K, de Haan AFJ, van Dissel J, van der Hoeven JG, Verweij PE. Influenza-associated aspergillosis in critically ill patients. Am J Resp Crit Care Med 2017; Apr 7. doi: 10.1164/rccm.201612-2540LE.

Table 4.6.7.1 Triazole resistance frequency in unselected clinical *A. fumigatus* isolates in five University Medical Centers, 2013 to 2016.

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

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

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

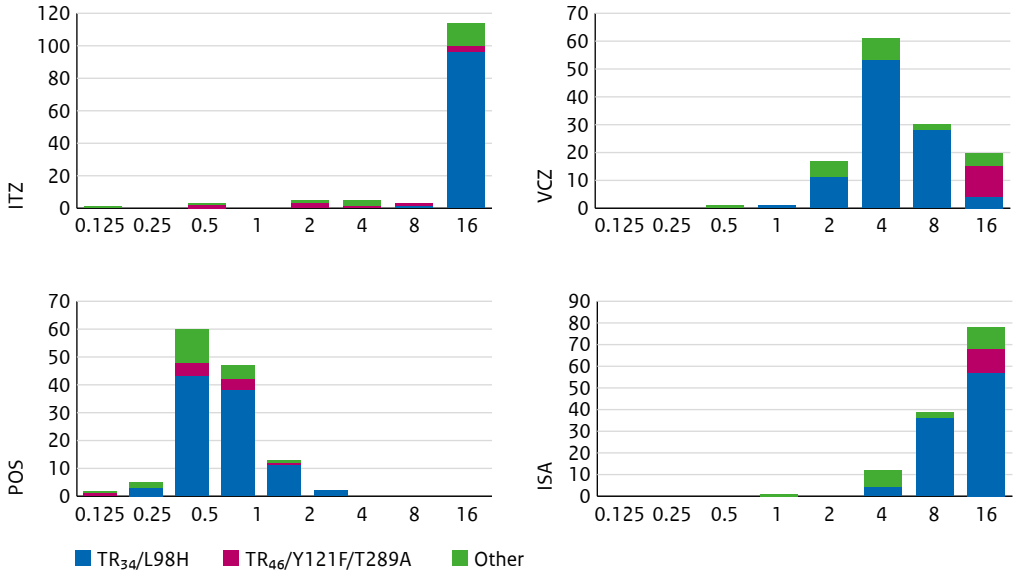
Table 4.6.7.2 In vitro activity of medical triazoles against 131 azole-resistant *A. fumigatus* isolates recovered in 2016.

Drug	R-breakpoint (mg/L)*	Susceptible	Intermediate	Resistant
ITZ	>2	3%	4%	93%
VCZ	>2	2%	12%	86%
POS	>0.25	2%	4%	94%
ISA	>1	1%	0%	99%

ITZ, itraconazole; VCZ, voriconazole; POS, posaconazole; ISA, isavuconazole.

* *EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/Antifungal_breakpoints_v_8.0_November_2015.xlsx.*

Figure 4.6.7.1 MIC distributions of four medical triazoles according to the underlying resistance mutation.



X-axis indicates the MIC (mg/L) and the Y-axis the number of isolates.
ITZ, itraconazole; VCZ, voriconazole; POS, posaconazole; ISA, isavuconazole

5 Antimicrobial Stewardship Monitor

5.1 Introduction

In response to the recommendation by the SWAB, the Dutch Health Care Inspectorate (IGZ), and the Minister of Health, Dutch hospitals have started to establish antimicrobial stewardship teams (A-teams). 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 Antimicrobial Stewardship Monitor is a SWAB-initiated program to measure the progress and impact of the national implementation of antimicrobial stewardship. Since 2016 the Antimicrobial Stewardship Monitor reports in NethMap on 1) the stewardship activities employed by A-teams aimed at measuring and improving the quality of antimicrobial use and 2) the quality of antibiotic use in hospitals in the Netherlands.

5.2 Methods

In 2016, an electronic questionnaire was sent to all Dutch hospitals, irrespective of the presence of an A-team to collect information on: hospital characteristics, organization of the antimicrobial stewardship team/program, resources, monitoring of quality of antibiotic use and educational activities. Results are presented as % of hospitals replying to the question. Subsequently, based on the outcomes of the questionnaire, three stewardship indicators were selected for inclusion in the Antimicrobial Stewardship Monitor to represent the quality of antibiotic use in hospitals. These were: 1. prescribe restricted antibiotics according to local guideline; 2. switch from intravenous to oral therapy (IV-oral switch); and 3. perform infectious disease specialist bedside consultations in patients with a *Staphylococcus aureus* bacteremia. All Dutch A-teams were asked to report on these stewardship activities in order to compute process indicator performance scores. Such scores, deemed quality indicators are described as a percentage between 0 and 100 where the numerator represents the number of patients to whom care is delivered as defined, and the denominator represents the eligible target population.

5.3 Results

42 of 88 hospitals returned the questionnaire, resulting in a response rate of 47%. The mean number of hospital beds was 609, with a range of 28 to 1350 beds. 14% of the hospitals were academic teaching hospitals, 55% non-academic teaching hospitals and 31% non-teaching hospitals. 37 of 42 responding hospitals had an A-team and 4 were preparing one. All operational A-teams consisted of at least one hospital pharmacist and one medical microbiologist. 97% of the A-teams had at least one internist and 70% at least one infectious disease specialist. Authorisation from the Hospital Board of Directors had been granted to 81% of the A-teams, whereas only 39% of the Hospital Boards of Directors provided a budget for the A-teams. IT budget had officially been allocated to 10% of the A-teams. 46% of the A-teams could apply for IT support if needed and no IT support was available for 44% of the A-teams. All hospitals used an Electronic Medical Record system. 95% of the systems facilitated consultation of radiological and laboratory data, 92% clinical data, 92% medication data, and 87% microbiology data. 69% of the A-teams made an annual report summarizing the quality of antimicrobial use, practice improvement initiatives and describing the goals for the following year. The mean time dedicated by the A-team to antimicrobial stewardship related activities was 15 hours per week (range 1-47 hours), corresponding with 0.68 FTE (range 0.1-1.5).

5.3.1 Monitoring of appropriateness of antibiotic use

Appropriate antibiotic use was monitored in several ways in the various hospitals. Table 5.3.1.1 provides an overview of the monitoring activities in the different hospitals with A-teams in place. Table 5.3.1.2 and table 5.3.1.3 provide specific interventions for monitoring the use of restricted antibiotics and bedside consultations, respectively.

Table 5.3.1.1 Percentage of hospitals receiving information about antimicrobial susceptibility/use and monitoring stewardship activities (n=37 hospitals).

Data acquisition	
Receipt of reports on cumulative antimicrobial susceptibility > 1/yr	67%
Receipt of reports on quantitative use of antimicrobials > 1/yr	73%
Point prevalence study	66%
Monitoring stewardship activities	
Use of restricted antimicrobials	77%
Guideline adherence	71%
Continuously	21%
Occasionally	50%
IV-oral switch	76%
Bedside consultation *Staphylococcus aureus* bacteremia	53%
Streamlining	71%
Therapeutic drug monitoring	63%
Correct use of diagnostics	58%

Table 5.3.1.2 Interventions performed to monitor the use of restricted antibiotics (n=39 hospitals).

Postprescription telephonic feedback	21 (54%)
Prospective audit and feedback	16 (41%)
Monitoring correct use of microbiologic diagnostics	11 (28%)
Automatic electronic alert	10 (26%)
Preauthorization	5 (13%)
Formulary restriction	4 (10%)
Order forms	4 (10%)
Stop orders	3 (8%)
Bedside consultation	2 (5%)
Other	1 (3%)
No monitoring	9 (23%)

Table 5.3.1.3 Patient categories for which the hospital agreed to perform bedside consultation by an infectious disease specialist and for which A-teams monitored the performance (n=38 hospitals).

Patient category	Bedside consultation indicated [n, % of total hospitals]	Monitoring of bedside consultation [n, % of hospitals with indication for consultation]
No bedside consultation	12 (32%)	Not applicable
Staphylococcus aureus bacteremia	26 (68%)	20 (77%)
Endocarditis	7 (18%)	4 (15%)
Prosthetic joint infection	6 (16%)	2 (33%)
Vascular prosthesis infection	6 (16%)	2 (33%)
Invasive fungal infection	4 (11%)	2 (50%)
Other	5 (13%)	0 (0%)

5.3.2 Education

Education on antimicrobial stewardship to residents was provided in 57% of the hospitals. This had a structural character in 62%, and attendance was compulsory in 40%. The subjects discussed included antibiotic resistance, specific infectious diseases and several aspects of the use of antibiotics, like IV-oral switch and streamlining. Medical specialists were targeted in educational programs in only 22% of the hospitals and always on a voluntary basis.

5.3.3 Quality of antibiotic use in hospitals

As a start this year, the quality of antibiotic use could be evaluated in a limited number of 11 hospitals. Ten of these had an A-team able to systematically record and monitor their activities and to provide stewardship outcomes over 2016. Of these, 8 provided data on at least one of the three selected stewardship objectives: 8 on restricted antimicrobials, 4 on IV-oral switch, and 4 on bedside consultation. Table 5.3.3.1 shows the number of prescriptions reviewed and bedside consultations performed. The numbers vary widely between hospitals, and in particular the low numbers indicate the lower part of the learning curve.

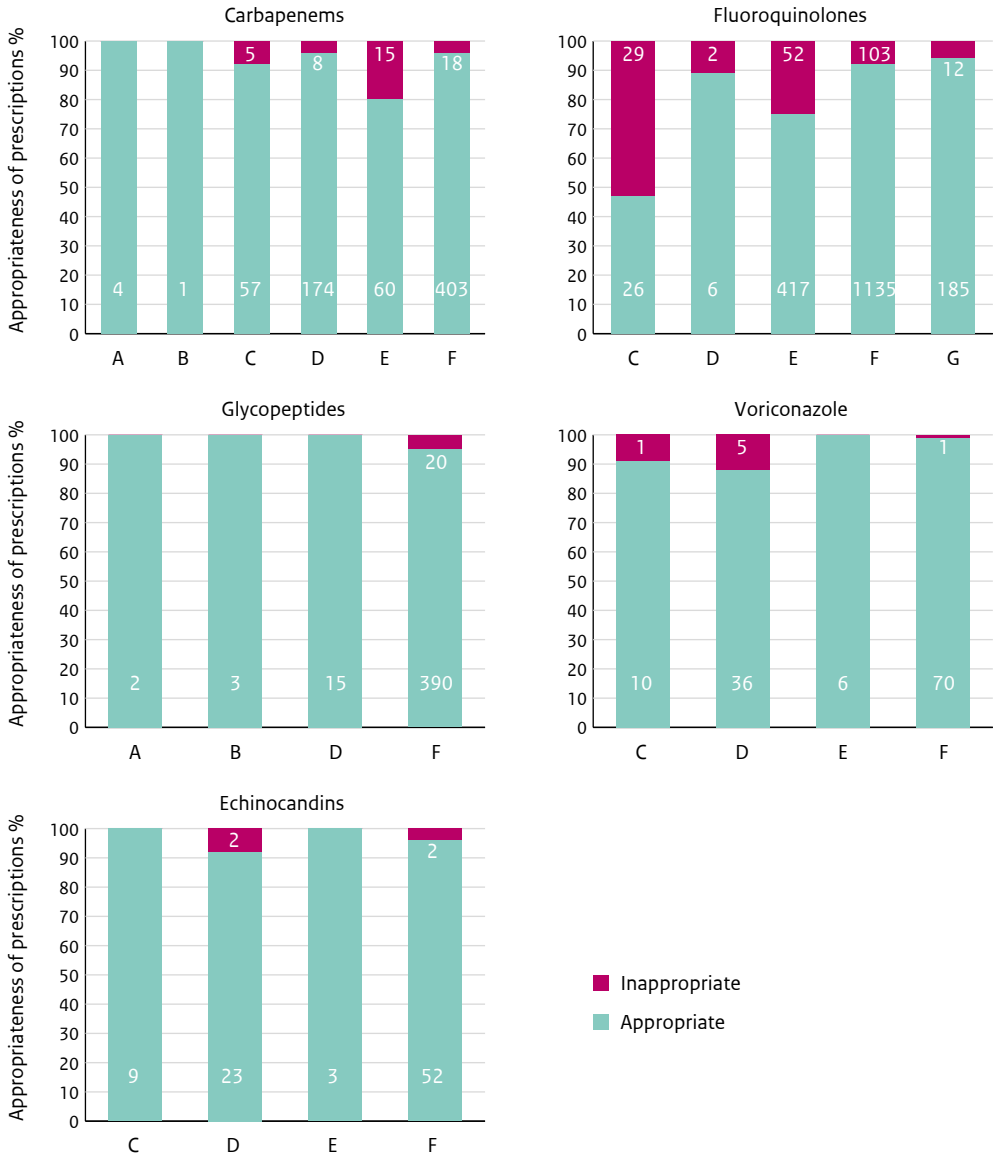
Table 5.3.3.1 Number of prescriptions reviewed and bedside consultation performed in 2016 by the participating hospitals (A-K).

Hospital	Restricted antibiotics	IV-oral switch	Bedside consultation
A	6		
B	4		
C	147	2784	
D	861	120	59
E	498		
F	3827		
G	209	610	78
H		486	
I			116
J	6400		
K	293		

5.3.3.1 Restricted antimicrobials

The quality of restricted antimicrobials could be aggregated for seven hospitals (3 academic and 4 peripheral hospitals) on the quality; the data of the other two indicators were considered too few to be meaningfully representative. It could be concluded however, that there was a large variation in approach and execution between hospitals. The frequency of monitoring ranged from repeated point prevalence surveys (2 hospitals) to continuous monitoring (5 hospitals, of which three during 12 months of 2016). The following antimicrobial drugs were monitored most frequently: carbapenems (6 hospitals), echinocandins (4 hospitals), fluoroquinolones (5 hospitals), glycopeptides (4 hospitals), and voriconazole (4 hospitals). Overall appropriateness, defined as a prescription following the local guideline or an expert's advice, was 94% for carbapenems, 96% for echinocandins, 90% for fluoroquinolones, 95% for glycopeptides, and 95% for voriconazole. Fluoroquinolones showed the highest variation in appropriateness: 47 to 92% (Figure 5.3.3.1). Only few hospitals could provide data about the total number of prescriptions, prophylactic or therapeutic nature of the prescriptions and whether or not the advice given to the prescriber was followed or not.

Figure 5.3.3.1 Appropriateness of restricted antimicrobials in 2016 in 6 hospitals (A-G). The number in the columns corresponds to the number of appropriate and inappropriate prescriptions.



5.4 Discussion

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 at least 42% of the hospitals. This inventory probably underestimates the presence of A-teams, since we have no information about the non-responders. It is important to stress that even in the absence of an A-team, the three core specialties perform aspects of antimicrobial stewardship in Dutch hospitals, although this is usually reactive (prescriber-initiated consultation or diagnostics driven) instead of proactive and often lacks structural documentation, precluding the possibility to give feedback to prescribers.

A-teams dedicate significant time to antimicrobial stewardship related activities (mean 0.68 FTE), however with a wide range: 0.1-1.5 FTE. Nevertheless, an independent report estimated that the implementation of a stewardship program requires 0.87-1.68 FTE, which further increases to 1.25-3.03 FTE in the following years. A likely explanation for this discrepancy is the limited budget that the Hospital Boards of Directors provide for antimicrobial stewardship: only 39% of (the responding) A-teams was financially supported.

Structurally monitoring the quality of use of restricted antimicrobials, the performance of bedside consultation (predominantly in patients with *Staphylococcus aureus* bacteremia), IV-oral switch and TDM were most often incorporated into the antimicrobial stewardship programs. This corresponded with the A-teams' preferences about which stewardship objectives to include in the Antimicrobial Stewardship Monitor. The appropriateness of (a selection of) restricted antimicrobials was relatively high (90-96%), although fluoroquinolones showed the highest variation in appropriateness (47-92%), consistent with last year's observations. We were not informed about several aspects of the monitoring (for example, days of therapy, nature of the prescription and selection of wards/patients), hindering interpretation and between-hospital comparison. The variation seen in IV-oral switch could also be explained by differences in patient selection. The SWAB-initiated project to facilitate automated data-extraction from electronic medical record systems will lead to a structured and uniform documentation and increased possibilities to report to the Antimicrobial Stewardship Monitor. It is encouraging to see that advices given to prescribers are well received and lead to the desired treatment modification.

MARAN 2017

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2016

June 2017

Colophon

This report is published under the acronym MARAN-2017 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 Authority (SDa). The information presented in MARAN-2017 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
MARAN-2017 is published in a combined back-to-back report with NETHMAP-2017. 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 websites of the Netherlands Veterinary Medicines Authority (www.autoriteitdiergeneesmiddelen.nl).
MARAN-2017 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 Authority (SDa), Utrecht

The following persons contributed to the writing of MARAN 2017
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, Alieda van Essen, 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, Raymond Heymans

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 2016 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 in 2016 (176 tonnes) showed a remarkable reduction (15%) compared to 2015 (206 tonnes). In relation to 2009, the index year used by the Ministry of Economic Affairs, in 2016 total sales decreased by 64.4%. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 69%. This year it was possible to allocate most sold antimicrobial veterinary medicinal products to the species the products are used in by using several alternative data sources. Most classes of antibiotics showed a decrease in sales in 2016, but some increased. In all but one sectors (veal calves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum.

Antimicrobial resistance

In 2016 *S.* Enteritidis (N = 438) followed by *S.* Typhimurium (N = 260) together with the monophasic variant of Typhimurium: 1,4,5,12:i:- (N = 229), were most frequently isolated from humans suffering from salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, besides *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. In poultry (including poultry products, broilers and layers), the number of *S.* Paratyphi B var. Java further reduced in 2016. Also *S.* Infantis, still predominant in 2015, was less frequently isolated in 2016. The prevalence of *S.* Enteritidis increased compared to 2015 and was the most predominant serovar in poultry in 2016. Highest resistance levels were observed in *S.* Kentucky (travel related), monophasic *S.* Typhimurium 1,4,[5],12:i:-, other *S.* Typhimurium, *S.* Paratyphi B var. Java and to a lesser extent in *S.* Infantis and *S.* Newport. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Enteritidis (23%), *S.* Typhimurium (18%) and *S.* Kentucky (11%). In 2016, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 28/1954 (1.4%), among nine different serovars, predominantly isolated from human sources. No carbapenemase producing *Salmonella* were found in 2016.

As a result of prioritization and changes in legislation, since 2014 the focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly in isolates from poultry (including broilers, laying hens and ducks) and poultry meat.
Resistance rates in *C. jejuni* from broilers was somewhat lower, whereas rates in poultry meat did not substantially change in 2016, compared to 2015. Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates. Resistance rates for quinolones in *C. coli* isolates from broilers, laying hens and poultry meat decreased since 2015. Levels of resistance of *C. jejuni* for tetracycline and the quinolones were substantially higher in broilers than in ducks and laying hens. In *C. jejuni* from milk sheep and milk goats, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with a slight decrease in 2016), 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 levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

After a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim since 2009 in STEC O157 isolates from humans, in 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%). Resistance for the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates.

In 2016, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and pigs and stabilized in veal calves and dairy cattle. In isolates from chicken meat resistance levels were substantially lower than in isolates from turkey meat. The levels of resistance were similar to 2015 in both types of poultry meat. Resistance levels for almost all tested antibiotics were much higher in samples of imported chicken and turkey meat than in samples from retail. Resistance to third-generation cephalosporins was low (< 1%) in all tested animal species. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from broilers and to a lesser extent in white veal calves, but substantially decreased in *E. coli* from white veal calves. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulphonamides and trimethoprim were still reasonably high in broilers, turkey, pigs and veal calves.
Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Susceptibility testing of enterococci is considered of lesser priority than *E. coli*, also in the new EU legislation. Therefore, from 2016, no enterococci from faecal samples were tested, but in 2016 *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples. The poultry meat samples were taken at retail.
In chicken meat, highest resistance levels were observed for erythromycin (55.4% for *E. faecalis* and 57.1% for *E. faecium*) and tetracycline (66.1% and 25.0% respectively). In addition, a high level of resistance was observed for quinu/dalfopristin in *E. faecium* (42.9%). In turkey meat, highest resistance levels were observed for erythromycin (65.1% for *E. faecalis* and 58.8% for *E. faecium*) and tetracycline (88.9% and 76.5% respectively). A high resistance percentage was also observed for quinu/dalfopristin in *E. faecium* (58.8%).

ESBL/AmpC-producing *Escherichia coli* represented 0.3% of the randomly isolated *E. coli*, the lowest proportion observed since 2007. In spite of the above, selective culturing in livestock faeces indicated that the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* marked a general tendency to increase in livestock, excluding broilers and layers. Currently an explanation for this phenomenon is lacking.
A follow up of the 2009 study on within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers showed a significant decrease from 66% in 2009 to 38% in 2016. The proportion of fresh chicken meat with ESBL/AmpC-producing *E. coli* isolates decreased to 24% (67% in 2014, 39.4% in 2015). In imported chicken meat the proportion was much higher with 61.2%. The most prevalent ESBL/AmpC gene in *E. coli* from livestock and meat was $bla_{\text{CTX-M-1}}$ in almost all animal species followed by $bla_{\textbf{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-14}}$.

The prevalence of ESBL/AmpC-producing *Salmonella* in 2016 was 1.7%, confirming the decreasing trend observed in the period 2013-2015. Most represented ESBL/AmpC genes were $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul, $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky, and $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium. The majority of ESBL-producing *Salmonella* isolates from humans were highly multidrug resistant, with most of the isolates showing a resistant phenotype to 5-8 antibiotics (67%).

No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance in livestock. Only $bla_{\text{OXA-48}}$-like genes were detected in two faecal samples from veal calf and slaughter pig associated with *Shewanella* spp.. In a retrospective study in companion animals, horses and as well as in a prospective study in companion animals no carbapenemase-producing *Enterobacteriaceae* were detected.

The colistin resistance gene *mcr-1* was incidentally found in *E. coli* from livestock (0.5%). In retail meat *mcr-1* was most frequently identified in turkey (8.3%), but also in chicken (0.7%).

It can be concluded that the sales of antibiotics for animals further decreased in 2016. Moreover, in all but one sectors (veal valves, dairy cattle, pigs, broilers and turkeys) a reduction in consumption was realized. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum. This reduction in usage is reflected in the resistance data of 2016 where resistance levels decreased in *E. coli* from most animal species. Also the occurrence of ESBL/AmpC-producing *E. coli* in poultry meat was substantially lower than in previous years. This suggest that the measure 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. Additional resistance determinants of public health concern such as carbapenemase or the colistine resistance gene *mcr-1*, were not detected or found at low levels, respectively. The ongoing reduction of antibiotic use in livestock in the past seven years is reflected by the ongoing reduction of antibiotic resistance in animals and the food thereof.

2
Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2016

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all antimicrobial veterinary medicinal products on package level sold in 2016 in the Netherlands, as extracted from the Vetindex and supplemented with antimicrobial veterinary medicinal products (AVMP) data of non FIDIN members. The data are estimated to cover approximately 98% of all sales in the Netherlands. Actual use can be different from the quantities sold as a result of stock piling and cross border use. Monitored use in the major livestock farming sectors (pigs, broilers, turkey, veal calves, dairy- and other cattle) covered 97.3% of sales in 2016.
The European Medicines Agency (EMA) collects harmonised systemic antibiotic usage data based on overall sales of veterinary antimicrobial agents through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project since September 2009. Sales figures from 1999 to 2008 were recalculated and adjusted according to the ESVAC protocol. Data as from 2011 are calculated according to the SDa method for all antimicrobial veterinary medicinal products, which means only active base substance mass (excluding mass of salts and esters) is calculated, including (unlike the ESVAC reports) topical applications like ointments, eye drops and sprays. The sales data in this report involves total sales, for all animals, not stratified by individual animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported on in the next chapter.

The average number of food-producing animals present in the Dutch livestock farming sector (pigs, poultry, veal calves, other cattle and sheep) shows annual variations (Table ABuse01). In pigs, a decrease is noted in line with international market developments, and in dairy cattle a major increase occurred due to the abandoning of milk quota. This increase will be reversed the coming year because of phosphate production limitations. All in all this indicates that the reported reduction in sales of antimicrobials over the years can be interpreted as true reduction in usage.

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
Piglets (less than 20 kg)	4,809	4,649	4,797	4,993	4,920	5,115	5,408	4,986
Sows	1,100	1,098	1,106	1,081	1,095	1,106	1,053	1,022
Fattening pigs	6,199	6,459	6,200	4,189	4,209	4,087	4,223	4,140
Other pigs	2,100	2,040	2,021	1,841	1,789	1,765	1,769	1,733
Turkeys	1,060	1,036	990	827	841	794	863	762
Broilers	52,323	54,367	57,811	43,912	44,242	47,020	49,107	48,378
Other poultry	46,383	48,218	40,442	52,356	54,345	56,924	58,636	57,172
Veal calves	886	921	906	908	925	921	909	956
Other cattle	3,112	3,039	2,993	3,045	3,064	3,230	3,360	3,353
Dairy cattle	1,562	1,518	1,504	1,541	1,597	1,610	1,717	1,794
Sheep	1,091	1,211	1,113	1,093	1,074	1,070	1,032	1,032
Fattening rabbits	271	260	262	284	270	278	333	318
Dows	41	39	39	43	41	43	48	45

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. Sales of antimicrobial veterinary medicinal products in 2016 (176 tonnes) showed a remarkable reduction (15%) compared to 2015 (206 tonnes). Total sales decreased by 64.45 % over the years 2009-2016. Some of the unexpected increases of 2015 were reversed.
Most classes of antibiotics showed a decrease in 2016, but some increased (Figure ABuse02). Increased sales were noted for 1st and 2nd generation cephalosporins (+12%), amphenicols (+7%), other (+6%) and macrolides (+4%). Reductions in sales were realized for 3rd and 4th generation cephalosporins (-85%), polymyxins (-35%), tetracyclines (-24%), quinolones (-20%), aminoglycosides (-15%), fluoroquinolones (-15%), combinations (-14%), macrolides (-17%), penicillins (-15%) and trimethoprim/sulfonamides (-7%).

Tetracyclines

The total mass of tetracyclines sold decreased considerably more than decrease in use. Since the sales of 2015 increased, in contrast to the decreased use in that year, this likely affected the 2016 figure.
The fraction of doxycycline had increased to 50% of the total sales of tetracyclines (42% in 2015, 41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

Trimethoprim/sulfonamides

The use of trimethoprim/sulfonamides decreased further in 2016, but regained the second rank in mass sold.

Figure ABuse01 Antimicrobial veterinary medicinal product sales 1999-2016 in kg (thousands)

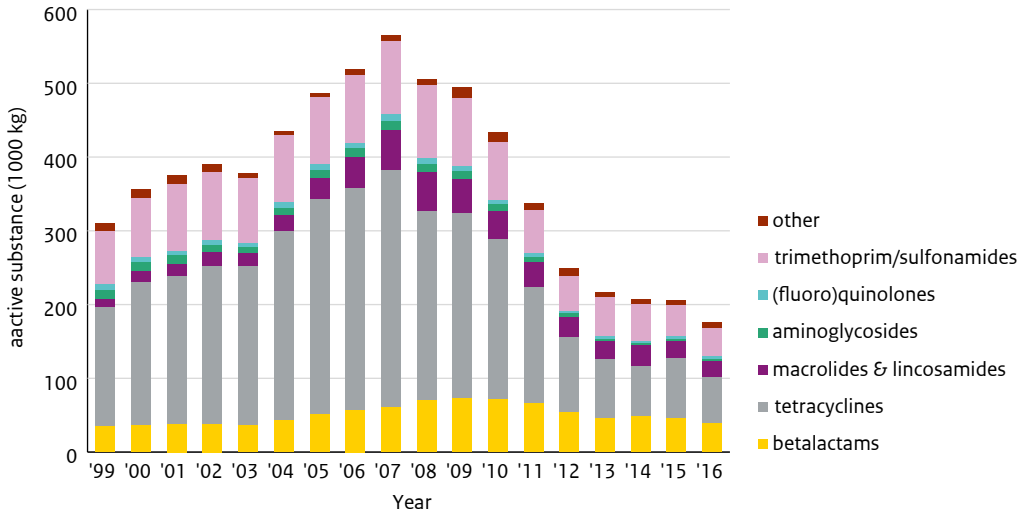
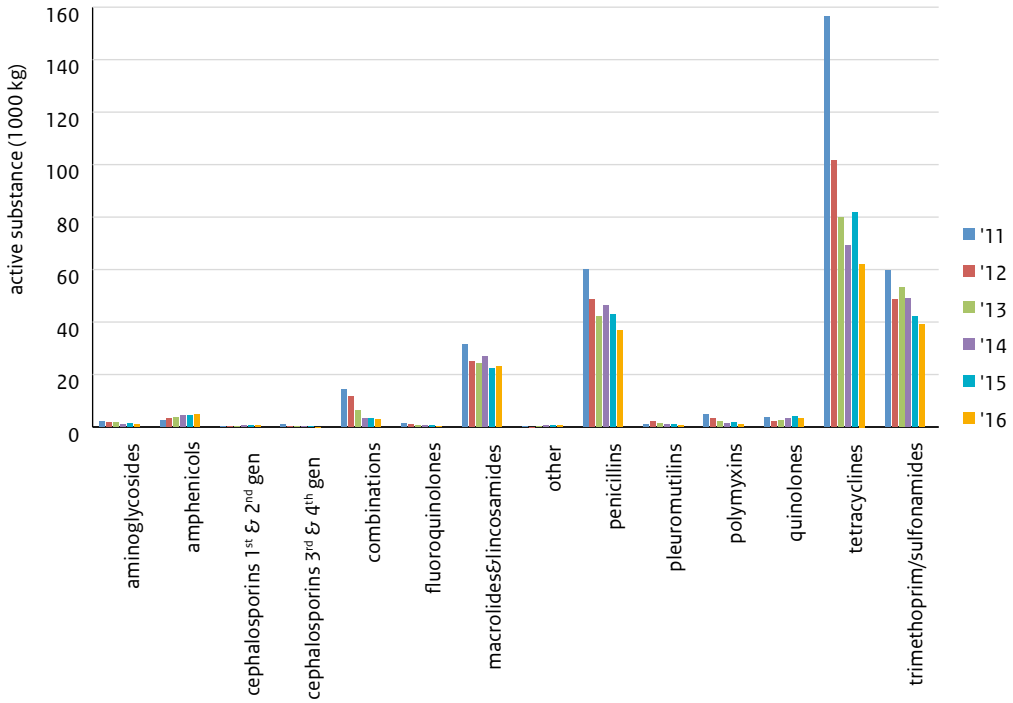


Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2016 in kg (thousands) (FIDIN, 2017).

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

Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmaco-therapeutic class 2011-2016 in kg (thousands)



Penicillins

Now third place in mass again, penicillin sales decreased 20% compared to 2015. 44% of the mass in this group consists of broad spectrum penicillins, compared to 70% previous years. These changes follow the introduction of, and are in line with, the guideline “Dry cow treatment”, endorsing selective use of dry cow treatment and the shift from broad spectrum dry cow treatments to small spectrum dry cow treatments.

(Fluoro)quinolones

The sales of fluoroquinolones decreased with 60 kg in 2016. An overall reduction of 78% was realized in comparison with 2011. 43.2% of the sales are applied in the monitored sectors. The sales of quinolones decreased also, compared with 2011 an overall decrease of 16% 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 presentations of veterinary medicinal product for companion animals were reported for the first time. Sales of these VMPs were stable with a slight decrease in 2015 and an increase in 2016. The sales of 3rd and 4th generation cephalosporins decreased in 2016 with 9 kg, a reduction of 99.8% was achieved since 2011. After this enormous reduction in sales, only 30% (was 83.5%) of 3rd and 4th generation cephalosporin use was not traceable to the monitored food producing animal sectors and companion animals. 12% of the mass sold was used in the monitored sectors.

Polymyxins

Colistin sales and use decreased in 2016, compared to 2011 a reduction of 79% was accomplished. This decrease was promoted by the withdrawal of all oral veterinary medical products of colistin combinations with other antimicrobial drugs.

2.2 Usage in pigs, veal calves, cattle, broilers, turkeys and rabbits 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 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.

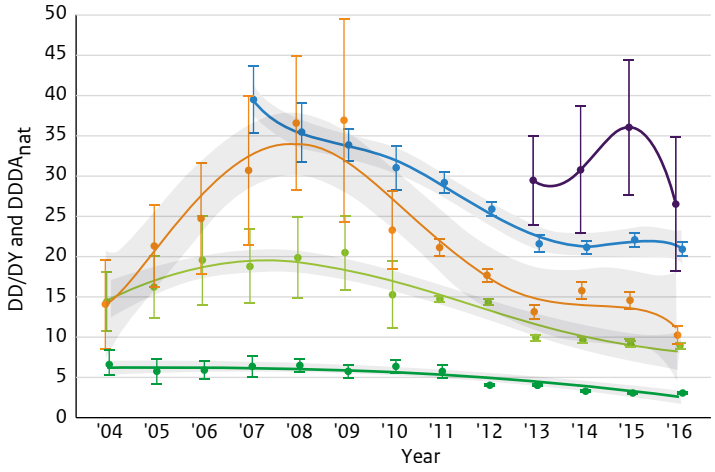
Since 2011, husbandry related consumption reports are prepared by the Netherlands Veterinary Medicines Authority (SDa) using consumption data from *all* farms in the largest sectors of food production animals: pigs, veal calves, broilers and (starting 2012) cattle. Since 2013 also turkeys provided the consumption data, and in 2016 rabbits also joined the monitoring. While the calculation method for treatable body mass (numerator) is the same, totalized for all farms per sector, the denominator represents the whole sector, and this measure is referred to as Defined Daily Doses Animal ($DDDA_{NAT}$). Table ABuse03 shows the animal populations veterinary medicinal products consumption data are reported for in 2012-2016 (pigs, veal calves, cattle, broilers, turkeys and rabbits). Table ABuse04 depicts the animal bodyweights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In all but one sectors (veal valves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low.

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. $DDDA_{NAT}$ 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 2016, 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
pigs	710,688	710,802	704,937	706,025	686,638
veal calves	156,602	159,547	158,828	156,751	164,890
diary cows	924,600	958,200	966,000	1,030,200	1,076,400
other cattle	597,900	573,800	649,000	649,800	600,100
broilers	43,846	44,242	47,020	49,107	48,378
turkeys	4,961	5,046	4,763	5,178	4,572
rabbits	872	830	860	1,004	948

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 2011-2016 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, except for turkey because of broad interval due to small number of farms).



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 ABuse06 depicts the animal bodyweights applied in the calculation of the denominator of $DDDA_F$ by the SDa.

This year the developments in colistin usage over the last four years was reviewed, in view of the international discussion about plasmid bound resistance and the report of ESVAC 2014. In general the usage is low in all sectors, range 0.2% - 3.2% of total use. In pigs the usage was the highest, 0.28 DDDA/year, this corresponds with 0.559 mg/PCU. Thus, colistin usage is below the lowest ESVAC-EMA benchmark for use on the sector level of 1 mg/PCU.

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	

Table ABuse05 Trends in $DDDA_{nat}$ in the Netherlands in livestock.

	Animalsector														
	Veal calfs*					Dairy cattle					Cattle				
Year	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Number of farms with prescriptions	2175	2125	2061	1978	1928	18053	18005	17747	17737	17529	14201	13644	13359	12971	12548
Pharmacotherapeutic group															
Aminoglycosides	0.81	0.53	0.34	0.19	0.23	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.01	0.01	0.01
Amphenicols	1.23	1.23	1.52	1.63	1.59	0.04	0.05	0.06	0.06	0.06	0.07	0.11	0.10	0.10	0.11
Cefalosporins 1st & 2nd generation	-	-	-	-	-	0.04	0.03	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.00
Cefalosporins 3rd & 4th generation	0.00	0.00	0.00	-	-	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Combinations	0.42	0.09	0.01	0.00	0.00	1.30	1.01	0.48	0.42	0.38	0.14	0.08	0.04	0.03	0.03
Fluoroquinolones	0.31	0.03	0.02	0.02	0.03	0.01	0.00	0.00	0.00	0.00	0.01				
Macrolides/lincosamides	3.91	3.84	3.72	3.88	3.54	0.07	0.06	0.10	0.10	0.07	0.13	0.22	0.20	0.16	0.17
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillins	2.80	2.11	2.15	2.33	2.25	1.86	2.20	2.00	1.87	1.86	0.22	0.19	0.18	0.16	0.16
Pleuromutilins	-	-	-	-		-	-	-	-	-	-	-	-	-	-
Polymyxins	0.73	0.36	0.15	0.19	0.07	0.06	0.02	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.00
Quinolones	0.27	0.30	0.49	0.58	0.66	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.03
Tetracyclines	12.61	10.87	10.66	11.01	10.47	0.43	0.42	0.39	0.37	0.35	0.55	0.59	0.47	0.42	0.44
Trimethoprim/sulfonamides	2.76	2.14	2.08	2.22	2.05	0.20	0.22	0.24	0.25	0.24	0.16	0.16	0.11	0.10	0.10
Total	25.85	21.50	21.15	22.05	20.88	4.06	4.03	3.30	3.11	3.01	1.37	1.40	1.15	1.00	1.07

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

Table ABuse05 Trends in $DDDA_{nat}$ in the Netherlands in livestock.

Animalsector															
	Pigs					Broilers					Turkeys				Rabbits
Year	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2013	2014	2015	2016	2016
Number of farms with prescriptions	6425	6588	6072	5824	5462	732	770	797	816	849	41	40	47	43	43
Pharmacotherapeutic group															
Aminoglycosides	-	-	0.01	0.01		0.58	0.03	0.03	0.02	0.01	1.24	0.40	0.71	0.69	9.66
Amphenicols	0.06	0.09	0.17	0.18	0.24	-	-	-	-	-	0.02	-	-	-	0.00
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	0.27	0.10	0.05	0.04	0.03	0.52	0.37	0.08	0.11	0.05	-	-	-	-	-
Fluoroquinolones	0.00	0.00	0.00	-	0.00	0.80	0.24	0.18	0.07	0.07	1.76	1.29	1.20	1.60	0.25
Macrolides/lincosamides	1.39	1.02	1.09	1.04	1.08	1.06	0.31	0.35	0.48	0.25	3.55	2.12	1.98	1.18	1.08
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.37
Penicillins	2.91	2.17	2.05	1.93	1.97	7.46	6.34	9.96	8.44	6.48	9.34	14.89	16.61	13.75	-
Pleuromutilins	0.35	0.12	0.09	0.08	0.07	-	-	-	-	-	-	-	0.12	-	1.38
Polymyxins	0.58	0.44	0.34	0.38	0.28	0.84	0.08	0.05	0.06	0.04	0.18	0.08	0.63	0.61	0.09
Quinolones	0.03	0.03	0.05	0.03	0.02	1.97	1.65	2.22	2.86	1.51	0.23	0.02	0.10	0.01	-
Tetracyclines	6.79	4.58	4.34	4.15	4.07	2.40	2.52	1.77	1.49	1.01	11.19	9.58	12.57	7.63	10.49
Trimethoprim/sulfonamides	1.92	1.40	1.33	1.20	1.10	1.97	1.46	1.45	1.07	0.78	1.80	2.37	2.01	0.95	1.62
Total	14.32	9.97	9.52	9.05	8.87	17.61	13.01	15.76	14.59	10.19	29.31	30.74	35.94	26.42	40.93

* *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	192
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 in pigs, veal calves, cattle, broilers and turkeys in the Netherlands in number of DDDvet per animal-year

A comparison of the number of DDDA with the internationally established ESVAC DDD_{vet} was conducted for the 2016 data, with the denominator of the $DDDA_{NAT}$.

The use is calculated excluding the locally administered veterinary medicinal products for mastitis and metritis, which are included in the Dutch system, but in the ESVAC system are only accounted for in the defined course dose (DCD_{vet}) calculation.

In general, both methods result in comparable consumption. In the Dutch system, veterinary medicinal products consisting of a combination of active substances result in only one treatment day, while in the ESVAC system the application of such product results in one treatment day for every active substance. This is noticeable in the group trimethoprim/sulfonamides in all sectors, except for turkeys. In turkeys predominantly a product with one sulfonamide is applied, with a much lower authorized dose in the Netherlands than the average dose in Europe. Figure Abuse04 and Table Abuse07 depict the results.

Figure ABuse04 Number of $DDDA_{NAT}$ versus DDD_{vet} per animal-year of systemic veterinary medicinal product only (excluding intramammary and intrauterine applications) in 2016

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

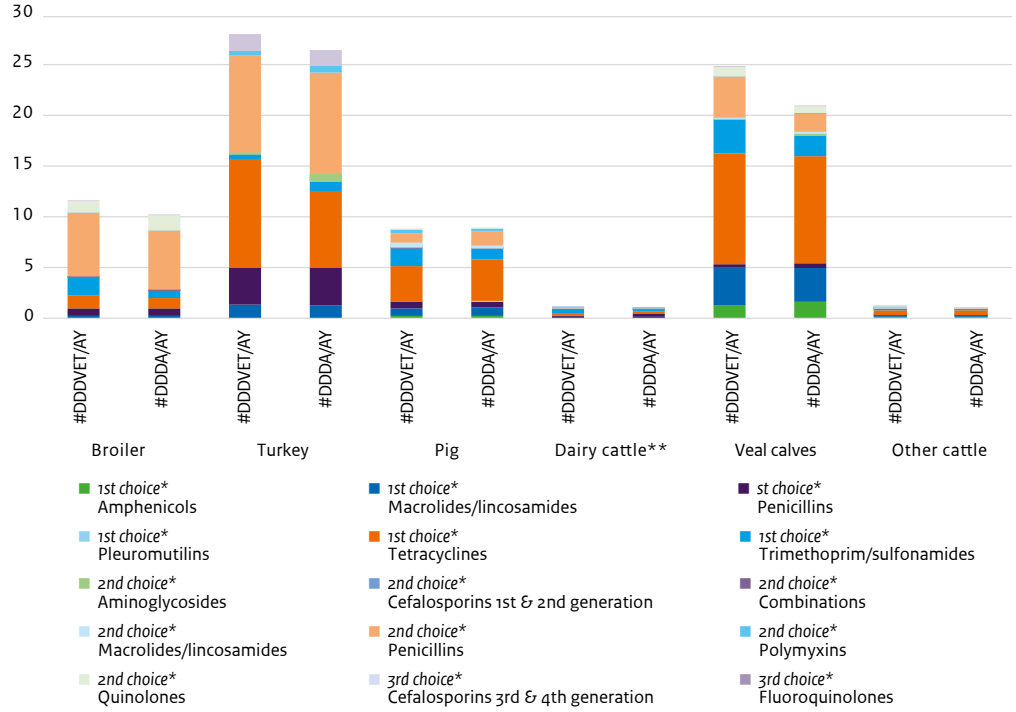


Table ABuse07 comparison number of $DDDA_{VET}$/animal year (AY) and DDDA/AY (=$DDDA_{NAT}$) in monitored sectors.

	Broilers		Turkeys		Pigs		Dairy cattle (excluding intramammary and intrauterine administrations)		Veal calves		Other cattle	
	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA	#DDD_{VET}	#DDDA
First choice*	4.02	2.74	16.12	13.46	6.91	6.88	0.95	0.87	19.51	17.94	0.95	0.89
% 1st choice of total	34.84%	26.87%	57.72%	50.95%	79.13%	77.54%	90.33%	90.13%	78.93%	85.90%	81.28%	85.20%
Amphenicols	-	-	-	-	0.18	0.24	0.04	0.06	1.22	1.59	0.09	0.11
Macrolides/lincosamides	0.24	0.25	1.28	1.18	0.81	0.82	0.03	0.05	3.81	3.35	0.17	0.15
Penicillins	0.68	0.70	3.64	3.70	0.57	0.58	0.15	0.26	0.26	0.48	0.05	0.08
Pleuromutilins	-	-	-	-	0.07	0.07	-	-	-	-	-	-
Tetracyclines	1.32	1.01	10.71	7.63	3.46	4.07	0.24	0.27	10.88	10.47	0.47	0.43
Trimethoprim/sulfonamides	1.78	0.78	0.49	0.95	1.81	1.10	0.47	0.24	3.34	2.05	0.17	0.10
Second choice*	7.47	7.38	10.21	11.36	1.82	1.99	0.10	0.09	5.18	2.92	0.22	0.15
% 2nd choice of total	64.55%	72.41%	36.55%	42.99%	20.87%	22.45%	9.34%	9.47%	20.97%	13.97%	18.68%	14.75%
Aminoglycosides	0.00	0.01	0.20	0.69	0.00	0.00	0.01	0.01	0.09	0.23	0.01	0.01
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	-	-	-	-
Combinations	1.08	1.51	0.01	0.01	0.02	0.02	0.00	0.00	0.85	0.66	0.04	0.03
Macrolides/lincosamides	0.09	0.05	-	-	0.08	0.03	0.04	0.04	0.00	0.00	0.03	0.03
Penicillins	-	-	-	-	0.41	0.26	0.01	0.01	0.12	0.19	0.01	0.02
Polymyxins	6.28	5.78	9.56	10.05	0.97	1.39	0.04	0.03	4.05	1.77	0.13	0.06
Quinolones	0.03	0.04	0.44	0.61	0.34	0.28	0.01	0.01	0.07	0.07	0.01	0.00
Third choice*	0.07	0.07	1.60	1.60	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
% 3rd choice of total	0.61%	0.72%	5.73%	6.06%	0.00%	0.00%	0.33%	0.40%	0.10%	0.13%	0.03%	0.05%
Cefalosporins 3rd & 4th generation	-	-	-	-	-	-	0.00	0.00	-	-	0.00	0.00
Fluoroquinolones	0.07	0.07	1.60	1.60	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Total	11.57	10.19	27.93	26.42	8.73	8.87	1.05	0.97	24.72	20.88	1.17	1.04

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

2.4 Usage of antimicrobial veterinary medicinal products in unmonitored sectors

Surveys were performed in companion animals and horses and the results were published in 2016. Both sectors showed relatively low prescription rates. The consumption of antimicrobials, based on purchased antimicrobial veterinary medicinal products by the veterinary practices in 2014, was for companion animals 2.6 DDDA/animal year, for horses 0.56 DDDA/animal year.

Data from antimicrobial use surveys in sheep and goats of 2012, mink in 2015, zoo-animals in 2016, other poultry (a.o. laying hens) in 2016 and the use of multi-species authorized products in horse and companion animals added up to roughly 10.000 kg mass of active substances. This mass represents the use of veterinary medicinal products apart from the mass used in the monitored sectors and the mass of veterinary medicinal products authorized only for companion animals or horses.

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers, in 2016 food producing rabbits have joined the regular monitoring as well. The rather steep decrease in sales of antibiotics licenced for therapy in the Netherlands in 2016 may be the result of an adjustment or compensation for the relatively high 2015 sales. The calculation of consumption is based on national conversion factors (DDDA's) of authorized drugs. This year it was possible to allocate most sold antimicrobial veterinary medicinal products to the species the products are used in by using several alternative data sources.

In all but one sectors (veal calves, dairy cattle, pigs broilers and turkeys) a reduction in consumption was realized. In other cattle increased consumption is noted, albeit this consumption is low.

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.

3 Resistance data

This chapter describes susceptibility test results as determined in 2016 for the food-borne pathogens *Salmonella* enterica, *Campylobacter* spp. and *Escherichia coli* O157, and the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*. Reduced susceptible and resistant isolates were defined using epidemiological cut-off values (www.eucast.org) 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) should not be automatically classified as clinically resistant. For the purpose of this report we designate all non-wild-type susceptible isolates as "resistant", and specify this per antibiotic if necessary.

3.1 Food-borne pathogens

3.1.1 *Salmonella*

This chapter presents resistance percentages of *Salmonella* isolates, sampled from humans suffering from clinical enteral infections, 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 2016 *S.* Enteritidis (N = 438) followed by *S.* Typhimurium (N = 260) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 229), were most frequently isolated from humans suffering from salmonellosis.
2. In pigs, the monophasic variant of *S.* Typhimurium dominated. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated.
3. In poultry (including poultry products, broilers and layers), the number of *S.* Paratyphi B var. Java further reduced in 2016. Also *S.* Infantis, still predominant in 2015, was less frequently isolated in 2016. The prevalence of *S.* Enteritidis increased compared to 2015 and was the most predominant serovar in poultry in 2016.
4. Highest resistance levels were observed in *S.* Kentucky (travel related), the monophasic *S.* Typhimurium, other *S.* Typhimurium, *S.* Paratyphi B var. Java and to a lesser extent in *S.* Infantis and *S.* Newport.
5. Ciprofloxacin resistance was most common amongst isolates from humans and poultry. Predominant serovars were *S.* Enteritidis (23%), *S.* Typhimurium (18%) and *S.* Kentucky (11%).
6. In 2016, the percentage cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 1.7%, among eleven different serovars, predominantly isolated from human and poultry sources.
7. In 2016 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.

Human isolates tested (N = 1473 in 2016) were a selection of all isolates sent to the RIVM by regional public health and other clinical laboratories. These strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N = 36) and cattle (N = 36) were a random selection sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections in animals. Those from poultry (N=122) (and broilers, N = 4; layers, reproduction animals, N = 39) 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 = 385 from animal feed and food products; other animals from animal husbandry (e.g. horses, sheep, goats, ducks) and pets, samples from the environment etc.) have also been serotyped and tested. In addition, NVWA tested 135 *Salmonella* isolates obtained from raw meats, vegetables and seeds. The results of these isolates were not included in Tables S02, S03, S04 and S05, but are depicted in Table S06.

Traditionally, *S.* Enteritidis and *S.* Typhimurium are the most frequently isolated serovars from human clinical infections. This did not change in 2016: *S.* Enteritidis (28.7%) followed by *S.* Typhimurium (17%) together with the monophasic variant of Typhimurium, (*S. enterica subspecies enterica* 1,4,5,12:i:-) (15%), were most frequently isolated from humans suffering from salmonellosis. Referring to *S.* Enteritidis a large European outbreak was caused by consumption of imported eggs from Poland in Dutch

restaurants. Also noticeable is the multi-country outbreak of *S.* Bovismorbificans which could be traced back to a of a raw ham product from Belgium.
S. Typhimurium and its monophasic variant were predominantly associated with pigs and cattle, but was also found in poultry. *S.* Enteritidis was mainly isolated from poultry and layers (Table S01). In pigs, *S.* Typhimurium and its monophasic variant were most predominant. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. In poultry (predominantly layers), *S.* Enteritidis was by far the most prevalent serotype (62.2% of isolates), followed by monophasic variant of Typhimurium (9.6%). The presence of *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis was substantially reduced (6.0% and 4.8% respectively).
Depending on the serotype, reported travel contributed up to 39% of the cases of human salmonellosis over the years 2013-2016. Relative high contributions (≥25%) were noted for the serovars Typhi/ Paratyphi A,B (including var. Java),C, Livingstone, Stanley, Tennessee, Weltevreden, Corvallis and Virchow. It should be noted that the contribution of travel as depicted in Table S01 is only indicative of the true contribution, because travel is underreported by an estimated factor of about two.

Resistance levels

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 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 usage of colistin in veterinary medicine has been under discussion and measurements have been taken to reduce the use in animals. Moreover, the recent finding of a plasmid mediated colistin resistance gen (*mcr-1*) resulted in even more attention for this compound. However, like in former years, colistin resistance is not reported in *Salmonella*. That is because a general epidemiological cut-off value is lacking for colistin, the results are 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 for colistin). As a result, colistin resistance would have been over reported in *Salmonella*.

Table S01 Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2013-2016	2015	2016	2015	2016	2015	2016
N Total			1204	1528	54	48	54	45
N tested	Tested		1140	1473	51	36	54	36
Enteritidis	802	13%	283	438				1
Typhimurium	635	5%	233	260	28	10	30	15
Typhimurium (monofasisch)	518	5%	176	229	22	28	7	13
Infantis	181	9%	52	37				
Paratyphi B var. Java	101	20%	19	34				
Dublin	87	4%	21	28		1	15	9
Derby	81	8%	18	20	2	2		
Agona	59	15%	12	13				
Senftenberg	59	24%	3	5				
Typhi/Paratyphi A,B,C	57	28%	22	31				
Brandenburg	54	4%	8	11		2	1	3
Kentucky	53	17%	11	36				
Napoli	52	9%	16	31				
Newport	48	22%	14	23				
Bovismorbificans	39	8%	5	42		1		
Livingstone	37	30%	4	5	1	1		
Stanley	36	25%	21	14				
Saintpaul	34	11%	13	13				
Chester	33	17%	14	16				
Schwarzengrund	33	5%	5	9				
Heidelberg	32	10%	4	5				
Mbandaka	27	17%	2	6				
Tennessee	27	29%		1				
Anatum	26	15%	4	1				
Montevideo	26	24%	5	4				
Goldcoast	25	12%	11	8	1	1	1	2
Rissen	23	11%	10	5		2		
Thompson	22	16%	8	9				
Braenderup	21	19%	9	12				
Oranienburg	21	15%	18	5				
Weltevreden	21	29%	4	10				
Hadar	20	21%	15	5				
Corvallis	19	39%	7	9				
Bredeney	18	25%	5	4				
Give	18	10%	4	4				
Panama	18	14%	7	4				
Muenchen	16	22%	8	2				1
Bareilly	14	7%	5	6				
Virchow	14	29%	6	9				
Indiana	13	9%	4	3				
Kedougou	13	0%	1					
Goettingen	12	0%	5	3				

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2015	2016	2015	2016	2015	2016	2015	2016
N Total	1204	1528	54	48	54	45	54	45
N tested	1140	1473	51	36	54	36	54	36
Enteritidis	41	155	8	10	20	84	212	63
Typhimurium	9	8	2		5	2	39	44
Typhimurium (monofasisch)	10	24	9	13		3	20	47
Infantis	32	12	20		3	2	58	95
Paratyphi B var. Java	27	15	14			2	38	25
Dublin	1	1	1				5	14
Derby	9		1		1		20	38
Agona	5		2		2		25	18
Senftenberg	1		1				69	29
Typhi/Paratyphi A,B,C								
Brandenburg	2		2				26	45
Kentucky	1						4	6
Napoli	1						2	3
Newport	1	1					1	4
Bovismorbificans	1		1				1	1
Livingstone	2		1				44	146
Stanley							3	3
Saintpaul	1						3	8
Chester	1		1				1	7
Schwarzengrund	2						5	16
Heidelberg	18		8				14	4
Mbandaka	2	1			2	1	36	17
Tennessee		1		1			34	43
Anatum	1	2	1			1	16	68
Montevideo	1	2				1	10	31
Goldcoast							2	1
Rissen							3	8
Thompson	3				2		1	4
Braenderup	1				1		1	2
Oranienburg							8	7
Weltevreden							10	6
Hadar		1					4	7
Corvallis	1	2	1	1			1	2
Bredeney							3	18
Give		2					5	6
Panama		2				2	5	4
Muenchen	1						2	3
Bareilly							3	
Virchow	2						7	5
Indiana		2				2	10	3
Kedougou	5						1	19
Goettingen	2				1			

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2013-2016	2015	2016	2015	2016	2015	2016
N Total			1204	1528	54	48	54	45
N tested	Tested		1140	1473	51	36	54	36
Javiana	11	9%	6	5				
Mikawasima	11	0%	7	4				
Kottbus	10	20%	2	5				
Putten	10	n.a.						
Blockley	9	8%	10					
Cerro	9	0%		1				
Poona	9	18%	2	6				
London	8	11%	3	1				
Jerusalem	7	n.a.						
Ohio	6	0%		1				
OVERIGE	278	197%	82	95				1

Table S02 shows MIC-distributions and resistance percentages of 1954 *Salmonella's* from different sources tested for susceptibility in 2016. Highest levels of resistance were observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, chloramphenicol and trimethoprim. The levels of resistance to ciprofloxacin and cefotaxime/ceftazidime have slightly increased compared to 2015, and seem to fluctuate a little since 2013, but are still higher than in 2012. No resistance to the carbapenem antibiotic meropenem was detected, indicating that carbapenemase producers were not present in the tested isolates (see also chapter 4.2). Similar to 2015 low levels of resistance were found for tigecycline (1.0%) and azithromycin (0.5%) almost exclusively in human isolates.

Table S03 presents resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2016. Resistance profiles varied considerably among serovars. High resistance levels (86.9-88.3%) were observed in the monophasic *S.* Typhimurium and in *S.* Kentucky (66.7-91.7%), and to a lesser extent (40.7-45.9%) in *S.* Typhimurium.
Most serovars have acquired resistance against a number of antimicrobials. The most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT). High resistance levels for quinolones (ciprofloxacin and nalidixic acid) were regularly found in *Salmonella*, especially in *S.* Kentucky (travel related), and to a lesser extent in *S.* Infantis, *S.* Newport, *S.* Typhimurium, *S.* Paratyphi B variant Java and *S.* Enteritidis. Highest percentage of fluoroquinolone resistance (18 and 13%) were found amongst human isolates and in poultry (including broilers and layers), reflecting the usage of quinolones in humans and the poultry production chain.

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* spp, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella*

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2015 and 2016 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2015	2016	2015	2016	2015	2016	2015	2016
N Total	1204	1528	54	48	54	45	54	45
N tested	1140	1473	51	36	54	36	54	36
Javiana							1	1
Mikawasima							1	1
Kottbus	1		1				2	
Putten	5		1				2	6
Blockley								
Cerro							8	5
Poona							4	1
London	1		1				12	5
Jerusalem	2	4			1	4	3	2
Ohio	6		1				4	5
OVERIGE	10	14	4	1	1	6	144	240

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, 15.1% of *Salmonella* isolates (N =296/1954), demonstrated a resistant phenotype for ciprofloxacin (Table S02). The dominant serovars of ciprofloxacin resistant isolates were S. Enteritidis (23%), S. Typhimurium (18%) and S. Kentucky (11%), mainly from human sources.
In isolates from retail meat (Table S06) the overall ciprofloxacin resistance percentage was 27%. The majority of the isolates was obtained from chicken (N = 33) or turkey meat (N = 7). In chicken meat S. Infantis (N = 19) was most predominant ciprofloxacin resistant serotype followed by S. Paratyphi Java (N = 4). In turkey meat S. Saintpaul (N = 4) was the predominant ciprofloxacin resistant serotype (data not shown).

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). In 2016, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 28/1954 (1.4%), among nine different serovars. Twenty-six isolates were derived from humans: predominantly S. Kentucky (N = 9), S. Typhimurium (N = 4), monophasic S. Typhimurium (N = 3) and S. Infantis (N = 4). The remaining two isolates were derived from poultry sources (S. Paratyphi B variant Java and S. Infantis).
In isolates derived from retail meat (Table S06) the overall cefotaxime resistance was 7.1%. Almost all cefotaxime resistant isolates were obtained from chicken meat (S. Heidelberg (N = 2), S. Paratyphi B variant Java (N = 1) and S. Minnesota (N = 1) or turkey meat (S. Saintpaul (N = 3). One cefotaxime resistant isolate (S. Molade) was obtained from crocodile meat (data not shown).
In addition, at NVWA eight cefotaxime resistant *Salmonella* isolates identified in raw meat from poultry (N=4), turkey (N=3) and crocodile (N=1). Including these isolates the overall cefotaxime resistance in *Salmonella* is 1.7%.

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N=1954) tested for antibiotic susceptibility during 2016.

Salmonella	MIC (%) distribution mg/L																	R%	95% CI
N = 1954	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							43.7	31.0	1.5		0.1			23.7					23.8	22-25.7
Cefotaxime					97.4	1.1			0.1	1.4									1.4	0.9-2
Ceftazidime						97.1	1.6	0.1	0.5	0.4	0.4								1.2	0.8-1.7
Gentamicin						90.2	7.1	0.7	0.1	0.3	0.8	0.4	0.6						2.0	1.4-2.7
Tetracycline								73.6	1.7	0.2	0.1	1.8	2.4	20.2					24.4	22.5-26.3
Sulfamethoxazole										41.5	24.9	7.0	1.1	0.1	0.1			25.4	25.4	23.5-27.3
Trimethoprim					75.5	17.1	1.0						6.4						6.4	5.4-7.6
Ciprofloxacin	24.9	58.2	1.7	0.7	6.2	5.0	1.3	0.1	0.2	0.7	1.0								15.1	13.6-16.7
Nalidixic acid									80.1	4.6	2.3	1.7		0.5	10.8				13.1	11.6-14.6
Chloramphenicol										89.0	4.1	0.2	0.2	0.8	5.7				6.9	5.8-8
Azitromycin*								0.8	36.5	59.3	3.0	0.3	0.1	0.2					0.5	0.2-0.9
Colistin**							73.2	19.3	7.1	0.4									-	-
Meropenem		92.8	7.2																0.0	0-0
Tigecyclin					48.8	43.2	7.0	1.0	0.1										1.0	0.6-1.5

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoints are identical

* *tentative set ECOFF during the EURL AMR WP meeting on 25 April 2015 in Lyngby (DK).*

** *Because of differences in natural susceptibility for colistin between serovars there is no general Salmonella ECOFF available for colistin. For this reason the percentage of resistance is not depicted*

Table S03 Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2016 (N tested).

	Enteritidis (467)	Typhimurium (305)	1,4,[5],12:i:- (282)	Infantis (80)	Dublin (40)	Paratyphi B var Java (37)	Kentucky (36)	Derby (35)	Bovismorbificans (32)	Napoli (32)	Brandenburg (29)	Newport (29)
Ampicillin	2.1	45.6	86.9	7.5	0.0	18.9	66.7	2.9	6.3	0.0	0.0	13.8
Cefotaxime	0.0	1.3	1.1	6.3	0.0	5.4	25.0	0.0	3.1	0.0	0.0	0.0
Ceftazidime	0.0	0.0	1.1	6.3	0.0	5.4	25.0	0.0	3.1	0.0	0.0	0.0
Gentamicin	0.0	2.0	2.1	3.8	0.0	0.0	50.0	0.0	3.1	0.0	0.0	0.0
Tetracycline	1.1	40.7	88.3	18.8	0.0	5.4	72.2	5.7	3.1	0.0	3.4	13.8
Sulfamethoxazole	1.1	45.9	87.2	20.0	2.5	21.6	72.2	22.9	3.1	0.0	0.0	13.8
Trimethoprim	0.4	12.5	8.9	15.0	0.0	16.2	8.3	2.9	3.1	0.0	0.0	13.8
Ciprofloxacin	14.3	17.4	8.5	21.3	0.0	16.2	91.7	5.7	3.1	0.0	3.4	20.7
Nalidixic acid	13.9	14.8	6.0	21.3	0.0	13.5	91.7	2.9	0.0	0.0	3.4	13.8
Chloramphenicol	0.0	26.2	8.9	3.8	2.5	2.7	0.0	2.9	3.1	0.0	0.0	13.8
Azithromycin	0.9	0.0	1.1	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.4	0.3	1.4	7.5	2.5	0.0	2.8	0.0	0.0	0.0	3.4	0.0

S. Typhimurium

Table S01 shows that *S.* Typhimurium represents 17.0% (260/1528) of all human *Salmonella* isolates as characterized by the RIVM in 2016. This is a bit less than in 2015 (19.4%), but slightly more than 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.

Resistance in *S.* Typhimurium was very high for ampicillin, tetracycline and sulfamethoxazole, for chloramphenicol in human and cattle isolates, and also for trimethoprim in pig isolates (Table S04). About 26% 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) is substantially higher than 2015 (12%) and more similar to the proportion in previous years. Resistance to the clinical important drug cefotaxime was only seen in isolates from humans at a low level (1.5%). Resistance to fluoroquinolones was frequently present in isolates from humans (19.2%) and pigs (12.5%) and (9.0%) in isolates from cattle. These figures indicate a clear increase of fluoroquinolone resistance in *S.* Typhimurium isolates derived from humans and animals compared to 2015. Resistance to tigecycline was absent in isolates derived from humans, pigs and cattle. The only tigecycline resistant isolate (MIC: 2 mg/L) was collected from a sheep carcass.

Table S04 Resistance percentages of *S.* Typhimurium (N tested) isolated from different sources in 2016.

	S. Typhimurium (305)			
	Humans (266)	Cattle (11)	Pigs (8)	Other sources* (20)
Ampicillin	46.2	36.4	62.5	35.0
Cefotaxime	1.5	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0
Gentamicin	1.9	9.1	0.0	0.0
Tetracycline	38.7	72.7	62.5	40.0
Sulfamethoxazole	44.4	72.7	75.0	40.0
Trimethoprim	10.9	0.0	50.0	25.0
Ciprofloxacin	19.2	9.1	12.5	0.0
Nalidixic acid	16.5	9.1	0.0	0.0
Chloramphenicol	27.1	36.4	25.0	10.0
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	5.0

** Other sources includes broilers, laying hens, goats and feed products.*

Resistance levels 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 levels for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated from 2014 to 2016 (Figure S01).
Resistance levels in *S.* Typhimurium isolates from animal samples (cattle and pigs shown in figure S01) vary considerably over the years. Levels seemed to decrease from 2013, but an increase was seen in 2016. However, these levels 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 raw eggs and, to a lesser extent, of poultry meat products. 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 poultry (broilers and laying hens) and were similar to the most predominant MLVA types in 2013,2014 and 2015. However, in 2016, the most predominant (N=139) *S.* Enteritidis MLVA type (02-09-07-03-02) was part of the outbreak associated with the consumption of Polish eggs. In contrast to 2014 and 2015, resistance to ciprofloxacin and nalidixic acid was found in laying hens, at approximately the same levels as in the human samples (13 to 14%, Table S05). However, the number of samples from laying hens was very low (15), so the reliability of the given percentage of resistance in these strains is low. Sources of human infection with *S.* Enteritidis are considered to be consumption of contaminated eggs and poultry

food products and travel abroad. *S.* Enteritidis prevalence varies over the years, but is traditionally much higher in layers than in broilers.
Compared to many other *Salmonella* serovars, resistance in *S.* Enteritidis is much lower (Table S03). The trends in resistance of *S.* Enteritidis over the years are summarized in Figure S02. Resistance levels in human isolates showed a small decrease for all antimicrobials, compared to 2015. As seen for *S.* Typhimurium, resistance levels for isolates from laying hens and other sources seem to vary considerably over the years due to the relatively small number of samples per year. It should be realized that these resistance percentages are not very reliable.

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999-2016.

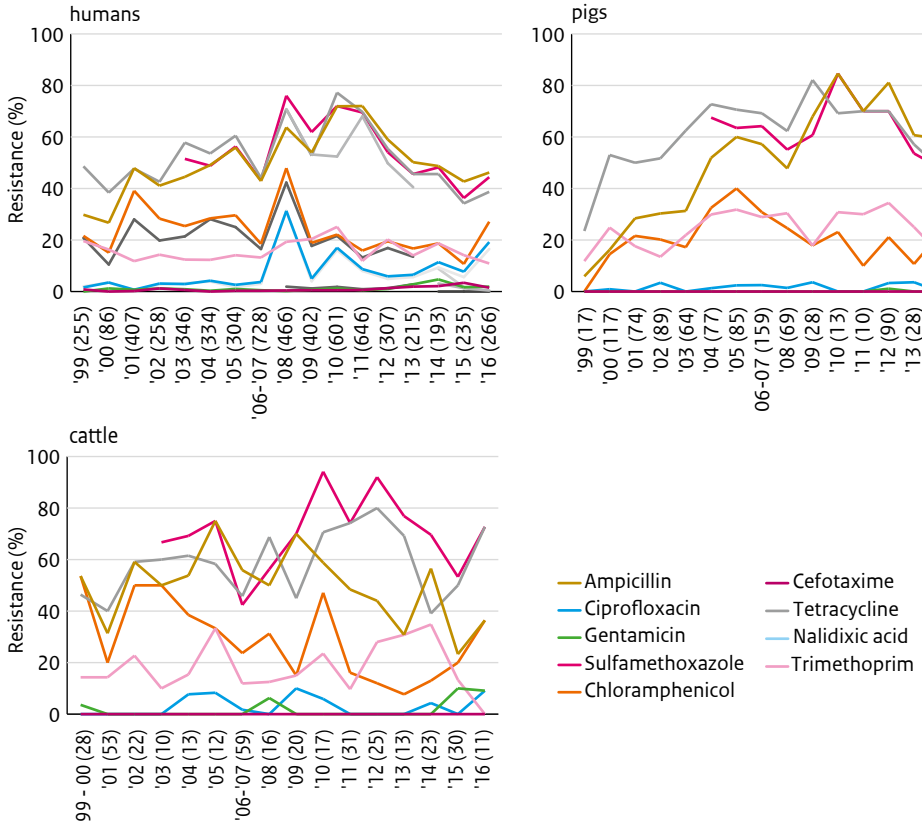


Table S05 Resistance percentages of *S.* Enteritidis (N tested) isolated from different sources in 2016.

	S. Enteritidis (467)		
	Humans (392)	Laying hens (15)	Other sources* (60)
Ampicillin	2.1	0.0	0.0
Cefotaxime	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Tetracycline	1.1	0.0	0.0
Sulfamethoxazole	1.1	0.0	0.0
Trimethoprim	0.4	0.0	0.0
Ciprofloxacin	14.3	13.3	13.3
Nalidixic acid	13.9	13.3	13.3
Chloramphenicol	0.0	0.0	0.0
Azithromycin	0.9	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	0.4	0.0	0.0

** Other sources includes mainly broilers (n = 55), but also isolates from cattle, feed and food products.*

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

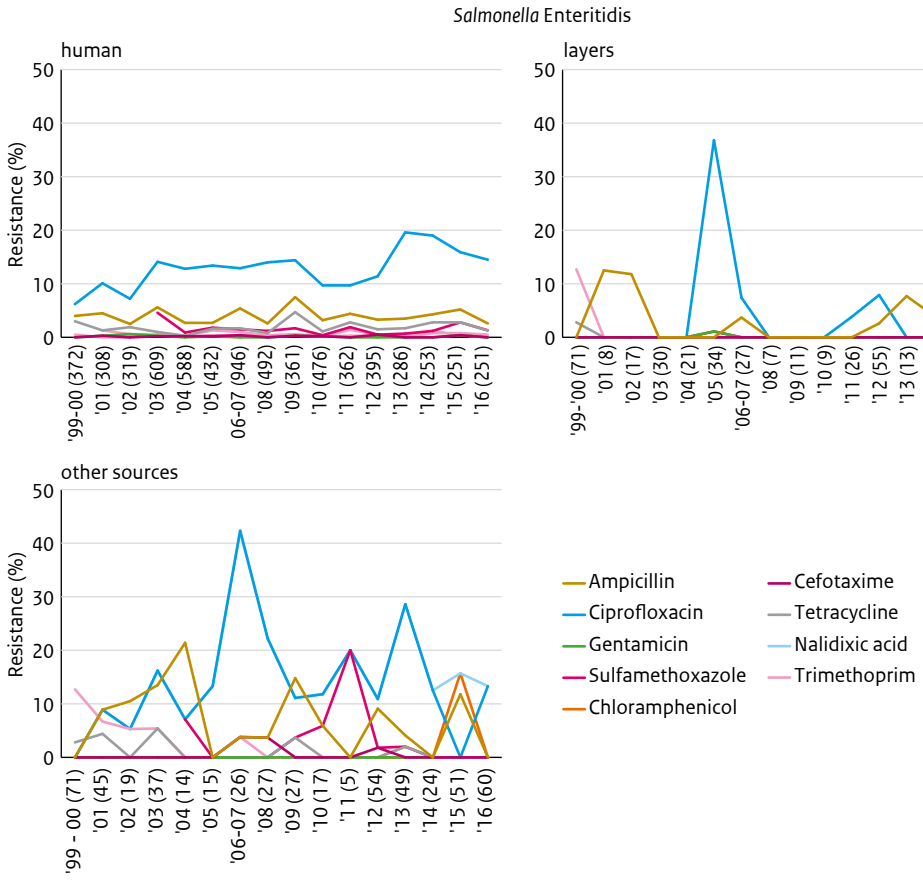
The prevalence of *S.* Java further decreased in 2016. As a consequence, *S.* Java was not the most predominant serovar isolated in broiler production anymore, as it was in the period before 2015. Sixteen *S.* Java strains from poultry were included for susceptibility testing (Figure S03). Resistance levels of most antimicrobials increased, compared to 2015. Since 2012, the resistance levels seem to fluctuate, and a real increasing or decreasing trend cannot be seen. The resistance percentage to trimethoprim was at 100%, like in former years. NB: due to an error in MARAN 2016 resistance percentages of animal isolates (especially for trimethoprim) were wrongly depicted in Figure S03. Resistance against chloramphenicol was not detected in the samples from 2016. The resistance level for the quinolones ciprofloxacin and nalidixic acid were for both 50.0%.
In 2016, 34 *S.* Java strains were isolated from human infections. All strains tested were trimethoprim susceptible and therefore not considered to be related to the clone spreading in Dutch poultry and probably travel related.

Salmonella from chicken meat, pork, other meat sources and herbs and seeds

Resistance data of *Salmonella* isolates from raw meat, herbs and seeds are presented (Table S06, Figure S03). In 2016 *S.* Infantis (40%) was the dominant serovar found in samples from chicken meat, followed by *S.* Enteritidis (18%) *S.* Paratyphi B variation Java (16%). In general, isolates from pork were resistant against a fewer number of antimicrobials than isolates from chicken meat and other raw meat sources (Java excepted). Resistance levels to the quinolones (ciprofloxacin and nalidixic acid) in chicken meat isolates were very high (61.8% and 60.0% respectively); levels in meat from other species were reduced, compared to 2015. Resistance levels to tigecycline were lower than in 2015, and not detected in isolates from herbs and seeds. Resistance to cephalosporins (cefotaxime and ceftazidime) halved in

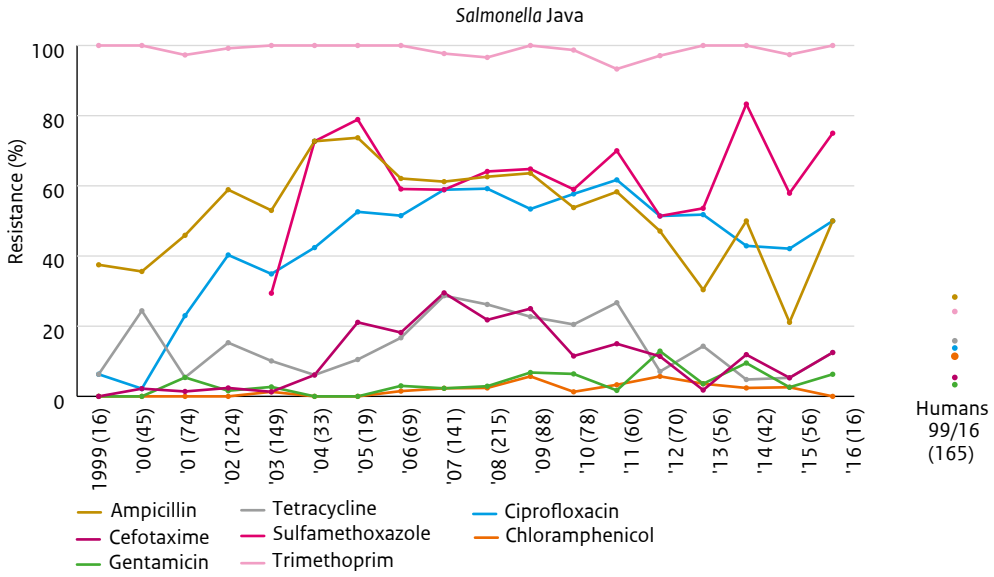
chicken meat and other raw meat since 2015 (7.3% for both in chicken meat and 9.8% for both in other raw meat products), and was absent in pork meat.
Resistance levels to quinolones (ciprofloxacin and nalidixic acid) in herbs and seeds were lower than in 2015 (8.7%); resistance to cephalosporins (cefotaxime and ceftazidime) was not detected in these samples. Eighteen different *Salmonella* serotypes were found among 23 samples from herbs and seeds. Among those were three of the twelve most prevalent serotypes described earlier in Table S03:
S. Enteritidis (n=1), S. Kentucky (n=1) and S. Typhimurium (n=1).
All percentages in TableS06 should be interpreted with care, because of the relatively low number of samples.

Figure S02 Trends in resistance (%) of S. Enteritidis isolated from humans, layers and other sources from 1999-2016.



The overall resistance levels of *Salmonella* from poultry products over the years are shown in Figure S04. Resistance levels fluctuated since 2001, with an increasing trend for ciprofloxacin and tetracycline. In 2013 a substantial reduction was observed for most antimicrobials. However, after 2013 the level tended to increase again for sulfamethoxazole, ciprofloxacin, tetracycline, ampicillin and cefotaxime, with a slight decrease for most of them in 2016. The increase in 2014/2015 could reflect the relative high proportion of strains from imported poultry products included. It should be noticed that the fluctuating resistance levels during the years, could be influenced by the varying proportions of imported products sampled per year.

Figure S03 Trends in resistance (%) of S. Paratyphi B var. Java isolated from poultry sources from 1999-2016 and humans (Separate data on the rigt indicate all human S. java isolates from 1999-2016).

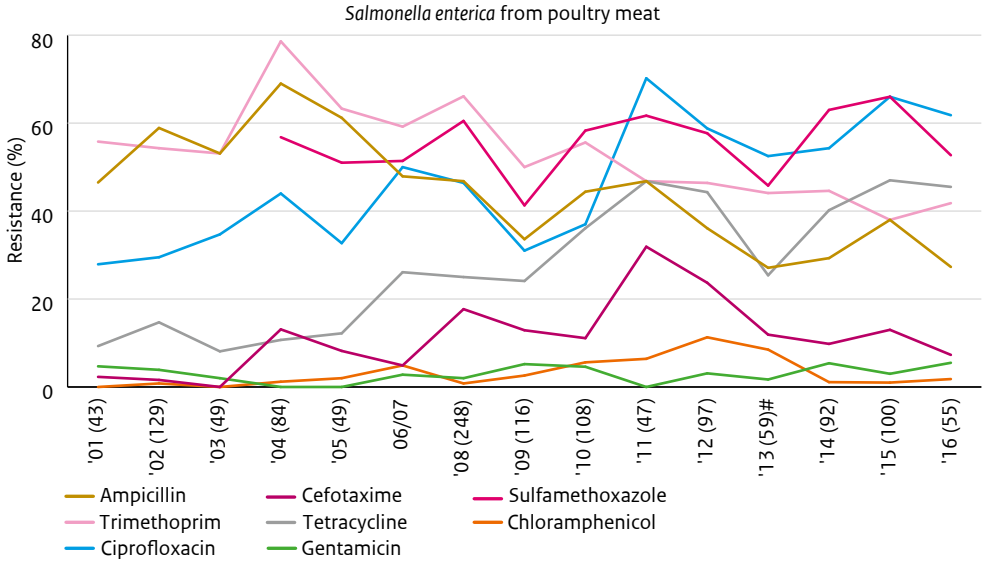


Due to an error in MARAN 2016 resistance percentages of animal isolates (especially for trimethoprim) were wrongly depicted.

Table S06 Resistance (%) of *Salmonella enterica* isolated from different types of raw meat, herbs and seeds in the Netherlands in 2016.

	Chicken N = 55	Pork N = 16	Other meat N = 41	Herbs and seeds N = 23
Ampicillin	27.3	31.3	19.5	0.0
Cefotaxime	7.3	0.0	9.8	0.0
Ceftazidime	7.3	0.0	9.8	0.0
Gentamicin	5.5	0.0	0.0	0.0
Tetracycline	45.5	31.3	19.5	8.7
Sulfamethoxazole	52.7	31.3	22.0	13.0
Trimethoprim	41.8	6.3	14.6	0.0
Ciprofloxacin	61.8	0.0	19.5	8.7
Nalidixic acid	60.0	0.0	19.5	8.7
Chloramphenicol	1.8	0.0	7.3	0.0
Azithromycin	0.0	0.0	2.4	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.8	0.0	4.9	0.0

Figure S04 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meat in the Netherlands from 2001-2016.



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

3.1.2 Campylobacter

This chapter describes the 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 focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly at poultry (and poultry meat products). In addition to broilers, laying hens and ducks were included in the surveillance of 2016. In 2016 also *C. jejuni* isolates from milk goats and milk sheep were tested for resistance.

Table C01 presents the MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated at WBVR from caecal samples of broilers, laying hens and ducks in 2016. Resistance percentages of *C. jejuni* and *C. coli* isolated from different faecal and meat sources are shown in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and broiler 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 2006 onwards in Table C03.

Highlights

1. As a result of prioritization and changes in legislation, since 2014 the focus of the surveillance of antimicrobial resistance in *Campylobacter* is mainly in isolates from poultry (including broilers, laying hens and ducks) and poultry meat.
2. Resistance rates in *C. jejuni* from broilers was somewhat lower, whereas rates in poultry meat did not substantially change in 2016, compared to 2015.
3. Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates.
4. Resistance rates for quinolones in *C. coli* isolates from broilers, laying hens and poultry meat decreased since 2015.
5. Levels of resistance of *C. jejuni* for tetracycline and the quinolones were substantially higher in broilers than in ducks and laying hens.
6. In *C. jejuni* from milk sheep and milk goats, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels than in poultry.
7. Ciprofloxacin resistance in *Campylobacter* isolates from human patients is still high (with a slight decrease in 2016), 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 levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Resistance levels

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. The remaining six antimicrobials ciprofloxacin and nalidixic acid (quinolones), gentamicin and streptomycin (aminoglycosides), erythromycin (macrolides) and tetracycline (tetracyclines), represent antimicrobial classes, which are all important in human medicine for treatment of campylobacteriosis.

In 2016, the highest resistance levels of *C. jejuni* and *C. coli* in broilers, laying hens and ducks were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table CO1). Table Co2 shows that resistance percentages for the antimicrobials were at high levels for broilers, ducks, poultry and turkey meat (both *C. jejuni* and *C. coli*), a bit lower for isolates from laying hens (only for C. *jejuni*) , and at the lowest levels (around 10%) for *C. jejuni* isolates from faecal samples of milk goats and milk sheep. Resistance in *C. jejuni* from broilers and poultry meat seems to have stabilized to very low levels for erythromycin, streptomycin and gentamicin: resistance to erythromycin and gentamicin could not be detected in broilers, as was the case for gentamicin and streptomycin in poultry meat. Resistance to tetracycline showed a slight decrease since 2013, although in 2016 the resistance percentage in poultry meat was a bit higher than in 2015. However, the resistance percentage of *C. jejuni* to tetracycline was still high (46.5% in broilers and 42.3% in poultry meat). Resistance to ciprofloxacin showed more fluctuation over the years and was over 60% since 2014 (Figure Co1).

More fluctuation over the years was observed in *C. coli* from broilers and poultry meat than in C. *jejuni*, probably due to the relatively low number of isolates in the survey (Figure Co2). However, resistance in *C. coli* from broilers stabilized to low levels for erythromycin, streptomycin and gentamicin, and was not detected in poultry meat samples in 2016. Resistance percentages for ciprofloxacin in broilers have been fluctuating a lot since 2001, with 65.1% resistant isolates in 2016. Resistance percentages for ciprofloxacin in poultry meat showed a substantial decrease in 2016 to 56.5%, after having been between 78% and 83% since 2010. However, because of the low number of *C. coli* isolates tested in 2016 (N = 23) these results should be interpreted with care. Resistance levels to tetracycline in broilers and poultry meat seem to follow the same trend as ciprofloxacin resistance, at approximately equal percentages (Figure Co2).

Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates (Table Co1 and Co2). Table Co2 shows that resistance against gentamicin was not detected in any of the *C. coli* isolates and in the *C. jejuni* isolates only in 4.8% of the turkey meat samples. Resistance against streptomycin and erythromycin was also at low levels, except for the streptomycin resistance percentage in *C. jejuni* isolates from turkey meat (14.3%) and the *C. coli* isolates from laying hens and ducks (6.9% and 6.3% respectively).

Table C01 MIC distribution (in %) for *Campylobacter jejuni* (N = 309) and *C. coli* (N = 146) isolated from caecal samples of broilers, layers and ducks in 2016.

C. *jejuni*	MIC (%) distribution mg/L												R%	95% CI
(N = 309)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	42.1	5.5	1.0	0.0	0.0	0.3	17.8	19.7	13.6				51.5	45.7 - 57.1
Nalidixic acid				0.0	10.0	36.6	4.5	0.0	0.0	0.0	48.9		48.9	43.1 - 54.5
Erythromycin				86.4	12.6	1.0	0.0	0.0	0.0	0.0	0.0		0.0	0 - 0
Gentamicin	76.4	23.6	0.0	0.0	0.0	0.0	0.0	0.0					0.0	0 - 0
Streptomycin		10.0	61.5	27.5	0.0	0.0	0.3	0.3	0.3				1.0	0 - 2
Tetracycline			58.6	2.6	0.0	0.0	0.0	1.0	3.6	4.9	29.4		38.8	33.2 - 44.3

C. *coli*	MIC (%) distribution mg/L												R%	95% CI
(N = 146)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	34.2	5.5	0.0	0.0	0.0	8.9	36.3	13.7	1.4				60.3	52.1 - 68.3
Nalidixic acid				0.0	3.4	25.3	11.0	0.0	0.0	6.2	54.1		60.3	52.1 - 68.3
Erythromycin				76.0	19.2	1.4	0.0	0.0	0.0	1.4	0.0		3.4	0.4 - 6.4
Gentamicin	18.5	77.4	4.1	0.0	0.0	0.0	0.0	0.0					0.0	0 - 0
Streptomycin		0.0	13.7	78.8	2.1	0.0	0.7	3.4	1.4				5.5	1.7 - 9.2
Tetracycline			37.0	7.5	0.0	0.0	0.0	0.0	0.7	2.7	52.1		55.5	47.2 - 63.7

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 EUCAST clinical breakpoints.

For tetracycline (only C. coli), ciprofloxacin and erythromycin the ECOFF and clinical breakpoint are identical.

Table C02 Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of broilers, layers, ducks, milk goats and sheep and from meat samples of poultry and turkey in 2016

	C. jenuni								*C. coli*			
	Broilers	Layers	Ducks	Poultry meat	Turkey meat	Milk	goats	Milk sheep	Broilers	Layers	Ducks	Poultry meat
N	170	71	68	52	21	382	43	17	43	87	16	23
Ciprofloxacin	60.6	32.4	48.5	65.4	38.1		9.3	11.8	65.1	56.3	68.8	56.5
Nalidixic acid	59.4	29.6	42.6	65.4	38.1		9.3	11.8	65.1	56.3	68.8	56.5
Erythromycin	0.0	0.0	0.0	3.8	0.0		0.0	5.9	4.7	3.4	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0	4.8		0.0	0.0	0.0	0.0	0.0	0.0
Streptomycin	1.2	1.4	0.0	0.0	14.3		2.3	0.0	2.3	6.9	6.3	0.0
Tetracycline	46.5	25.4	33.8	42.3	38.1		9.3	5.9	65.1	52.9	43.8	52.2

Quinolones

The increasing trend in resistance to the quinolones of *Campylobacter* spp. isolates from animal origin (Figures Co1 and Co2) as well as from human patients (Figure Co3) is a public health concern. After a period of decreasing ciprofloxacin resistance in *C. jejuni* isolates from broilers (52.2% in 2013), resistance increased to 64.3% in 2014 and 69.6% in 2015. In 2016 a slight decrease to 60.6% was seen. The resistance level of *C. jejuni* from poultry meat is comparably high and also showed an increase to 63.4% in 2014 and 66.0% in 2015, and stabilized at 65.4% in 2016. Ciprofloxacin resistance rates in *C. jejuni* isolates from laying hens were relatively high, but showed a slight decrease from 36.4% in 2015 to 32.4% in 2016. The resistance levels for ducks, goats, sheep and turkey meat cannot be compared to former years, because these samples were collected in 2016 for the first time.

High levels of ciprofloxacin resistance were also observed in *C. coli* isolates from broilers with 51.3% in 2014 and 71.4% in 2015, but like the *C. jejuni* isolates a slight decrease in 2016 to 65.1%. The quinolone resistance for *C. coli* isolates from poultry meat showed a substantial decrease from 78.0% in 2015 to 56.5% for ciprofloxacin and from 84.0% in 2015 to 56.5% in 2016 for nalidixic acid. Also ciprofloxacin resistance in laying hens decreased from 69.3% in 2015 to 56.3% in 2016. The resistance levels for fluoroquinolone in human campylobacter isolates were also high (58.4%), but were also decreased compared to 2014 (60.7%) and 2015 (61.4%).

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

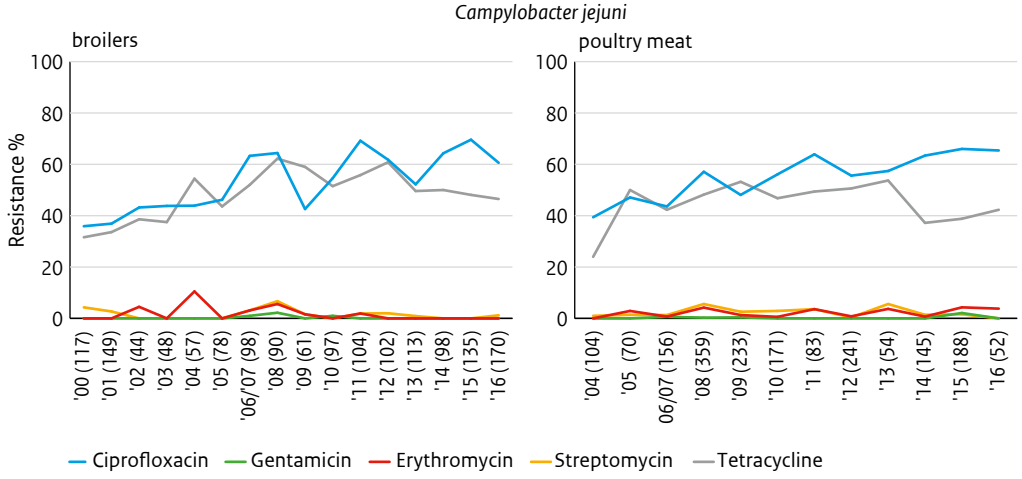


Figure C02 Trends in resistance (%) of *Campylobacter coli* isolated from broilers and poultry meat in the Netherlands.

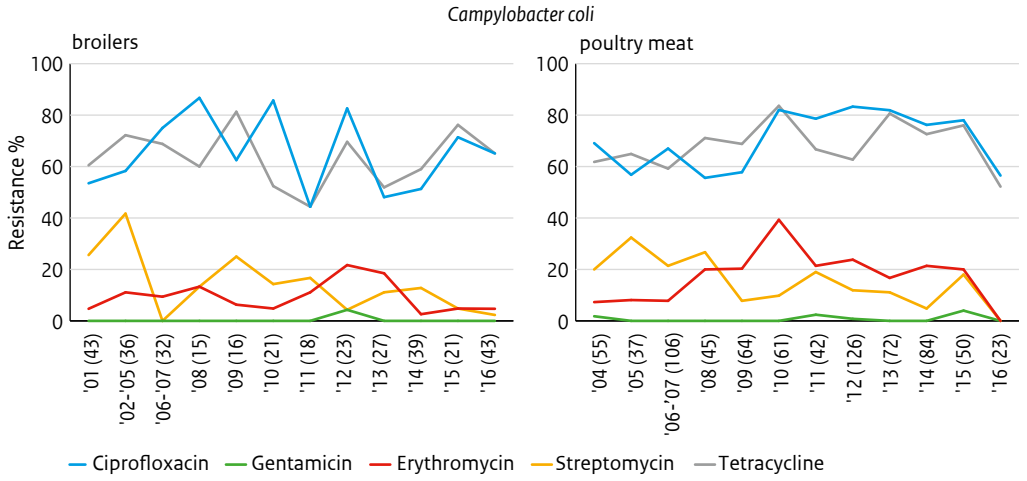


Table C03 Domestically acquired and travel related resistance in C. *jejuni* and C. *coli* isolated from humans from 2006 - 2016 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2006-2011							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	15261	49.9	1127	48.8	786	65.0	83	59.0
Tetracycline	9612	20.2	795	30.6	257	30.0	42	23.8
Erythromycin	12606	2.2	968	6.7	596	4.0	66	12.1

	2012-2016							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	14179	58.4	986	62.9	846	73.8	106	70.8
Tetracycline	8310	39.2	569	57.1	393	59.0	52	61.5
Erythromycin	12286	2.0	800	14.0	736	3.8	95	25.3

	Campylobacter spp. (R%)					
	2016	2015	2014	2013	2012	2006/11
Fluoroquinolone	58.4	61.4	60.7	57.6	59.4	49.3
Tetracycline	42.3	42.2	44.3	38.5	35.4	21.2
Erythromycin	2.7	2.9	3.4	3.2	3.0	2.6

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. The level of resistance to macrolides reported in animals and humans is low for C. *jejuni*, on average 1.3% of strains from broilers, layers, turkey, poultry meat and turkey meat in 2016 and 2.0% of human isolates from 2012-2016 were classified resistant. It should be noted that for human isolates more sensitive 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*).

In 2016, like in former years, erythromycin resistance was low in *C. jejuni* isolates, with no resistance in broilers, laying hens, ducks, milk goats and turkey meat, and 3.8% in poultry meat and 5.9% in milk sheep (Table Co2). Erythromycin resistance in *C. coli* was also low in broilers (4.7%) and laying hens (3.4%), and could not be detected in ducks and poultry meat, which is remarkable, because the resistance percentage of poultry meat isolates in 2015 was 20.0%. Again, this difference could be the effect of the inclusion of imported meat products.

Broiler chickens, laying hens, ducks, poultry meat and turkey meat

In *Campylobacter* spp from poultry, resistance profiles were determined for isolates recovered from animals (broilers, laying hens, ducks) as well as from chicken and turkey meat samples. In laying hens, the antibiotic use is on average considerably less than in broilers.

As shown in Table Co2, levels of resistance of *C. jejuni* for tetracycline and the quinolones were substantially higher in broilers than in laying hens. Resistance levels of *C. jejuni* isolates from ducks for these antimicrobials was lower than in broilers, but higher than in laying hens. However, resistance rates for the quinolones of *C. coli* isolates from broilers, laying hens and ducks were comparable, and reasonably high. The resistance rate for tetracycline in *C. coli* isolates was highest for broilers (65.1%), and somewhat lower for laying hens (52.9%) and ducks (43.8%).

Resistance rates for tetracycline and the quinolones in *C. jejuni* isolates from poultry meat were at the same level as for the isolates from broilers. The resistance percentages for the *C. coli* isolates from broilers were a little higher than for the isolates from meat. Resistance rates for *C. jejuni* isolates for erythromycin, gentamicin and streptomycin were at low levels, except for streptomycin resistance in turkey meat isolates (14.3%). Resistance in *C. coli* isolates streptomycin was 6.9% in laying hens and 6.3% in ducks.

In general, higher resistance rates were observed for most antimicrobials in *C. coli* from broilers, laying hens and ducks compared to *C. jejuni* from the same animals. The difference in resistance of *Campylobacter* spp. isolates from animals and meat products may be due to the inclusion of foreign poultry products in the survey.

Milk sheep and milk goats

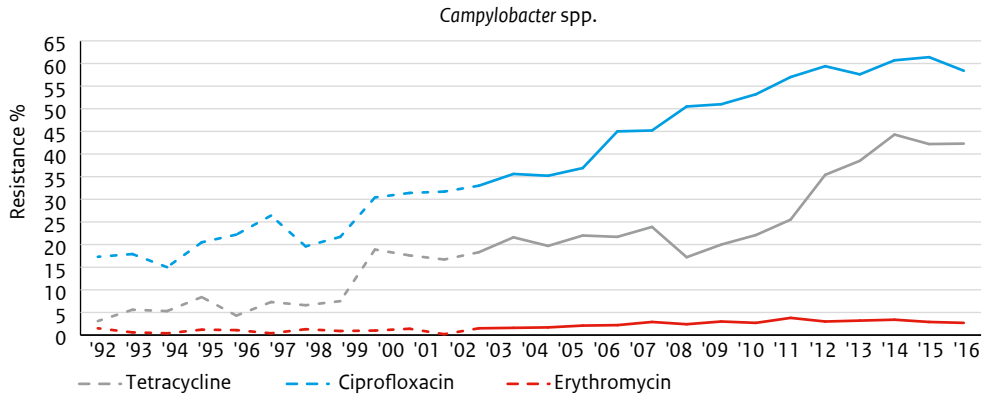
In 2016 for the first time *C. jejuni* isolates from milk goats and milk sheep were tested for antimicrobial resistance. Like in the other animal species, resistance percentages were highest for ciprofloxacin, nalidixic acid and tetracycline, but at much lower levels (9.3% for both quinolones in goats and 11.8% for both quinolones in sheep, for tetracycline 9.3% in goats and 5.9% in sheep) (Table Co2). Although no information is available on the usage of antimicrobials in goats and sheep the low resistance rates probably reflect relatively low use in these animals.

Campylobacter in humans

Data on resistance levels are available for ciprofloxacin, erythromycin and tetracycline and are summarized in Table C03 and Figure C03. The trends as shown in Figure C03 indicate a continuously increasing trend of ciprofloxacin and tetracycline resistance in *Campylobacter* spp. isolated from human patients, with a slight decrease for tetracycline since 2015 and for ciprofloxacin since 2016. 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. After a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim since 2009 in STEC O157 isolates from humans, in 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%).
2. Resistance for the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates.

Shiga-toxin producing *E. coli* O157 (STEC O157) isolates from humans were tested for susceptibility. MIC results for all *E. coli* O157 isolates from humans are presented in Table STEC01 and the trends over time in Figure STEC01. In 2016, no *E. coli* non-O157 isolates were tested from animals or beef products.

Human STEC O157 isolates

Resistance rates of human isolates showed a tendency to increase for ampicillin, tetracycline, sulfamethoxazole and trimethoprim since approximately 2009 (Figure STEC01). In 2016, a decrease was found for ampicillin (from 14.3% to 10.7%), sulfamethoxazole (from 15,6% to 14.7%) and trimethoprim (from 14.3% to 8.0%). After finding low resistance levels for quinolones in 2013 (4.2%) and 2014 (2.4%), resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016. As in former six years, no ESBL-producing isolates were detected.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2016.

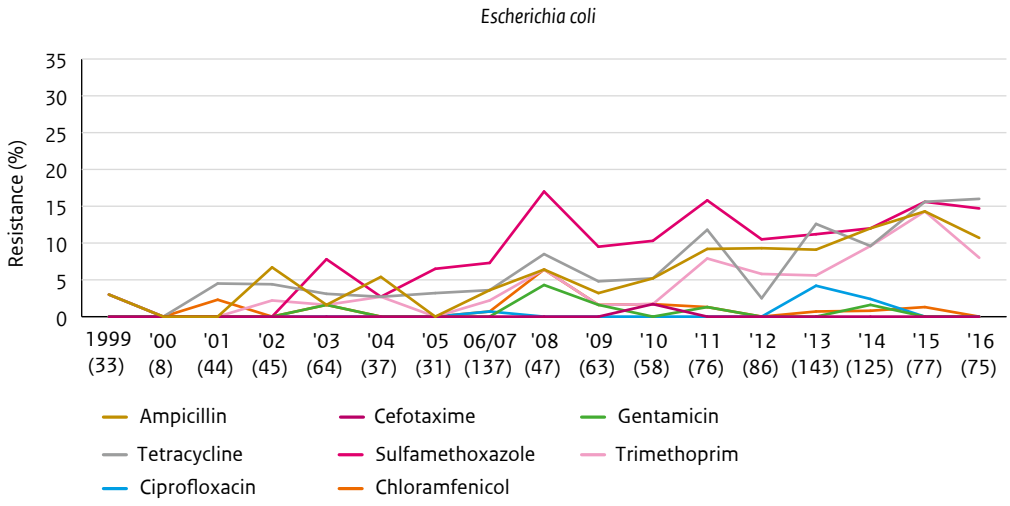


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=75) isolated from humans the Netherlands in 2016.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 77	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								2.7	86.7					10.7					10.7	3.5 - 17.7
Cefotaxime					100														0.0	0 - 0
Ceftazidime						100													0.0	0 - 0
Gentamicin						81.3	16.0	2.7											0.0	0 - 0
Tetracycline								41.3	42.7				1.3	14.7					16.0	7.5 - 24.4
Sulfamethoxazole										85.3								14.7	14.7	6.4 - 22.8
Trimethoprim					92.0								8.0						8.0	1.7 - 14.2
Ciprofloxacin	72.0	28.0																	0.0	0 - 0
Nalidixic acid									100.0										0.0	0 - 0
Chloramphenicol										93.3	6.7				0.0				0.0	0 - 0
Azithromycin*								6.7	70.7	22.7									0.0	0 - 0
Colistin							100.0	0.0											0.0	0 - 0
Meropenem		100																	0.0	0 - 0
Tigecycline					100														0.0	0 - 0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints..

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 thereof. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. For this purpose, *E. coli* and *Enterococcus* species (*E. faecium* and *E. faecalis*) are included as indicator organisms for the Gram-negative and the Gram-positive flora, respectively. As a result of less priority for including enterococci in the surveillance, no enterococci from faecal samples were tested in 2016, but *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples.

Isolation of bacteria from the intestine of randomly picked food-producing animals at slaughter aims to detect the development of resistance at the bacterial population level in food animals as prescribed by EFSA [1]. Since 1998 this monitoring is conducted in slaughter pigs and broilers. From 2005 onwards, resistance in isolates from both dairy cattle, veal calves and meat samples have been included. In the years 2010 and 2011 samples of individual dairy cattle were taken 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 of veal calves were collected at slaughterhouses and resistance levels were reported separately for white veal calves 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 taken 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 animal in the Netherlands.
The EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in 2014 and 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 2016

1. In 2016, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and pigs and stabilized in veal calves and dairy cattle.
2. In isolates from chicken meat resistance levels were substantially lower than in isolates from turkey meat. The levels of resistance were similar to 2015 in both types of poultry meat.
3. Resistance levels for almost all tested antibiotics were higher in samples of imported chicken and turkey meat than in samples from retail.
4. Resistance to third-generation cephalosporins was low (< 1%) in all tested animal species.
5. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from broilers and to a lesser extent white veal calves, but substantially decreased, in *E. coli* from white veal calves.
6. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulphonamides and trimethoprim were still reasonably high in broilers, turkey, pigs and veal calves.
7. Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.

Resistance levels

Resistance levels of a total of 1492 *E. coli* isolates obtained from broilers, pigs, dairy cows, veal calves, laying hens and ducks are presented as MIC-distributions in Table Eco01 and as resistance percentages per animal species in Table Eco02. Trends in resistance levels from 1998 to 2016 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.
Resistance percentages of 321 *E. coli* isolates collected from raw chicken and turkey meat products are presented in Table Eco03. Trends in resistance of *E. coli* in the Netherlands from 2002 to 2016 isolated from raw meat products of poultry and turkey are presented in Figure Eco03.

For most drugs or drug classes there were notable variations in resistance levels between the different animal species (Table Eco02). Highest levels were present in broilers, slaughter pigs and white veal calves, lower levels for rosé veal calves, laying hens and ducks, and hardly any resistance was observed in isolates from dairy cattle. In general, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These include the most frequently used drug classes in veterinary medicine in The Netherlands.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1492) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2016.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1492	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	30.1	45.6	2.5		0.1		20.0					20.2	18 - 22.2
Cefotaxime					99.7	0.1			0.1	0.2									0.3	0 - 0.6
Ceftazidime						99.7	0.1	0.1		0.1									0.3	0 - 0.6
Gentamicin						68.3	27.3	2.8	0.1	0.2	0.5	0.5	0.2						1.5	0.9 - 2.1
Tetracycline								64.1	10.7	0.1	0.5	0.3	7.9	16.6					25.2	22.9 - 27.4
Sulfamethoxazole										77.8	0.1	0.1	0.2		0.2	0.1		21.4	21.8	19.6 - 23.9
Trimethoprim					33.6	45.0	2.7						18.7						18.7	16.6 - 20.7
Ciprofloxacin	78.6	11.6	0.1	0.5	6.0	1.9	0.9			0.2	0.1								9.6	8 - 11.1
Nalidixic acid									89.3	1.4	0.3	0.1	1.2	4.0	3.8				9.0	7.5 - 10.4
Chloramphenicol										88.3	4.6	1.1	0.9	1.6	3.6				7.1	5.7 - 8.4
Azithromycin*								5.5	47.4	44.6	2.2	0.1	0.1	0.2					0.0	0 - 0.6
Colistin							99.9	0.1											0.0	0 - 0
Meropenem		99.9	0.1																0.0	0 - 0
Tigecycline					78.5	19.7	1.8												0.0	0 - 0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (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, rosé veal calves, layers and ducks in the Netherlands in 2016.

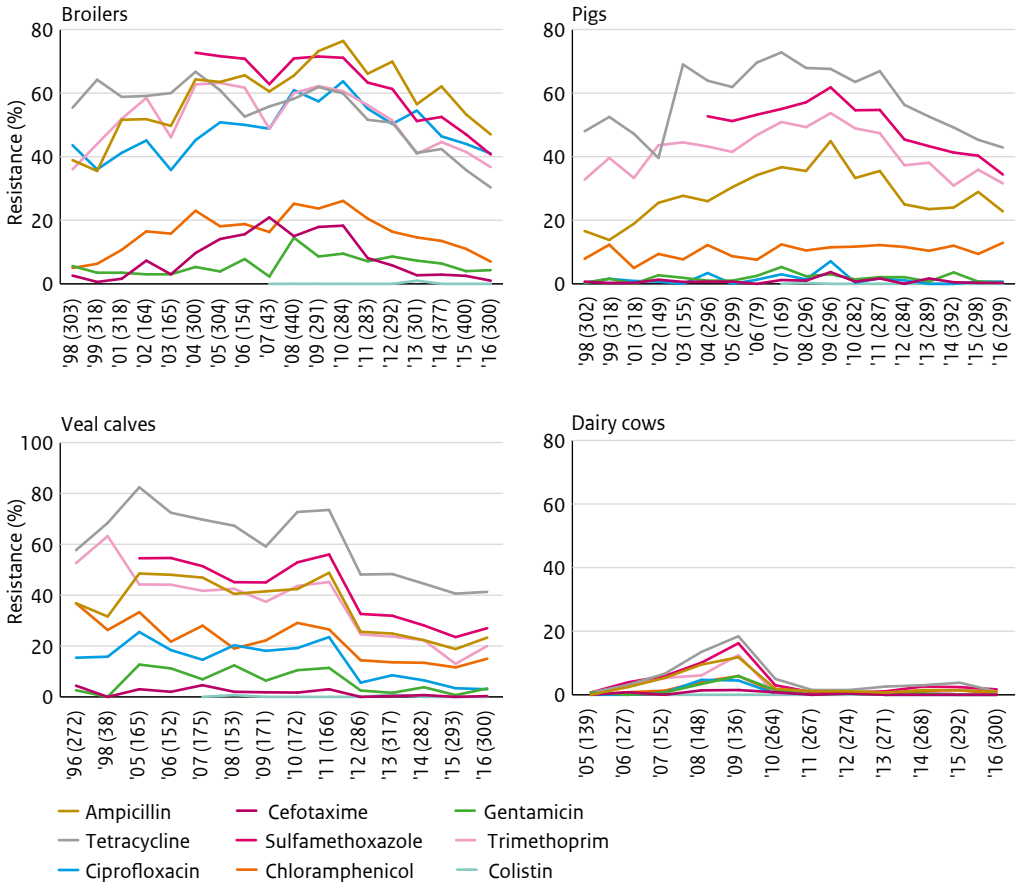
Faecal samples	Broilers	Pigs	Dairy cows	Veal calves		Layers	Ducks
	N = 300	N = 299	N = 300	White, N = 181	Rosé, N = 119	N=193	N=100
Ampicillin	47.0	23.1	1.0	31.5	10.9	5.2	8.0
Cefotaxime	1.0	0.3	0.0	0.0	0.8	0.0	0.0
Ceftazidime	1.0	0.3	0.0	0.0	0.8	0.0	0.0
Gentamicin	4.3	0.0	0.0	5.0	0.8	0.0	0.0
Tetracycline	30.3	42.8	1.0	56.4	18.5	8.8	13.0
Sulfamethoxazole	40.7	34.4	1.7	36.5	12.6	2.6	9.0
Trimethoprim	36.7	31.8	1.0	28.2	7.6	2.1	7.0
Ciprofloxacin	41.0	0.7	0.0	6.1	0.0	3.1	3.0
Nalidixic acid	39.3	0.7	0.0	4.4	0.0	3.1	0.0
Chloramphenicol	7.0	12.7	0.3	22.7	3.4	0.5	0.0
Azithromycin	0.0	0.3	0.0	2.2	0.0	0.0	0.0
Colistin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Quinolones

The highest resistance levels to quinolones were found in *E. coli* from broilers: 41.0 % resistance to ciprofloxacin and 39.3% resistance to nalidixic acid. Although resistance rates to quinolones were still high, these rates show a tendency to decrease in comparison with previous years (in 2013 for both drugs 54%; in 2014, 46 and 45%; and in 2015, 44 and 42% respectively). In 2016, high level resistance (MIC >1 mg/L) to ciprofloxacin in broilers was detected in 1.0% (3/300) of the isolates, which is a bit lower than in former years. Resistance to ciprofloxacin in 2016 was 6.1% in *E. coli* isolates from white veal calves, 3.1% in laying hens, 3.0% in ducks, remained low in slaughter pig isolates and was undetectable in isolates from rosé veal calves and dairy cows.

Resistance to quinolones in *E. coli* from meat was tested for chicken and turkey meat samples. In 2016 not only retail samples from The Netherlands (partially also from Germany or Belgium) were collected but also samples from imported meat (outside EU). Resistance levels were high to very high in chicken and turkey imported meat products. Resistance in chicken products at retail was a bit lower than in 2015: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 26.1% and 25.0%, respectively. The resistance percentages of *E. coli* in meat products were somewhat higher for ciprofloxacin than for nalidixic acid. This is probably due to the increase of plasmid mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid.

Figure Eco01 Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2016.

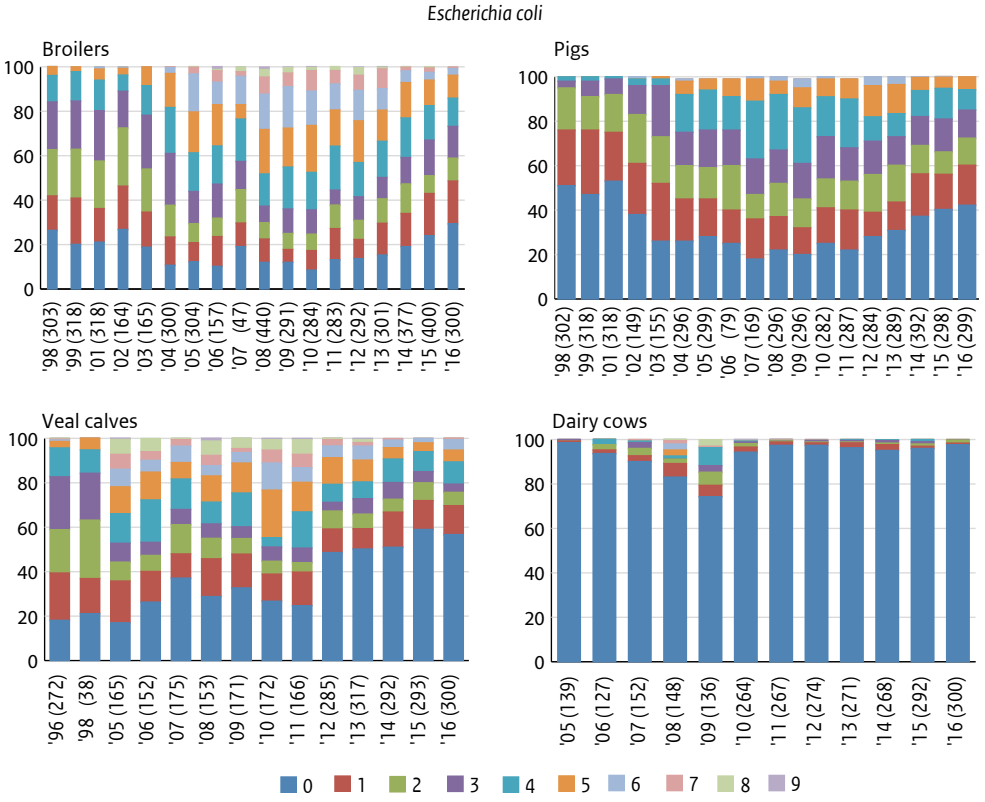


Cefotaxime resistance

Resistance to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL/AmpC producing *E. coli*, was detected in broilers, pigs and rosé veal calves. Cefotaxime resistance was not detected in dairy cows, white veal calves, laying hens and ducks. Resistance levels in *E. coli* were 1.0% in broilers, 0.3% in pigs and 0.8% in rosé veal calves for both, cefotaxime and ceftazidime. The 1.0% cefotaxime resistance in broilers was a further decrease in occurrence compared to 2013, 2014 and 2015 (2.7%, 2.9%, and 2.5% respectively) (Figure Eco01).

Resistance percentages to third generation cephalosporins in chicken and turkey meat samples were much higher for the imported samples, compared to the retail samples (Table Eco03). Resistance to cefotaxime in commensal *E. coli* obtained from all chicken meat samples (from import and retail) showed a slight increase, compared to 2015 (from 4.3% to 7.2%) (Figure Eco03). These figures are strongly

Figure Eco02 Resistance (%) to 0-9 antimicrobial classes among *E. coli* strains from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2016.



influenced by the high cefotaxime resistance of *E. coli* from imported meat (Table Eco03). Resistance to cefotaxime in all turkey meat samples decreased from 2.5% in 2015 to 1.4% in 2016. The reduction in cefotaxime resistance, determined in randomly selected *E. coli* isolates cultured on non-selective media, suggests that the concentration of *E. coli* resistant to Extended Spectrum Cephalosporins (ESC) on meat decreased. This is strengthened by the fact that the prevalence of cefotaxime resistant *E. coli* in fresh chicken meat samples using selective media decreased from 67% in 2014 to 39% in 2015 and 26.4% in 2016 (see chapter 4). The mentioned decrease of cefotaxime resistance in randomly selected *E. coli* from poultry meat is an important finding because it suggests that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is reduced. In contrast, selective culturing revealed a clear and unexplained increase in the prevalence of ESBL/AmpC-producing *E. coli* in faecal samples of veal calves. The prevalence in pigs and dairy cattle also showed an increasing tendency. In broilers and layers a decrease was ESBL/AmpC-producing *E. coli* was observed (see chapter 4).

Broiler chickens

Commensal *E. coli* isolated from caecal samples from broiler chickens showed resistance to all commonly tested antimicrobials (Table Eco02). Overall, resistance levels were lower than in 2015, but level of resistance to ampicillin (47.0), tetracycline (30.3%), sulfamethoxazole (40.7%), trimethoprim (36.7%) and the quinolones ciprofloxacin (41.0%) and nalidixic acid (39.30%) remained high. Cefotaxime resistance decreased from 2.5% in 2015 to 1.0% in 2016.

Slaughter pigs

Resistance against tetracycline, sulfamethoxazole, trimethoprim and ampicillin remained high in 2016 in *E. coli* isolates from pigs and was 42.8%, 34.4%, 31.8% and 23.1%, respectively. Resistance levels of these four antibiotics showed an ongoing tendency to decrease since 2011. In 2015 a slight increase was shown for ampicillin and trimethoprim, but in 2016 resistance levels of these antibiotics were again decreased (Figure Eco01). Resistance to the 3rd generation cephalosporins was the same as in 2015 (0.3%), indicating that ESBLs are present, but in low concentrations.

Veal calves

Resistance data on white and rosé veal are reported separately. 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. In both production systems most antibiotics are administered during the starting period. On average, in white veal calves, more antibiotics are used than in rosé calves. This results in a clear difference in resistance levels between the two husbandry types. As seen in former years, a much higher resistance level was recorded for white than for 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 also the year in which the sampling strategy changed (see the description at the beginning of chapter 3.2). The changed strategy from sampling at farm to sampling at slaughterhouse might have influenced the results from 2012 and onwards. In 2016, the ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40%. This ratio better reflects the proportions of slaughtered calves in The Netherlands in 2016. This explains part, but not all of the slight increase in resistant rates of *E. coli* in veal calves in 2016 compared to 2015. In 2016, resistance against the 3rd generation cephalosporins in *E. coli* isolates from white veal calves was under the detection level (TableEco02).

Dairy cattle

Resistance in *E. coli* isolated from dairy cattle is very low compared to resistance levels observed in pigs, broilers and veal calves, reflecting the low use of antibiotics in this husbandry system. Resistance rates decreased compared to 2015, and overall rates remained below 2%. No resistance to 3rd generation cephalosporins was detected.

Laying hens

In laying hens resistance percentages of *E. coli* were substantially lower than in broilers, for all antibiotics. This is most likely a result of the difference in antimicrobial usage between the two farm types. The highest resistance percentage was observed for tetracycline (8.8%). *E. coli* isolates from laying hens were not tested in 2015, but compared to 2014 resistance percentages were substantially decreased (ampicilline from 13.7% to 5.2%, tetracycline from 14.2% to 8.8%, sulfamethoxazole from 5.8% to 2.6% and trimethoprim from 5.8% to 2.1%).

Ducks

There are no historical data available on antibiotic usage in ducks. Table Eco02 shows that in *E. coli* isolated from ducks resistance was observed for the same antimicrobials as in laying hens, but resistance percentages were a bit higher. Highest resistance percentages were measured for tetracycline, sulfamethoxazole, ampicilline and trimethoprim (13.0%, 9.0%. 8.0% and 7.0% respectively).

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. The data with the determined level of multidrug resistance over the years are shown in Figure Eco02.

The data from 2016 indicate a decreasing trend in the level of multidrug resistance in broilers and pigs, but a slight increase in veal calves. The increase in calves might have been caused by the changed ratio in samples from white and rosé calves (see before). However, levels of multidrug resistance (resistant to three or more classes of antibiotics) remained still quite high among *E. coli* originating from broilers (41.0%), pigs (27.6%) and veal calves (24.3%). In dairy cattle multidrug resistance in *E. coli* again was rare with only 0.3% (1 out of 300) of the isolates showing resistance to three or more classes of antimicrobials.

The overall increasing tendency of the number of completely susceptible *E. coli* isolates, especially in broilers and pigs (Figure Eco02), is ongoing and might be the best indicator to reflect the long term effect of the more prudent use of antibiotics on the level of multidrug resistance in the intestinal flora.

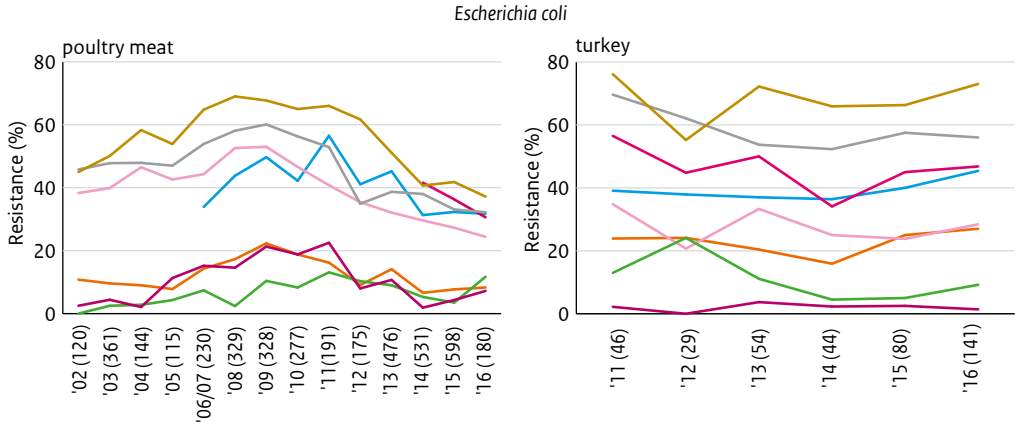
3.2.2 *E. coli* in raw meat products of food-animals

Resistance percentages of *E. coli* isolated from raw meat products from chicken and turkey, sampled at retail and from import products by the Dutch Food and Consumer Product Safety Authority (NVWA), are shown in Table Eco03. The trends in resistance are presented in Fig Eco03. The resistance rates in trends in resistance of isolates from chicken and turkey meat are for the first time given separately for import products and products in retail (which can include meat produced in The Netherlands, but also other EU countries). After a tendency to decrease from 2010 to 2014, resistance rates in chicken meat products seem to have stabilized or even increased in 2015 and 2016. In turkey meat, resistance rates have been at a constant high level since 2011. Cefotaxime resistance in *E. coli* isolates from chicken products showed, after a rapid decrease from 10.7% in 2013 to 1.9% in 2014, a slight increase to 4.3% in 2015, and a further increase to 7.2% in 2016. Fluctuations in the resistance rates might be caused by year-to-year differences in the proportion of foreign chicken and turkey products included in the survey.

Table Eco03 Resistance (in %) of *E. coli* isolated from raw chicken and turkey meat products in the Netherlands in 2016.

Meat products	Chicken import	Chicken retail	Turkey import	Turkey retail
	N = 46	N = 134	N = 9	N = 132
Ampicillin	63.0	28.4	55.6	74.2
Cefotaxime	21.7	2.2	11.1	1.5
Ceftazidime	19.6	1.5	11.1	1.5
Gentamicin	39.1	2.2	11.1	9.1
Tetracycline	50.0	26.1	66.7	55.3
Sulfamethoxazole	58.7	20.9	55.6	46.2
Trimethoprim	45.7	17.2	33.3	28.0
Ciprofloxacin	58.7	22.4	88.9	42.4
Nalidixic acid	43.5	20.9	55.6	28.0
Chloramphenicol	21.7	3.7	66.7	24.2
Azithromycin	6.5	0.0	0.0	4.5
Colistin	0.0	0.7	0.0	8.3
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from raw chicken and turkey meat products in the Netherlands from 1998-2016.



3.2.3 *Enterococcus faecalis* and *E. faecium*

In this chapter, information on resistance in *Enterococcus* species, as indicator organism for the occurrence and trends in resistance in Gram-positive bacteria from food-producing animals in the Netherlands, is presented. From 2013 onwards, as a result of less priority for including enterococci in the surveillance, poultry, pigs and cattle and meat thereof were sampled once every three years. From 2016, no enterococci from faecal samples were tested, but in 2016 *Enterococcus faecalis* and *E. faecium* were isolated from chicken and turkey meat samples. The poultry meat samples were taken at retail.

Highlights

1. In chicken meat, highest resistance levels were observed for erythromycin (55.4% for *E. faecalis* and 57.1% for *E. faecium*) and tetracycline (66.1% and 25.0% respectively). In addition, a high level of resistance was observed for quinu/dalfopristin in *E. faecium* (42.9%).
2. In turkey meat, highest resistance levels were observed for erythromycin (65.1% for *E. faecalis* and 58.8% for *E. faecium*) and tetracycline (88.9% and 76.5% respectively). A high resistance percentage was also observed for quinu/dalfopristin in *E. faecium* (58.8%).

Resistance levels

In 2016 resistance rates have been determined for 56 *E. faecalis* and 28 *E. faecium* strains isolated from chicken meat samples as well as for 63 *E. faecalis* and 17 *E. faecium* isolates from turkey meat samples. Resistance percentages for *E. faecalis* and *E. faecium* isolated from these products are presented in Table Ento1. Trends over the years in chicken meat are shown in Figure Ento1.

Chicken meat

High resistance levels in *E. faecalis* as well as in *E. faecium* were observed for erythromycin (55.4% and 57.1%) and tetracycline (66.1% and 25.0%), (Table Ento1). In *E. faecium*, a traditionally high level of resistance was observed for quinu/dalfopristin (42.9%), and low levels for ampicillin (7.1%) and daptomycin (14.3%) . Figure Ento1 shows a slight increase in resistance level for erythromycin since 2013 (no data for 2014 and 2015) in both enterococci species, and a decrease in resistance level for tetracycline. For daptomycin no data from earlier years were available for comparison. The resistance percentage for ciprofloxacin in *E. faecium* showed a substantial decrease since 2013 (from 23.6% to 7.1%).

Turkey meat

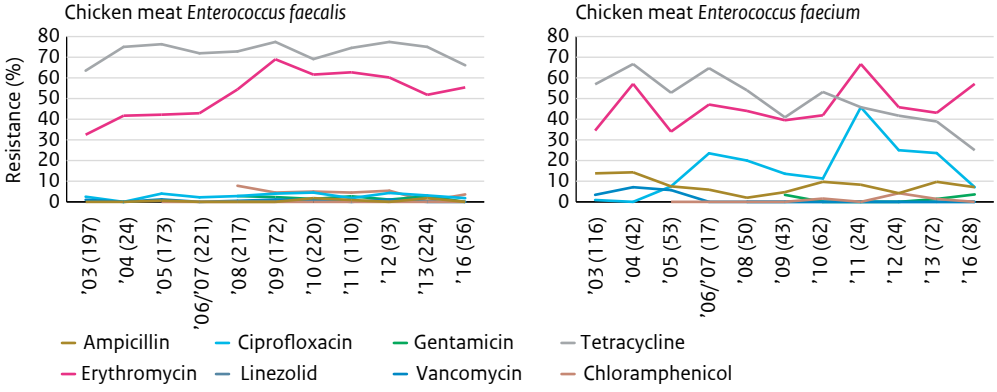
As in the chicken meat samples, also in the turkey meat samples high resistance levels were observed for erythromycin (65.1% and 58.8%) and tetracycline (88.9% and 76.5%), (Table Ento1). Also for these samples, a high resistance percentage was seen for quinu/dalfopristin in *E. faecium* (58.8%), and a lower level for ampicillin in *E. faecium* (17.6%). The data for *E. faecium* might be not representative, because of the low number of isolates (n=17).

Table Ent01 Resistance % of *Enterococcus faecalis* and *E. faecium* strains isolated from raw chicken and turkey meat in the Netherlands in 2016.

	Chicken meat		Turkey meat	
	E. faecalis (N = 56)	*E. faecium (N = 28)*	*E. faecalis (N = 63)*	*E. faecium (N = 17)*
Ampicillin	0.0	7.1	0.0	17.6
Chloramphenicol	3.6	0.0	7.9	0.0
Ciprofloxacin	1.8	7.1	4.8	0.0
Daptomycin	0.0	14.3	0.0	0.0
Erythromycin	55.4	57.1	65.1	58.8
Gentamicin	0.0	3.6	1.6	0.0
Linozelid	0.0	0.0	0.0	0.0
Quinu/dalfopristin*	-	42.9	-	58.8
Teicoplanin	0.0	0.0	0.0	0.0
Tetracycline	66.1	25.0	88.9	76.5
Tigecycline	0.0	0.0	0.0	0.0
Vancomycin	0.0	0.0	0.0	0.0

* *E. faecalis is intrinsic resistant to quinu/dalfopristin*

Figure Ent01 Trends in resistance percentages of *Enterococcus faecium* and *E. faecalis* isolated from veal calves in the Netherlands from 1998-2016.



4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2016

Highlights

1. ESBL/AmpC-producing E*scherichia coli* represented 0.3% of the randomly isolated *E. coli*, the lowest proportion observed since 2007.
2. In spite of the above, selective culturing in livestock faeces indicated that the prevalence (% of animal carriers) of ESBL/AmpC-producing *E. coli* marked a general tendency to increase in livestock, excluding broilers and layers. Currently an explanation for this phenomenon is lacking.
3. A follow up of the 2009 study on within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers showed a significant decrease from 66% in 2009 to 38% in 2016.
4. The proportion of fresh chicken meat with ESBL/AmpC-producing *E. coli* isolates decreased to 24% (67% in 2014, 39.4% in 2015). In imported chicken meat the proportion was much higher (61.2%).
5. The most prevalent ESBL/AmpC gene in *E. coli* from livestock and meat was $bla_{\text{CTX-M-1}}$ in almost all animal species followed by $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-14}}$.
6. The prevalence of ESBL/AmpC-producing *Salmonella* in 2016 was 1.7%, confirming the decreasing trend observed in the period 2013-2015. Most represented ESBL/AmpC genes were $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul, $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky, and $bla_{\text{CTX-M-9}}$ in S. Typhimurium.
7. The majority of ESBL-producing *Salmonella* isolates from humans were highly multidrug resistant, with most of the isolates showing a resistant phenotype to 5-8 antibiotics (67%).
8. No carbapenemase-producing *Enterobacteriaceae* were detected in in livestock and companion animals.
9. The colistin resistance gene *mcr-1* was present at low level in *E. coli* from livestock (0.5%) and in retail meat from turkeys (8.3%) and chicken (0.7%).

4.1 ESBL/AmpC-producing bacteria

4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2016

Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is routinely done by random isolation of a minimum of 170 isolated *E. coli*, each representing one epidemiological unit, from faecal samples of food-producing animals as prescribed by EFS*A* guidelines.[1] These isolates are tested for susceptibility to cefotaxime and ceftazidime. Proportions of resistant isolates are determined based on EUCAS*T* epidemiological cut-off values as described in Chapter 3. Since 1998, cefotaxime resistance was observed at low levels in all animal species. Figure ESBL01 shows the percentage of cefotaxime resistance in randomly picked *E. coli* isolated from non-selective media derived from broilers, slaughter pigs (1998-2016), veal calves and dairy cows (2005-2016). In broilers, after 2003 an apparent increase in

[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.

cefotaxime resistance was observed up to levels that varied between 15 – 20%, with the highest peak observed in 2007. The prevalence in broilers steadily declined to 2.7% in 2013, to reach a minimum of 1% in 2016. The strong decline observed in 2011, from 18.3% to 8.1%, was most likely the result of decreased usage of antibiotics in broilers since the spring of 2010 when the (off label) use of ceftiofur was ceased at Dutch hatcheries. In 2014, the decrease in usage stopped in broilers, which resulted in the levelling off observed in 2015 and the lowest registered prevalence so far in 2016.
From a total of 1492 randomly selected *E. coli* isolates that were tested in 2016, five displayed reduced susceptibility (MIC > 0.25 mg/L) to cefotaxime (see also 3.2.1). Three were isolated from broilers, one from a slaughter pig and one from a veal calf (Table ESBL01). In dairy cows no ESBL/AmpC-suspected *E. coli* isolates were found in 2016. Cefotaxime resistant isolates were screened for beta-lactamase gene families using PCR or the Check-Points CT101 miniaturised micro-array. Subsequently the genes were identified by dedicated PCR and sequence analysis. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutants in the chromosomal *ampC* genes. The results of this molecular typing are displayed in Table ESBL01.
In broiler isolates three plasmid meditated ESBL/AmpC genes were present: $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}52c}$, and $bla_{CMY\text{-}2}$. 2016 is the second year after 2015 in which $bla_{TEM\text{-}52c}$ was not found in cefotaxime resistant isolates from broilers derived from the monitoring program. $bla_{CTX\text{-}M\text{-}1}$ was also detected in a rosé veal calf isolate. Mutation in the chromosomal *ampC* gene was detected in one of the pig isolates (*ampC*-type 18). Variants of $bla_{CTX\text{-}M\text{-}14}$ (CTX-M-9 group) were not detected in 2016.
It can be concluded that by random isolation, only three plasmid mediated ESBL/AmpC genes were found in 1492 isolates in 2016 (0.3%), the lowest prevalence observed since 2007. This confirms the already promising results of 2015, when 0.9% ESBL/AmpC-producing isolates where detected, a major improvement compared to 2009 when ESBL/AmpC-producing isolates added up to 7.6%, before antibiotic usage reduction started in Dutch livestock.

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

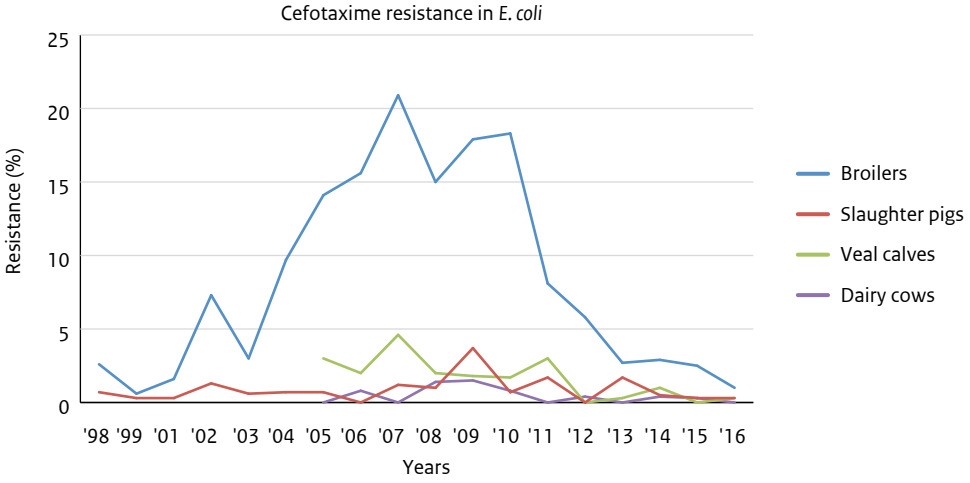


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2016.

Year	ESBLs isolated from					Total ESBL suspected (n)	ESBL-genes detected										Total *E.coli* (n)	% ESBL of total *E. coli*
	Broilers	Veal calves	Slaughter pigs	Dairy cows	Turkeys		CTX-M-1-group#	CTX-M-2	CTX-M-9-group!	TEM-52c	TEM-20	SHV-12*	SHV-2	CMY-2	chromosomal ampC	no gene found		
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
Total	266	27	31	8	6	338	133	19	3	38	2	42	9	44	20	28		

 # All were $bla_{CTX-M-1}$, only in 2011 one $bla_{CTX-M-3}$ gene was found in an isolate from veal calves.
Three combinations (all in broiler isolates) 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}.

** One combination of bla_{SHV-12} together with bla_{TEM-52} occured in 2012 in one broiler isolate.*

n.t. : not tested

Selective isolation of ESBLs in 2016

As of 2014 an active surveillance by selective culturing for ESBL/AmpC-producers in broilers was implemented together with the ongoing active surveillance in pigs and veal calves that started in 2011. Faecal samples taken for monitoring at slaughterhouse (slaughter pigs, white and rosé veal calves, broilers, layers, and ducks) and at farms (dairy cows) were also used for ESBL/AmpC-producing *E. coli* detection by selective methods. 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. This resulted in the screening of 1500 faecal samples (Table ESBL02).

In 2016, also 1395 meat samples (Table ESBL04) were analysed for ESBL/AmpC-producing *E. coli*. 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 *Enterobacteriaceae* morphology were selected for bacterial species identification, and confirmed *E. coli* were analysed for ESBL/AmpC-genes presence and screened for beta-lactamase gene families, as described above.

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

The prevalence of ESBL/AmpC-producing *E. coli* in faecal samples is shown in Table ESBL02. Suspected ESBL/AmpC isolates comprised all *E. coli* growing on MacConkey with 1 mg/L cefotaxime, including ESBL/AmpC negative isolates as well as isolates carrying mutations in the chromosomal *ampC* gene promoter. Confirmed ESBL isolates included only ESBL or AmpC gene-carrying isolates, most likely located on a horizontally transmissible plasmid. Each sample represented one slaughter batch of animals from one farm. Of the 1500 samples analysed for ESBL/AmpC-producing *E. coli*, 26.9% were positive, mainly due to the high prevalence in broilers (50.3%). A surprising increase in prevalence was observed in both white and rosé veal calves (33.9% and 28.7%, respectively) compared to 2015 (17.3% and 10%, respectively). As already noted in the past, ESBL/AmpC-producing *E. coli* levels in white veal calves were higher than in rosé veal calves. The slight reduction observed in ESBL/AmpC-producing *E. coli* in broilers in 2015 was confirmed also in 2016 (from 56.5% to 50.3%). Similar results were observed for layers, where ESBL/AmpC-producing *E. coli* prevalence dropped from 32.5% in 2014 to 28% in 2016. Prevalence in pigs was increased compared to 2015 (from 12.3% to 16.3%), as well as in dairy cows (from 9.3% to 13.2%). ESBL/AmpC-producing *E. coli* prevalence in ducks attested to 13%; the absence of previous data for this animal species does not allow for a comparison in time. In conclusion, 2016 marked a slight tendency to increase of ESBL/AmpC-producing *E. coli* carriership in livestock, excluding broilers and layers. An explanation for this phenomenon is not available yet.

ESBL/AmpC genes detected in animal faeces are reported in Table ESBL03. The increase in ESBL types variation observed in 2014 and 2015 compared to former years (MARAN 2011 and 2013) was confirmed in 2016, likely a consequence of the new surveillance method implemented in 2014, with a collection of faecal samples derived from a minimum of 150 to 400 different farms per animal species (MARAN 2014). Like in former years, $bla_{CTX\text{-}M\text{-}1}$ was the dominant ESBL-variant in all animal species examined (n=202 out of 367 genes), followed by $bla_{CMY\text{-}2}$ (n=52), $bla_{SHV\text{-}12}$ (n=31) and $bla_{TEM\text{-}52c}$ (n=30). Two $bla_{CTX\text{-}M\text{-}2}$ gene variants were reported in slaughter pig and white veal calf for the first time since 2014. The low variation in ESBL-types observed in broilers in the randomly isolated *E. coli* (Table ESBL01) mirrored the results of the selective culturing with 7 ESBL gene types compared to 2015. The increased ESBL/AmpC-producing *E. coli* prevalence observed in veal calves was associated with the highest gene variability (12 different ESBL genes). The more classical human associated genes $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$ were described in veal calves, conversely to 2015 where they were predominant in broilers. A similar phenomenon was observed in dairy cows, where increased prevalence in isolates matched an increase in ESBL-types with $bla_{CTX\text{-}M\text{-}1}$ being the predominant beta-lactamase gene. $bla_{CTX\text{-}M\text{-}55}$ was detected in dairy cows for the first time since gene typing was performed (MARAN 2011). Slaughter pig and layer hen isolates didn't show significant differences compared to previous years. Chromosomal *ampC* types confirmed their growing role in conferring cefotaxime resistance as already observed in 2015 with relatively high numbers in pig, layer, and dairy cow isolates (19%, 10%, and 13%, respectively). Conversely from previous years, no combination of ESBL gene types within the same isolate was detected.

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 2016.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	300	151	151	50.3
Layers	193	60	54	28.0
Ducks	100	13	13	13.0
Pigs	300	61	49	16.3
Veal calves				
white	183	64	62	33.9
rosé	122	35	35	28.7
Dairy cows	302	46	40	13.2
Total	1500	430	404	26.9

Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves and dairy cows in 2016. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Layers	Ducks	Slaughter pigs	Veal calves White	Veal calves Rose	Dairy cows	Total
CTX-M-1 group									
	CTX-M-1	66	33	4	35	31	14	19	202
	CTX-M-15	3				15	5	7	30
	CTX-M-32					1	4	5	10
	CTX-M-55	2	2			1	1	1	7
CTX-M-2 group									
	CTX-M-2				1	1		2	4
CTX-M-8/25 group									
	CTX-M-8				1				1
	CTX-M-9						1		1
CTX-M-9group									
	CTX-M-14		3		3	2	6	2	16
	CTX-M-27					1			1
	CTX-M-65			1		3	1	2	7
TEM									
	TEM-52c	19		1	5	3	1	1	30
	TEM-52cVar	6	3		2				12
	TEM-225		1						1
SHV									
	SHV-12	27				2	1	1	31
CMY									
	CMY-2	28	12	7	2	2	1		52
Chromosomal ampC									
	ampC-type-3		6		12	2		4	24
	ampC-type-3-like							2	
Total		235			56	28	15	33	367

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

Prevalence of ESBL suspected isolates in fresh raw meat are shown in Table ESBL04. Meat preparations (except for imported frozen poultry meat with approximately 1% salt) were not screened in 2016. Out of 1395 fresh and import meat samples, 133 were tested positive for ESBL/AmpC-producing *E. coli* (9.5%) and six samples were tested positive for *E. coli* with chromosomal mutations in the AmpC promotor region. The decreasing trend observed in imported poultry meat since 2012 (83%) and continued in the past two years (67% and 60% in 2014 and 2015, respectively) was confirmed in 2016, with a prevalence of 61.2% in imported chicken meat. Turkey meat showed a decrease in ESBL/AmpC-producing *E. coli* prevalence depending on the source, with 15% prevalence in fresh meat (22.5% in 2015) and 62.5% in imported meat (66.7% in 2015). While cattle and lamb meat showed ESBL/AmpC prevalence comparable to 2015 (between 2.0% and 2.7%) incidence in fresh calf meat was higher than 2014 and 2015 (from 0% to 4.4%). The first year of fresh goat meat sampling revealed a relatively high ESBL/AmpC-producing *E. coli* prevalence (7.7%).

All 139 isolates were selected for molecular typing and confirmed by MALDI-TOF as *E. coli*. Table ESBL05 shows the different ESBL/AmpC gene types detected in meat. Most of ESBL/AmpC genes found in beef and veal were also found in faecal samples of veal calves ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$, and $bla_{\text{CMY-2}}$) strongly suggesting faecal contamination during slaughter and/or meat processing. Chicken meat displayed more ESBL/AmpC gene variability than broiler faecal samples, with $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ not detected in the latter. Chromosomal *ampC* types were detected mainly in turkey meat isolates, together with a great variety of ESBL gene types. The dominant human $bla_{\text{CTX-M-15}}$ was not detected in chicken meat although it was detected in broiler faecal samples (Table ESBL03). Other frequent ESBL/AmpC gene types were $bla_{\text{CMY-2}}$ and $bla_{\text{SHV-12}}$ typically found in respective livestock, with an increase in $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ detection compared to 2015.

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat in the Netherlands in 2016.

Animal source		N screened	N ESBL/AmpC suspected	N ESBL/AmpC tested at WBVR	N ESBL/AmpC positive	% ESBL/AmpC positive
Beef						
	fresh meat	299	7	5	5	2.0
Veal						
	fresh meat	205	11	9	9	4.4
Pork						
	fresh meat	273	1	0	0	n.d.
Chicken						
	fresh meat	208	55	51	50	24.0
	import	49	32	30	30	61.2
Turkey						
	fresh meat	187	35	33	28	15.0
	import	8	5	5	5	62.5
Lamb						
	fresh meat	112	3	3	3	2.7
Sheep						
	fresh meat	28	1	1	1	3.6
Goat						
	fresh meat	26	2	2	2	7.7
Total		1395	152	139	133	9.5

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2016.

ESBL gene		Chicken	Turkey	Beef	Veal	Lamb	Sheep	Goat	Totaal
CTX-M-1 group									
	CTX-M-1	21	8	2	2	1		2	36
	CTX-M-3		1						1
	CTX-M-15		3	1	4				8
	CTX-M-32	1							1
	CTX-M-55	3	1		2				6
CTX-M-2 group									
	CTX-M-2	7							7
CTX-M-8/25 group									
	CTX-M-8	4	5						9
CTX-M-9 group									
	CTX-M-14		1						1
	CTX-M-27		1						1
	CTX-M-65		1						1
TEM									
	TEM-52c	2	5						7
	TEM-52cVar	3							3
SHV									
	SHV-12	6	5						11
CMY									
	CMY-2	33	2	2	1	2	1		41
Chromosomal ampC									
	ampC-type-3		3						3
	ampC-type-5		2						2
	ampC-type-11	1							1
Total		81	38	5	9	3	1	2	139

Chicken: 30 isolates were derived from imported frozen meat and 50 from fresh retail meat.
Turkey: 5 isolates were derived from frozen imported meat and 34 isolates from retail meat.

ESBL/AmpC-producing *Salmonella*

Surveillance of resistance to extended spectrum cephalosporins is also done in *Salmonella enterica* isolated in the Netherlands. In 2016 a selection of 2089 *Salmonella* isolates sent to RIVM for sero- or MLVA-typing were tested for susceptibility to cefotaxime and ceftazidime. In addition, NVWA tested 135 *Salmonella* mainly obtained from raw meat. In total, cefotaxime resistant *Salmonella* were isolated in 36 samples mainly from humans (n=26), poultry (n=6), and turkey (n=4) from which 35 isolates were further typed (Table ESBL06). The prevalence of ESBL/AmpC-producing *Salmonella* was 1.7%, confirming the decreasing trend observed in 2014 and 2015 (2.1% and 1.9%, respectively) and almost half of 2013 (4%). The predominance of *S.* Heidelberg observed in 2015 was not confirmed in 2016, as *S.* Kentucky, which is known to originate from Northern Africa, was the most prevalent (n=9), followed by Infantis, Saintpaul, Typhimurium and six other serovars identified to carry ESBL/AmpC genes (material and methods are the same as described above for *E. coli*. One *S.* Minnesota isolate from poultry meat was not included in the molecular analysis.

ESBL/AmpC genes detected in *Salmonella* are reported in Table ESBL06. The most represented genes were: i) $bla_{\text{CMY-2}}$, generally associated with *S.* Saintpaul and Heidelberg and also present in 2 other serovars; ii) $bla_{\text{CTX-M-14b}}$ in *S.* Kentucky; and iii) $bla_{\text{CTX-M-9}}$ in *S.* Typhimurium. Compared to previous years, prevalence of $bla_{\text{CMY-2}}$ kept dropping from 58% (2014) to 35% (2015) to 28% (2016). Similarly, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ were less represented. $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-14b}}$ appeared to be highly predominant compared to previous years with an increase from 1-6% to 11-25%, respectively. No ESBL/AmpC gene combination was detected. In isolates from human sources a variety of ESBL/AmpC genes were found including $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-14}}$.

All cefotaxime resistant *Salmonella* isolates were highly multidrug resistant, as shown in Table ESBL07. The increased finding of multi-resistance observed in 2015 compared to 2014 (70% vs 23%) was confirmed in 2016 with most of the isolates being resistant to 5 - 8 antibiotics (67%). 3% of the isolates were resistant to 9 out of 10 antibiotics, but no resistance was detected against meropenem or azithromycin. Colistin resistance observed in 8.8% of isolates in 2015 dropped to 0% in 2016.

ESBL/AmpC gene types found in *Salmonella* since 2007 are summarized in Table ESBL08. Every year genes $bla_{\text{CMY-2}}$, $bla_{\text{TEM-52}}$, and those belonging to the $bla_{\text{CTX-M-1}}$-group have been found in *Salmonella* isolates from diverse sources. After detection in 2015, $bla_{\text{CTX-M-2}}$ was not detected in 2016. $bla_{\text{DHA-1}}$ was identified for the first time in a human isolate of *S.* Bovismorbificans. Overall, *Salmonella* isolates held less variability in ESBL/AmpC gene types than 2015.

In conclusion, ESBL/AmpC-producing *E. coli* are widespread in Dutch food-producing animals and in raw meat mainly of poultry origin. ESBL/AmpC- was 0.3% of the randomly isolated *E. coli* , the lowest observed since 2007. Selective culturing in faecal samples of food-producing animals showed a slight tendency to increase of animals carrying ESBL/AmpCs for veal calves compared to 2015.

The dominant ESBL/AmpC gene types were confirmed to be $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$, in all animal species independent of the source of isolation, whereas an increased detection of $bla_{\text{CTX-M-14}}$ was registered in both *E. coli* and *Salmonella*. The dominant human ESBL gene $bla_{\text{CTX-M-15}}$ was frequently found in veal calves and dairy cows faecal samples as well as in beef and veal samples. $bla_{\text{CTX-M-15}}$ was only rarely found in broilers and it was absent in chicken products (Table ESBL06), as already observed in 2015.

Table ESBL06 Beta-lactamases in *Salmonella* isolated in 2016

				CTX-M-1 group			CTX-M-9 group			TEM	CMY	DHA	
Serovar	Humans	Poultry	Turkey	CTX-M-1	CTX-M-15	CTX-M-55	CTX-M-9	CTX-M-14b	CTX-M-65	TEM-52Var	CMY-2	DHA-1	Total
1,4,5,12:i:-	3					3							3
Bovismorbificans	1											1	1
Bredeney	2										2		2
Heidelberg	1	2									3		3
Infantis	4	1		3					2				5
Kentucky	9							9					9
Paratyphi B variant Java	1	2			1					2			3
Saintpaul			4								4		4
Thompson	1										1		1
Typhimurium	4						4						4
Total	**26**	**5**	**4**	**3**	**1**	**3**	**4**	**9**	**2**	**2**	**10**	**1**	**35**

This table contains the results of seven extra Salmonella isolates derived from turkey meat (S. Saintpaul, N = 4) and poultry meat (S. Heidelberg, N = 2, S. Paratyphi variant Java, N = 1) at NVWA.
One ESBL-suspected isolate from poultry meat (S. Minnesota) collected at NVWA was not included in the analysis. previous years.

Table ESBL07 Resistance and multidrug resistance percentages of ESBL-producing *Salmonella* in the Netherlands in 2016.

Antimicrobials	R%
Ampicillin	100.0
Cefotaxime	100.0
Ceftazidime	88.9
Gentamicin	47.2
Tetracycline	69.4
Sulfamethoxazole	75.0
Trimethoprim	38.9
Ciprofloxacin	86.1
Nalidixic acid	63.9
Chloramphenicol	25.0
Azithromycin	0.0
Colistine	0.0
Meropenem	0.0
Tigecycline	5.6

Multi drug resistance	N = 35
0	0%
1	0%
2	3%
3	22%
4	6%
5	11%
6	31%
7	11%
8	14%
9	3%
10	0%

4.1.2 Decreased prevalence of ESBL/AmpC-producing *E. coli* in broilers parallel to a reduced usage of antimicrobials in the Netherlands

In 2009 a study on prevalence of ESBL/AmpC producing *E. coli* on Dutch broiler farms showed that all broiler farms included in the study (n=26) were positive for ESBL/AmpC-producing *E. coli* (Dierikx *et al,* 2013). The within-farm prevalence (based on 41 faecal samples) appeared to be >80% (for 85% of the farms) and >90% (62% of the farms). Antimicrobial use in animals has been drastically reduced from 2009 to 2016. In the routine national surveillance program a decline in ESBL/AmpC-producing *E. coli* in broilers was observed. In 2016 this study was repeated on the same farms with the aim to describe the prevalence of ESBL/AmpC-producing *E. coli* and compare with the results from 2009.

All 26 farms that were included in 2009 were asked to participate again. Farms were visited twice during a production cycle (at start and just before slaughter). From each house on the farm 41 cloacal swabs were collected and analysed by enrichment in LB broth supplemented with 1 mg/L cefotaxime and subsequent inoculation on MacConkey agar supplemented with 1 mg/L cefotaxime for the presence of ESBL/AmpC-producing *E. coli*. Ten % of the strains was analysed for the presence of ESBL/AmpC genes by PCR. PCR-positive isolates were sequenced for ESBL/AmpC allele variant identification. Information about cleaning and disinfection, farm management and antimicrobial treatment was collected on each farm.

In total, 20 of the original 26 farms agreed to participate again. For all comparative analyses, only the farms from 2009 that also participated in 2016 were selected. The differences in farm management between 2009 and 2016 were small. Most important was the transition towards the slower growing Hubbard-line (4% to 25%). Regarding antimicrobial use 62% (2009) of the farms vs 15% (2016) had an early treatment of chickens and 4% (2009) vs 60% (2016) did not use antimicrobials for the sampled flock. The proportion of farms on which animals with ESBL/AmpCs were found just before slaughter remained high: 100% in 2009 to 95% in 2016. The within-farm prevalence decreased significantly from 66% of the animals in 2009 (range: 24-100%) to 38% (range: 0-98%) in 2016. Remarkable is the fact that on farms, at the same locations, houses were present with and without detected ESBL/AmpC-producing *E. coli*. There was no significant difference in prevalence between farms without antimicrobial treatment (36%) and farms with one or more treatments (42%). Typing showed that bla_{CMY-2}, bla_{CTX-M-}1 and bla_{SHV-12} were the most prevalent types both in 2009 and 2016. Within farms and within flocks different ESBL/AmpC variants were detected at the same time of sampling.

In summary, the within-farm prevalence of ESBL/AmpC-producing *E. coli* in broilers decreased significantly from 66% in 2009 to 38% in 2016, parallel to a huge reduction in antimicrobial use on these farms. Given this reduction in prevalence, in 2016 a differentiation between high and low prevalent farms could be made, in contrast with high prevalent farms only in 2009. However, despite the reduced numbers of carriers found in 2016 compared to 2009, ESBL/AmpC-producing *E. coli* is still widespread in the Dutch broiler production industry. It can be concluded that risk factors should be investigated at house level instead of farm level. Finally, the diversity of ESBL/AmpC types within flocks suggests common driver and not the presence of one successful clone.

Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2016

Year	CTX-M-1-group#	CTX-M-2##	CTX-M-8	CTX-M-9-group*	TEM-52	TEM-20	SHV-12**	CMY-2***	ACC-1	DHA-1	Total ESBL	Total Salmonella tested	% ESBL of total Salmonella
2007	9	13			17	2	4	2			47	1514	3.1
2008	25	12	1	1	13	1		6	2		61	2149	2.8
2009	12	4		2	3		1	9			31	2232	1.4
2010	8	3		1	2		3	4			21	1715	1.2
2011	5	3		1	1		2	13			25	1444	1.7
2012	14	5		2	2			10	1		34	1795	1.9
2013	1	3	5	4	5	1		36			55	1369	4.0
2014	6		2	3	1			21			33	1688	2.0
2015	13	2		6	1			12			34	1761	1.9
2016	7			15	2			10		1	36	2117	1.7
Total	100	45	8	35	47	4	10	123	3	1	375	17784	2.1

 # 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).

in 2008 one combination of $bla_{CTX-M-2}$ with bla_{TEM-52} was found in S. Paratyphi B var. Java.

** 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).*

*** In 2007 three S. Concord were found containing both bla_{SHV-12} and $bla_{CTX-M-15}$.*

**** 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*

In 2016, one S. Minnesota isolate obtained from poultry meat at NVWA was not included in the molecular analysis.

4.2 Carbapenemases

4.2.1 Monitoring of carbapenemase producing Enterobacteriaceae in livestock

In 2015 a sensitive method was applied to screen for carbapenemase producers, extended spectrum beta-lactamases that can also hydrolyse carbapenems. This is important in an environment with a very low anticipated prevalence of carbapenem resistance. All faecal samples sent to the Wageningen Bioveterinary Research (WBVR) by the Dutch Food and Consumer Protection Authority (NVWA) for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in Buffered Peptone Water (BPW). After incubation the culture was centrifuged and DNA isolated from pellet. A commercial RT-PCR (Check-Points, CarbaCheck MDR RT) which 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 on commercial selective plates (ChromID CARBA and ChromID OXA (Biomerieux) and on HIS plates with 0.125 mg/L ertapenem (for *Shewanella*).

Carbapenemase screening in 2016 (n=1800) resulted in two bla_{OXA-48}-like positive samples in the RT-PCR (one slaughter pig and one veal calf faecal samples). bla_{OXA-48}-like genes are known to be chromosomally associated with *Shewanella* spp. 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 and 2015 (MARAN 2016). Considering that *Shewanella* spp. is the natural progenitor of this carbapenemase family (Zong, 2012), carrying bla_{OXA-48}-like genes on the chromosome, 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 and in food products will continue in 2017, to monitor potential carbapenemase gene spread among environmental and clinically relevant bacteria.

4.2.1 Monitoring of carbapenemase producing Enterobacteriaceae in companion animals

Within Europe, carbapenemase producing *Enterobacteriaceae* (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). *So* far, in the Netherlands CPE have not been detected. In order to detect introduction of CPE, a monitoring for CPE in Dutch companion animals was initiated in 2015. The screening for CPE comprised of a retrospective and a prospective study.

In the retrospective study, clinical isolates were obtained through the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University. Since CPE are frequently reported in combination with ESBLs, all available ESBL-suspected isolates stored since 2009 were included in the screening. ESBL-suspicion was based on susceptibility to 3rd generation cephalosporins (ceftiofur). In total, 418 isolates were screened, originating from dogs (n=281), cats (n=73), horses (n=49), cattle (n=9) and other animal species (n=6). The isolates were obtained from divers matrices, including urine (47%), wound/abdominal fluid (22%) or 'other' (32%; e.g. pus, tracheal swab/lavage, horse uterus secrete). All isolates were screened using a disk diffusion assay (imipenem: 10 mg, ertapenem: 10 mg, meropenem: 10 mg) and results were interpreted as described by Cohen-Stuart *et al*, 2010. Suspected carbapenemase-producing strains were screened by PCR for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48}. All screened isolates were negative for CPE.

In the prospective study, faecal samples of cats and dogs were screened for the presence of CPE. The inclusion criterion for dogs was antimicrobial treatment in the week prior to sampling or at the moment of sampling. For collection of samples and selection of patients, dermatology and internal medicine specialists from 4 referral clinics participated. Additional faecal samples that met the antimicrobial treatment criterion were obtained through VMDC. Since cats are not frequently treated with antimicrobials, no inclusion criterion was given. All available faecal samples from cats submitted to VMDC were included. In 2015, 201 and 101 faecal samples from cats and dogs, respectively, were screened. In 2016, 178 and 145 faecal samples from cats and dogs were screened, respectively. From each sample, 0.5 gram feces were suspended in 4.5 ml TSB broth, supplemented with 50 mg/L vancomycin. After enrichment, the suspension was inoculated on ChromID Carba-Smart agar (BioMerieux). In addition, DNA of the enrichment broth was isolated for molecular screening using the RT-PCR Check-MDR carba kit (Check-Points). All screened faecal samples were negative for CPE.

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 indicator *E. coli* from faecal samples from 2014 and 2015 indicated a decreasing trend in the occurrence of this gene. To gain more knowledge on the current spread of *mcr-1* in livestock, a prospective study was performed in 2016 as part of the national surveillance program on antibiotic resistance in animals to reveal the current spread of this gene in livestock. For this purpose purified DNA of pooled BPW cultures (five samples per pool) from a total of 1500 faecal samples were tested with conventional PCR for the presence of *mcr-1* 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 4 mg/L colistin. As a result *mcr-1* positive *E. coli* were identified in eight faecal samples (0.5%) in different animal species: veal calves (n=4), broilers (n=2), pig (n=1) and dairy cow (n=1). In 2016, no colistin resistant *E. coli* isolates were identified amongst the indicator *E. coli* isolated from 1500 faecal samples. However, in meat fourteen colistin resistant *E. coli* non-selectively isolated from retail meat were confirmed as *mcr-1* carriers. These isolates almost exclusively originated from turkey (n=11) and chicken meat (n=2). The remaining *mcr-1*-positive isolate was obtained from imported crocodile 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 in raw meat from chicken and turkey, but not in *Salmonella*.

References

Dierikx C, *et al.* Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother. 2013 Jan;68(1):60-7.

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

González-Torralba *et al;* Survey of Carbapenemase-Producing *Enterobacteriaceae* in Companion Dogs in Madrid, Spain. Antimicrob Agents Chemother, 2016: 60(4):2499-2501

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

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

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