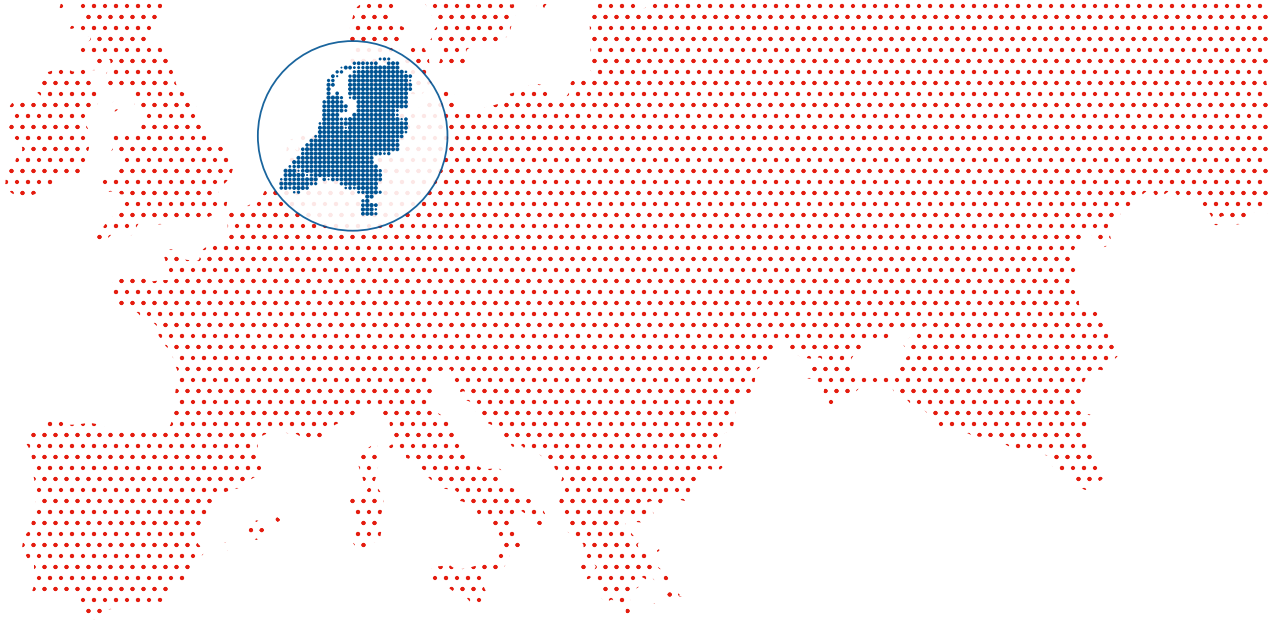
NethMap 2016

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

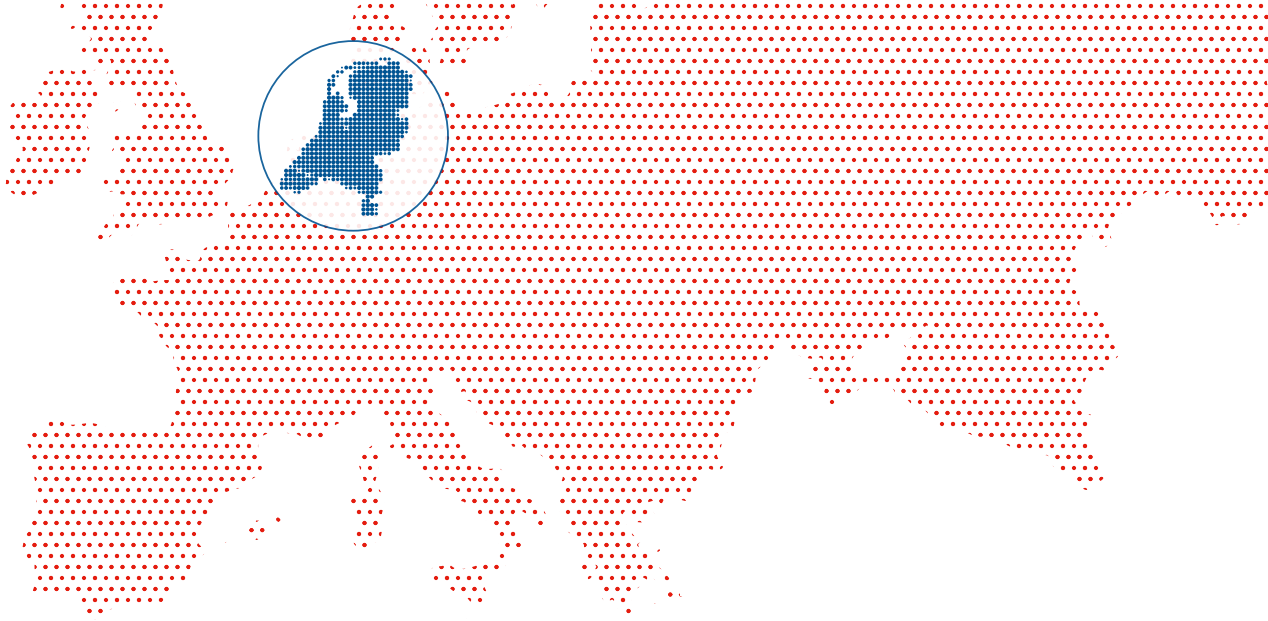


National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

SWAB

MARAN 2016

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2015



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NethMap 2016

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in The Netherlands
in 2015

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NethMap/MARAN-rapport

Wereldwijd neemt het aantal bacteriën dat resistent is tegen antibiotica toe. In Nederland is van de meeste bacteriën die in resistente vorm bij mensen is aangetroffen, het aantal de afgelopen jaren stabiel gebleven. Toch is er reden voor zorg. Het gebruik van antibiotica neemt langzaam toe. Ook zijn sommige resistente bacteriën, zoals Klebsiella, die resistent zijn voor 'laatste redmiddel-antibiotica' (carbapenems), in 2015 iets vaker aangetroffen, onder andere door een 'uitbraak' in een zorginstelling. Gezonde mensen hebben daar geen last van, maar kwetsbare mensen kunnen er ziek van worden. Verder blijken steeds meer bacteriën die bij mensen infecties kunnen veroorzaken, resistent tegen de antibiotica die als laatste redmiddel gebruikt worden. Dat betekent dat de keuze voor een antibioticum dat goed werkt steeds moeilijker wordt.

Om de ontwikkeling van resistentie tegen te gaan, moet het antibioticagebruik beter op de individuele patiënt en de infectie worden afgestemd. Daarnaast is het van belang dat zorgverleners zorgvuldig de hygiëne- en infectiepreventiemaatregelen naleven om te voorkomen dat resistente bacteriën zich verspreiden. Dankzij deze maatregelen is bijvoorbeeld het aantal MRSA-bacteriën in ziekenhuizen in de afgelopen jaren laag gebleven. Deze 'ziekenhuisbacterie' wordt overgedragen via direct huidcontact, vooral via handen, en is ongevoelig voor veel soorten antibiotica.

Het gebruik van antibiotica in Nederland die via de huisarts zijn verstrekt, is marginaal toegenomen (met ongeveer 1 procent ten opzichte van het voorgaande jaar). In Nederlandse ziekenhuizen is het totale gebruik eveneens licht gestegen (4-5 procent). Het gebruik van antibiotica voor dieren is, na jaren van forse daling, in 2015 zo goed als stabiel gebleven. Wel blijkt de mate waarin resistente bacteriën bij dieren voorkomen te zijn afgenomen.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2016, waarin diverse organisaties de gegevens over het antibioticagebruik en resistentie, zowel voor mensen als voor dieren, gezamenlijk presenteren.

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 2016 and earlier versions are also available from the website of the SWAB: www.swab.nl.

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

1 Introduction

This is NethMap 2016, the SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in The Netherlands in 2015 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. SWAB has initiated several major initiatives to achieve its goals. Among these are training programs on rational prescribing of antimicrobial drugs, development of evidence-based prescription guidelines, implementation of tailor-made hospital guides for antibiotic prophylaxis and therapy, a nationwide surveillance system for both antibiotic use and resistance and the development and implementation of a stewardship program.

CIb monitors and informs the government about potential national health threats with regard to antimicrobial resistance. 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 form the basis of the surveillance of resistance trends reported in NethMap.

NethMap 2016 extends and updates the information of the annual reports since 2003. Since the introduction of a more concise format two 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 as of last year. The reader is encouraged to visit www.isis-web.nl for tailored overviews of resistance development.

New in NethMap 2016 is chapter 5 reporting on antimicrobial stewardship. Together with infection prevention and control, antimicrobial stewardship programs are essential to curb antimicrobial resistance and ensure the treatment of infections in the future. In response to the recommendation by SWAB, IGZ and the Minister of Health, A-teams have been established in the majority of hospitals in the Netherlands (www.ateams.nl). In 2015, the SWAB has initiated an Antimicrobial Stewardship Monitor program to measure the progress and impact of the national implementation of antimicrobial stewardship. NethMap will report from this year on the quality of antibiotic use in hospitals in the Netherlands and the stewardship activities employed by A-teams aimed at measuring and improving the quality of antimicrobial use.

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 2016) and the veterinary sector (MARAN 2016).

2.1 Most important trends in antimicrobial use

In outpatients

- Compared to 2014, total antibiotic use in outpatients in 2015 marginally increased from 10.53 to 10.67 DDD/1000 inhabitant days (DID).
- Some remarkable shifts in the use of drugs are seen.
- The use amoxicillin increased substantially by 0.19 DID to 2.13 DID.
- The rise in use of azithromycin continued up to 0.80 DID.
- Use of nitrofurantoin now seems to stabilise at a level of 1.40 DID.
- Use of ciprofloxacin has stabilised at a level of 0.60 DID.

In nursing homes

- The mean use was 57.3 DDD/1000 residents/day but varied widely between individual settings with a minimum of 17 and a maximum of 121 DDD/1000 residents/day.
- The most frequently used antibiotics are combinations of penicillins (mainly amoxicillin with clavulanic acid), nitrofurantoin derivates and fluoroquinolones with 31%, 19% and 15% respectively.

In hospitals

- The in-patient use of antibiotics in 2014 increased by 5% when measured in DDD/100 patient-days (from 74.7 to 78.5) or 5.9% when measured in DDD/100 admissions (from 307.8 to 326)
- The increase in antibiotic use in 2014 is mainly due to increases in use of beta-lactam antibiotics. Cephalosporins show the highest total increase.

- University hospitals used the least antibiotics (76.3 DDD/100 patient-days), whereas large teaching hospitals the most (81.1 DDD/100 patient-days). General hospitals used 77.1 DDD/100 patient-days on average.
- Use of carbapenems remained stable at 1.5 DDD/100 patient-days. University hospitals account for most of the meropenem use.

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 2016.

In GPs

- For most antimicrobials, there are no significant shifts in resistance levels since 2011. The exceptions are trimethoprim and co-trimoxazole that show a decrease in resistance, although still between 20-30% for most species. There appears an increase in resistance to fosfomycin in some species.
- A distinction was made for patients aged below and above 12 years of age. In general, resistance rates in the older age group were slightly higher than in the younger age group.
- The percentage of highly resistant micro-organisms (HRMO) and multi-drug resistance remained relatively low (< 5%) in all Enterobacteriaceae.
- Resistance levels for *E. coli* were comparable between geographical regions for most antimicrobials. For co-amoxiclav, there was some geographical variation in resistance levels with highest levels found in the western and southern part in the Netherlands.
- The Gonococcal Resistance to Antimicrobials Surveillance (GRAS) reported no resistance to ceftriaxone.

In hospitals

- Compared to 2011, overall resistance rates for many antimicrobials were similar or slightly lower. One exception was *P. mirabilis* which showed increasing resistance for certain antimicrobials in ICUs (co-amoxiclav and ciprofloxacin) and for some empirical therapy combinations at inpatient urology departments compared to 2011.
- The percentage of HRMO was highest among *E. coli* and *K. pneumoniae* i.e. 8% (excl. ICU departments), 9-10% (ICU).
- CRE were a rare occurrence in the Netherlands and stable compared to the previous year, although one outbreak in a hospital occurred; 0.01% of *E. coli* and 0.19% of *K. pneumoniae* were non-susceptible to carbapenems. OXA-48 and NDM were the most prevalent carbapenemases detected.
- The prevalence of MRSA remains low and is 1% in blood isolates.
- Resistance to vancomycin remained rare in enterococci, although the percentage of VRE increased marginally up to 1% in clinical isolates in inpatient departments and a higher number of outbreaks were reported.

Table 2.2.1 Overview of surveillance programs in the Netherlands.

Surveillance program[1]	Origin of isolates	availability	Sources 2015	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-	37 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	E-test
Neisseria gonorrhoeae	STI centers	2006-	77% of STI center attendees	Decentral testing	E-test
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Agar dilution and
					BACTEC-Mgit 960 (liquid breakpoint)
Influenza antiviral drugs	community, GP, nursing home, hospital	2005-	NIVEL GP sentinels, SNIV nursing home sentinels, hospital/ regional laboratories	central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Neuraminidase enzym inhibition assay; for established molecular markers sequencing and/or single nucleotide polymorphism (SNP) PCR
Resistance among anaerobic pathogens	Hospital	2010-	1 lab	Central testing	E-test
Clostridium difficile	Hospital, nursing homes	2005-	23 hospitals	(de)central testing	E-test and ribotyping
azole resistance in Aspergillus fumigatus	Hospital	2011-	5 University hospitals	Central testing	EUCAST methodology

[1] *ISIS-AR: Infectious Disease Surveillance 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

- Resistance to penicillin (<1%) in pneumococci was still rare in the Netherlands.
- Resistance to penicillin in *N. meningitidis* was not found in 2015.
- For *C. difficile*, the prevalence of ribotype 027 was less prevalent than in the preceding 5 years (1% vs 3% the previous year) and no resistance was found to metronidazole and fidaxomycin.
- The overall frequency of azole resistance in *A. fumigatus* in 2015 was increased compared to in the previous years based on results from 4 UMCs.

2.3 Antibiotic use and resistance in veterinary sector

Antibiotic use

- Sales of antimicrobial veterinary medicinal product (206 tonnes) in 2015 decreased by 0.65% compared to 2014 (207 tonnes).
- Total sales decreased from 2009, the index year used by the Ministry of Economic Affairs, to 2015 by 58.4%. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 64%.
- Sales and consumption in the monitored animal sectors of antimicrobial drugs of critical importance for human healthcare (fluoroquinolones and cephalosporins of 3rd and 4th generation) were further reduced in 2015; a reduction of 98.8% was achieved since 2011.

Antimicrobial resistance

- Over the last decade, STEC O157 isolates from humans show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim, resulting in approximately 15% resistance for all four antibiotics in 2015
- Resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and veal calves and stabilized in pigs. Resistance to third-generation cephalosporins was low (< 1%) in most animal species. In broiler isolates the resistance level stabilised at 2.5%.
- ESBL-producing *E. coli* represented 0.9% of randomly isolated *E. coli*, the lowest proportion observed since 2007.
- Two variants of blaCTX-M-14 were found in broiler isolates, together with the reappearance of blaCMY-2 in both broilers and slaughter pigs, undetected in 2014. Selective isolation from livestock faeces indicated ESBL/AmpC producing *E. coli* prevalence of 56.5% in broilers, 12.3% in slaughter pigs, 17.3% in white veal calves, 10% in rosé veal calves and 9.3% in dairy cows. Classical human associated ESBL-types blaCTX-M-9, blaCTX-M-14, and blaCTX-M-15 were found in *E. coli* isolates from broiler faeces, together with blaCTX-M-55, not described before in Dutch broilers.
- ESBL/AmpC prevalence in *E. coli* isolates from prepared meat tended to be higher compared to raw meat, possibly due to cross-contamination during processing.
- ESBL/AmpC-prevalence in poultry meat decreased substantially compared to 2015. This decrease is most likely associated with the major reduction in antibiotic use in broilers since 2011 and the total ban on the use of ceftiofur at hatcheries in 2010.
- No carbapenemase producing Enterobacteriaceae were detected in the faecal samples from livestock in 2015.
- In 2015, the gene conferring resistance to colistin, mcr-1, was identified in eighteen isolates, all from poultry sources (chicken and turkey meat), but mcr-1 was not identified in randomly isolated *E. coli* from 1300 faecal samples.

In general, with a few exceptions, resistance levels appear to be decreasing and are in line with the reduction in antimicrobial use. An epidemiological analysis of this relationship indicates that drug use history and co-selection of resistance are key elements for perpetuation of resistance and that the recent Dutch policies of reducing total use of antimicrobials seems to have decreased resistance significantly, in particular in pig and veal calf production sectors. This substantiates the view that antibiotic use in general should be limited.

2.4 Implications for therapy

Overall, with a few exceptions, no major shifts in resistance rates have occurred in the Netherlands over the last five years. The resistance rates in 2015 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 cultures should be 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 that 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

Urinary tract infections

- Approximately 80% of Gram-negatives cultured were *E. coli*, *K. pneumoniae* and *P. mirabilis*. High levels of resistance to amoxicillin, trimethoprim and co-trimoxazole (all > 20%) make these agents less suitable for empirical treatment in UTI both in children and adults.
- The best suitable treatment options for uncomplicated UTI are nitrofurantoin (<3% resistance in *E. coli*) and fosfomycin (<2% resistance in *E. coli*, but >10% in *K. pneumoniae* and *P. mirabilis*). Of note, fosfomycin resistance appears to be increasing.
- Multi-drug resistance, defined as resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin remained relatively low below 5% but reduces the oral treatment possibilities of complicated UTI among selected GP patients.
- Antimicrobial susceptibility testing becomes increasingly important in the treatment of UTI.

In hospitals

Outpatient departments

- The levels of resistance preclude empirical treatment with oral agents for complicated UTI; culture, antibiograms and tailored therapy are necessary.
- Resistance rates are comparable to, or slightly higher than in GP patients, thus the treatment strategies will be largely similar

Unselected hospital patient departments

- In general, there are no major changes compared to 2014, except that aminoglycoside resistance appears to have decreased slightly 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

- High levels of resistance to amoxicillin, co-amoxiclav, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections.
- There are significant differences in resistant rates between hospitals as well as over time. This clearly indicates that empiric therapy should be based on the local epidemiology of resistance.
- Piperacillin/tazobactam, cefotaxime/ceftriaxone, ceftazidime and aminoglycoside resistance rates are all between 5 and 10%. This is in a range that warrants combination therapy or at least close monitoring for the severely ill. However, resistance to combinations of a beta-lactam and an aminoglycoside is 5% or lower. It should be realized however, that resistance to combinations is based on the effect of the drug alone and does not take into account any synergistic effects that may be present.

Specific micro-organisms

- The overall resistance frequency to azoles in *A. fumigatus* in 2015 increased to 10.7% in 4 university hospitals, requiring a reset in empirical therapy, guidelines and shows that susceptibility testing of this pathogen is now mandatory.
- In 2015, for the first time in years, there was an increase in the number of Mycobacterium tuberculosis complex strains isolated, in line with an increase in notification of TB of 6%.
- In gonococci, the diagnosis by molecular methods continues to increase, and in the near future may reach a level that surveillance of resistance becomes a significant problem. Although ceftriaxone resistance has not been found in 2015, as opposed to many other countries, the probability that resistance is missed because of this will soon reach an unacceptable level.

2.5 Antibiotic stewardship

The Antimicrobial Stewardship Monitor, developed by SWAB, will be published yearly in NethMap as from this year and report on the quality of antibiotic use in hospitals in the Netherlands and the stewardship activities employed by A-teams aimed at measuring and improving the quality of antimicrobial use. Since the formation of antimicrobial stewardship program in hospitals is not yet complete, we here present a summary of data obtained in a pilot study conducted in 5 hospitals.

- The appropriateness of glycopeptides prescription was generally high: 97% (range: 83-100%)
- Carbapenem prescriptions followed the local guideline or an expert's advice in 90% (range: 84-97%) of the cases
- Fluoroquinolone prescription was appropriate in 79% (range: 68% to 100%)
- A-teams that currently have successfully implemented an antimicrobial stewardship program often lack a systematic registration system incorporated in the daily work flow, implying that A-teams have insufficient data to analyze where and how to intervene. This requires further support.

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. For *K. pneumoniae*, data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) for 2014 show large differences in the national percentages of carbapenem resistance in invasive (i.e. mostly from bloodstream infections) isolates ranging from 0% to 62.3%. For *E. coli*, EARS-Net data for 2014 show a different epidemiological situation with a much lower EU/EEA population-weighted mean percentage (0.1%) of carbapenem resistance in invasive isolates, and national percentages ranging from 0% to 1.2% . 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 2011 and 2014 for both *E. coli* and *K. pneumoniae*.

In the Netherlands, CRE were a rare occurrence in 2015 and stable compared to the previous year, although one outbreak in a hospital occurred; 0.01% of *E. coli* and 0.19% of *K. pneumoniae* were non-susceptible to carbapenems. 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 2015 did not increase further for most antibiotics. Yet, there is a continuing concern as for some HRMO, an increased number of outbreaks were reported.

To control the occurrence and spread of HRMO, an integrated approach at regional, local and national level, in human healthcare as well as in the open population, the environment, food-producing animals and the food chain, is needed. In 2015, the Ministry of Health set targets to be achieved in collaboration with all stakeholders in above mentioned areas.

A major pillar of this approach is the development and implementation of a nationwide integrated surveillance system on antibiotic resistance, antibiotic use, and healthcare associated infections. The output of this system will support national, regional and local control measures.

Conclusions

The data presented in NethMap 2016 demonstrate the importance of an adequate surveillance system 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 more integrated into one nationwide surveillance system.

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

In comparison to 2014, total antibiotic use in outpatients in 2015 marginally increased from 10.53 to 10.67 DID. (Table 3.1)

Nevertheless, some remarkable shifts in the choice of drugs are observed. Use of amoxicillin increased substantially by 0.19 DID to 2.13 DID. The rise in use of azithromycin continued up to 0.80 DID.

Use of nitrofurantoin now appears to stabilise at a level of 1.40 DID, after years of increase. The same holds true for ciprofloxacin, which has stabilised at a level of 0.60 DID and tetracyclines at a level of 2.25 DID. After years of decline, the use of amoxicillin with clavulanic acid stabilised at 1.56 DID. (Figure 3.1)

Discussion

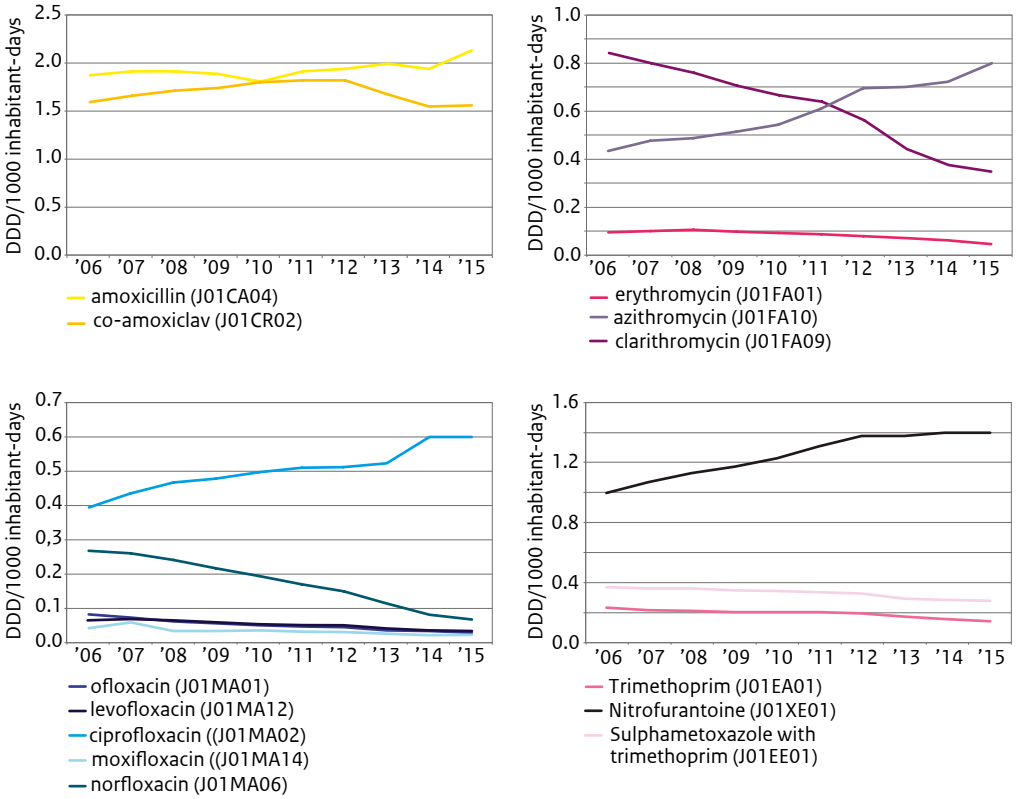
After years of increase in antibiotic use in outpatients in the Netherlands, until 2011, a slight but steady decrease in use was seen over the next three years. In 2015, use marginally increased compared to the year before. Increase in use of amoxicillin and stable use of amoxicillin with clavulanic acid at the same time probably shows that prescribers carefully choose the smallest spectrum antibiotic suitable for the targeted infection. Stabilisation in the use of nitrofurantoin and ciprofloxacin is promising, as they are valuable first-line treatments for uncomplicated and complicated urinary tract infections respectively.

Table 3.1 10-years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1000 inhabitant-days). 2006-2015 (Source: SFK).

ATC Group*	Therapeutic group	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	2.37	2.57	2.66	2.67	2.67	2.60	2.49	2.33	2.23	2.25
J01CA	Penicillins with extended spectrum	1.87	1.91	1.91	1.89	1.81	1.91	1.94	1.99	1.94	2.13
J01CE	Beta-lactamase sensitive penicillins	0.50	0.46	0.42	0.39	0.37	0.35	0.33	0.31	0.30	0.23
J01CF	Beta-lactamase resistant penicillins	0.31	0.32	0.36	0.38	0.38	0.39	0.41	0.41	0.44	0.43
J01CR	Penicillins + beta-lactamase-inhibitors	1.59	1.66	1.71	1.74	1.80	1.82	1.82	1.67	1.55	1.56
J01D	Cephalosporins	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
J01EA	Trimethoprim and derivatives	0.23	0.22	0.21	0.21	0.20	0.20	0.19	0.17	0.16	0.14
J01EE	Sulphonamides + trimethoprim	0.37	0.36	0.36	0.35	0.35	0.34	0.33	0.29	0.28	0.28
J01FA	Macrolides	1.39	1.39	1.36	1.33	1.31	1.34	1.34	1.22	1.18	1.20
J01FF	Lincosamides	0.09	0.10	0.11	0.12	0.14	0.15	0.16	0.17	0.18	0.19
J01GB	Aminoglycosides	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03
J01MA	Fluoroquinolones	0.87	0.91	0.89	0.86	0.85	0.82	0.80	0.76	0.79	0.77
J01XE	Nitrofuran derivatives	1.00	1.07	1.13	1.17	1.23	1.31	1.38	1.37	1.40	1.40
J01XX05	Methenamine	0.03	0.03	0.02	0.03	0.04	0.03	0.04	0.03	0.03	0.02
J01	**Antibiotics for systemic use (total)**	**10.73**	**11.10**	**11.24**	**11.21**	**11.23**	**11.37**	**11.34**	**10.80**	**10.53**	**10.67**

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

Figure 3.1 a-d Use of antibiotics for systemic use in primary health care, 2006-2015 (Source:SFK).



3.2 Hospital care

Methods

Data on the use of antibiotics in Dutch hospitals were collected by means of a questionnaire distributed to all Dutch hospital pharmacists. Data were received from 68 out of 91 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

In comparison to 2013, the inpatient use of antibiotics further increased in 2014: +5% when calculated as DDD/100 patient-days (from 74.7 to 78.5) (table 3.2); or +5.9% when calculated as DDD/100 admissions (from 307.8 to 326.0) (Table 3.2).

The use of beta-lactam antibiotics is the major driver of the observed increase. Within the group of beta-lactams, the largest share was for the cephalosporins with growth percentages of + 0.7 DDD/100 patient days, + 0.3 DDD/100 patient-days, and + 0.7 DDD/100 patient-days for the first, second, and third-generation cephalosporins, respectively. The use of combinations of penicillins with betalactamase-inhibitors and carbapenems remained stable. Fluoroquinolones and nitrofurantoin derivatives showed a little increase of +0.3 DDD/100 patient-days for each group.

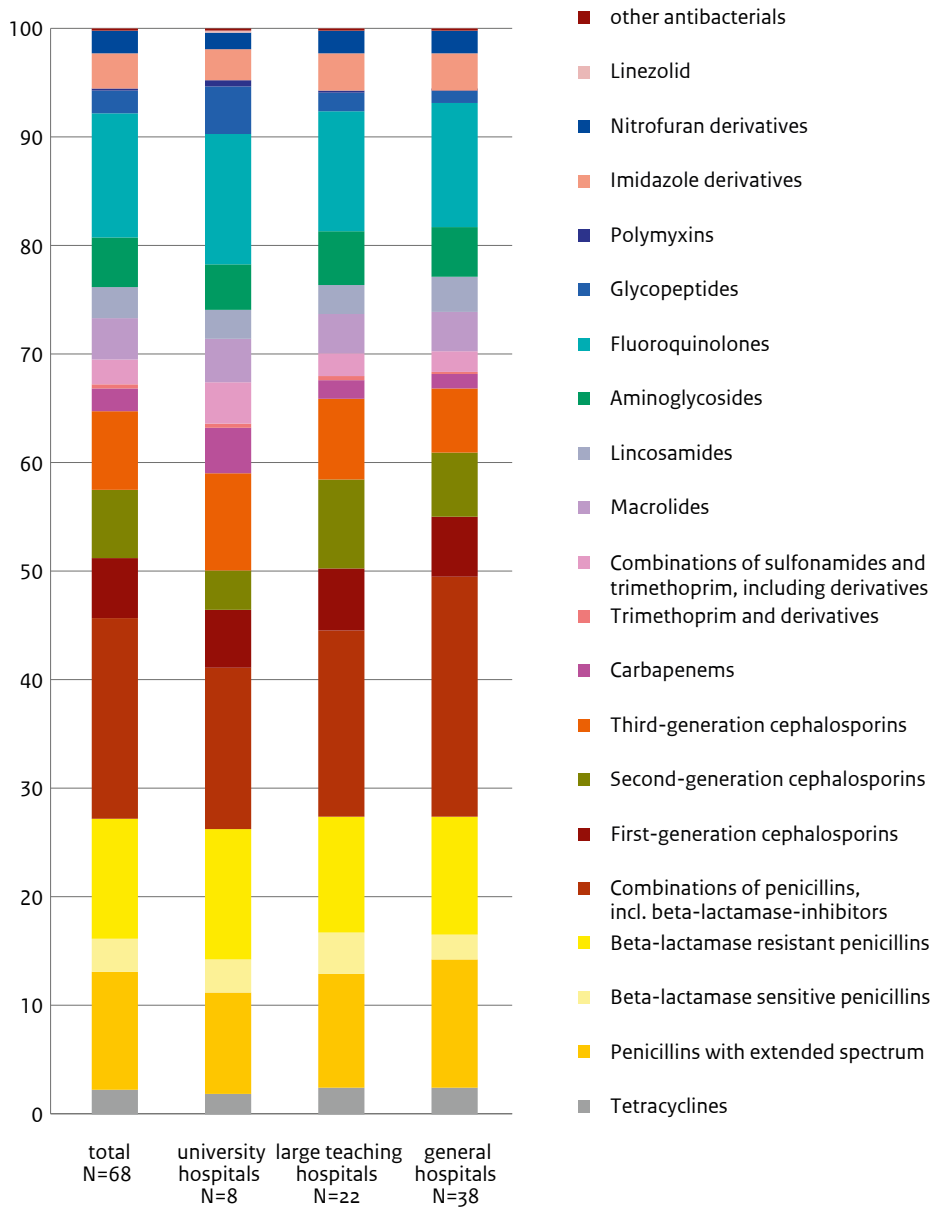
Considering site of care, university hospitals used the lowest amount of antibiotics (75.8 DDD/100 patient-days), whereas large teaching hospitals reported the highest overall antibiotic use (81.1 DDD/100 patient-days). Figure 3.2 and 3.5 show the use per antibiotic subgroup for these different types of hospitals in 2014. The use of combinations of penicillins (mainly amoxicillin with clavulanic acid) is still the highest in general hospitals with 22.1% versus 17.2% and 14.8% in large teaching hospitals and university hospitals, respectively. Carbapenems and glycopeptides used is most situated in university hospitals, whereas most nitrofuran derivates comes from general hospitals. Large teaching hospitals reported the most cephalosporin use.

Table 3.2. Ten years use of antibiotics for systemic use (J01) in hospitals. 2005-2014 (Source: SWAB). expressed in DDD/100 patient-days.

ATC Group*	Therapeutic group	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	1.6	1.6	1.4	1.7	1.6	1.7	1.8	1.7	1.7	1.9
J01CA	Penicillins with extended spectrum	6.7	7.6	7.3	6.5	7.6	7.3	7.3	7.6	8.0	8.4
J01CE	Beta-lactamase sensitive penicillins	1.4	1.4	1.2	1.3	1.6	1.5	1.5	1.7	1.9	2.4
J01CF	Beta-lactamase resistant penicillins	5.8	5.9	5.7	6.4	6.6	6.8	6.7	7.1	8.1	8.7
J01CR	Combinations of penicillins. incl. beta-lactamase-inhibitors	13.9	15.1	14.5	16.2	16.5	16.0	15.8	15.0	14.8	14.5
J01DB	First-generation cephalosporins	2.1	2.0	2.6	2.6	3.0	3.0	3.5	3.6	3.7	4.4
J01DC	Second-generation cephalosporins	2.9	3.8	2.8	3.0	3.6	3.4	3.7	4.1	4.7	5.0
J01DD	Third-generation cephalosporins	2.4	2.7	3.0	3.2	3.5	3.7	3.9	4.4	5.0	5.7
J01DH	Carbapenems	0.6	0.6	0.8	1.0	1.1	1.2	1.4	1.5	1.7	1.6
J01EA	Trimethoprim and derivatives	0.6	0.8	0.5	0.4	0.4	0.5	0.4	0.3	0.3	0.3
J01EE	Combinations of sulfonamides and trimethoprim. including derivatives	2.3	2.1	2.3	2.4	2.0	2.0	1.9	1.8	1.9	1.9
J01FA	Macrolides	2.8	2.5	2.8	2.7	2.6	2.7	2.9	2.8	2.6	2.9
J01FF	Lincosamides	1.9	2.0	2.1	2.1	2.4	2.3	2.3	2.2	2.3	2.3
J01GB	Aminoglycosides	2.6	2.5	2.6	3.9	4.2	4.1	3.9	3.3	3.5	3.6
J01MA	Fluoroquinolones	7.3	8.0	7.6	8.8	9.3	9.0	9.2	8.9	8.6	9.0
J01XA	Glycopeptides	0.8	0.7	1.0	1.1	1.3	1.3	1.3	1.4	1.5	1.6
J01XB	Polymyxins	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.2	0.2
J01XD	Imidazole derivatives	1.5	1.7	1.8	1.7	1.8	1.9	2.2	2.3	2.6	2.6
J01XE	Nitrofuran derivatives	1.0	1.0	1.1	1.2	1.1	1.2	1.2	1.2	1.3	1.6
J01XX08	Linezolid	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	other antibacterials	0.2	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0.1
J01	**Antibiotics for systemic use (total)**	**58.5**	**62.3**	**61.6**	**66.8**	**70.9**	**70.2**	**71.3**	**71.3**	**74.7**	**78.5**
	expressed in DDD/100 admissions:										
J01	**Antibiotics for systemic use (total)**	**316.9**	**335.9**	**337.5**	**344.7**	**321.29**	**315.9**	**306.37**	**295.7**	**307.84**	**326.0**

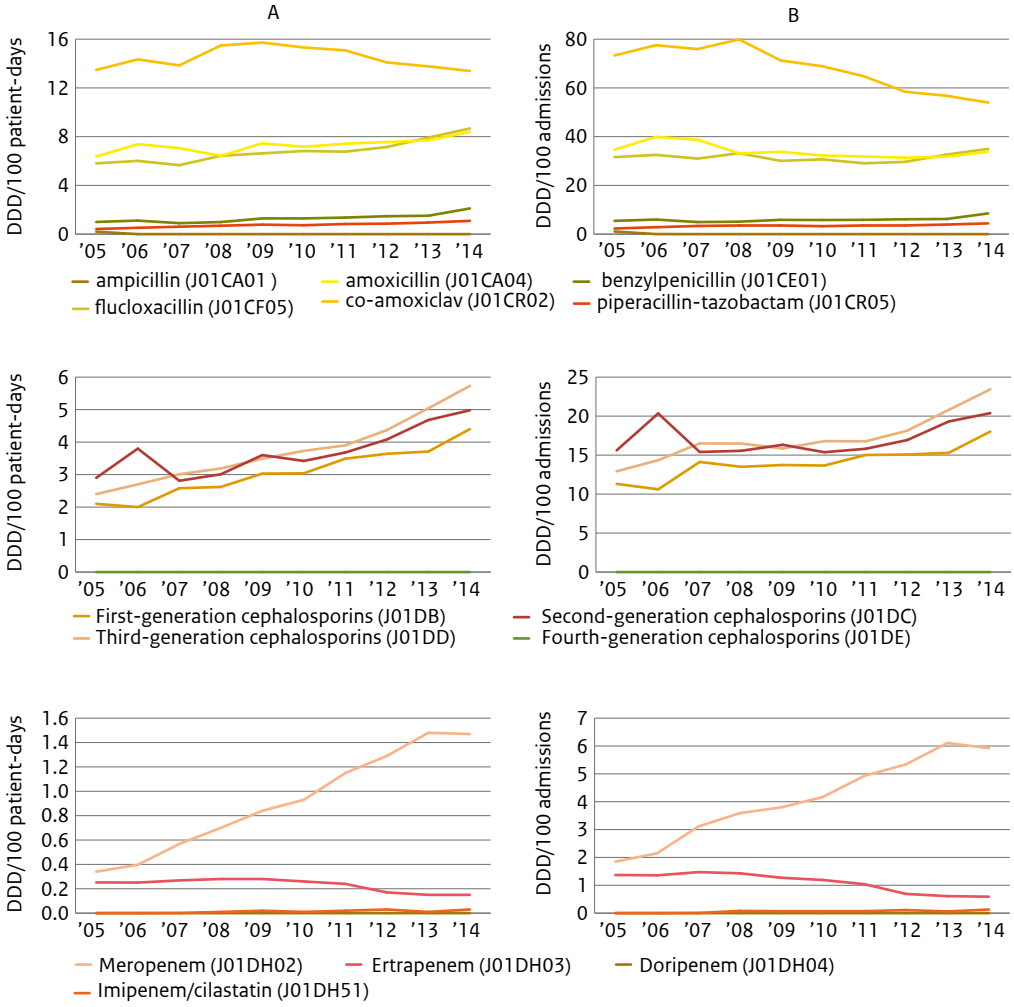
** From the 2014 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.2 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2014 (Source:SWAB)



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 3.4 use of antimycotics (J02), antimycobacterials (J04) and antivirals (J05) in university hospitals is provided from the years 2007 to 2014, expressed in DDD/100 patient-days. In 2014, the use of antimycotics increased further, both

Figure 3.3 Use of beta-lactams in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2005-2014 (Source:SWAB).



amphothericin B as well as azole antifungals. The use of antimycobacterials remained stable and the use of antivirals slightly decreased, compared to 2013.

In 2015 PREZIES data were received from 43 hospitals, including 11610 patients of which 3915 received antibiotics, with a total of 5024 prescriptions. Antibiotic use divided by surgical vs medical prophylaxis and hospital vs community acquired infections is depicted in figure 3.6. Most often used antibiotics were amoxicillin with clavulanic acid (19%), ciprofloxacin (12%) and cefuroxim (8%). Cefazolin was used in 52% cases of surgical prophylaxis. Use for medical prophylaxis was more diverse.

Figure 3.4 Use of macrolides, aminoglycoside, fluoroquinolones and glycopeptides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2005-2014 (Source:SWAB).

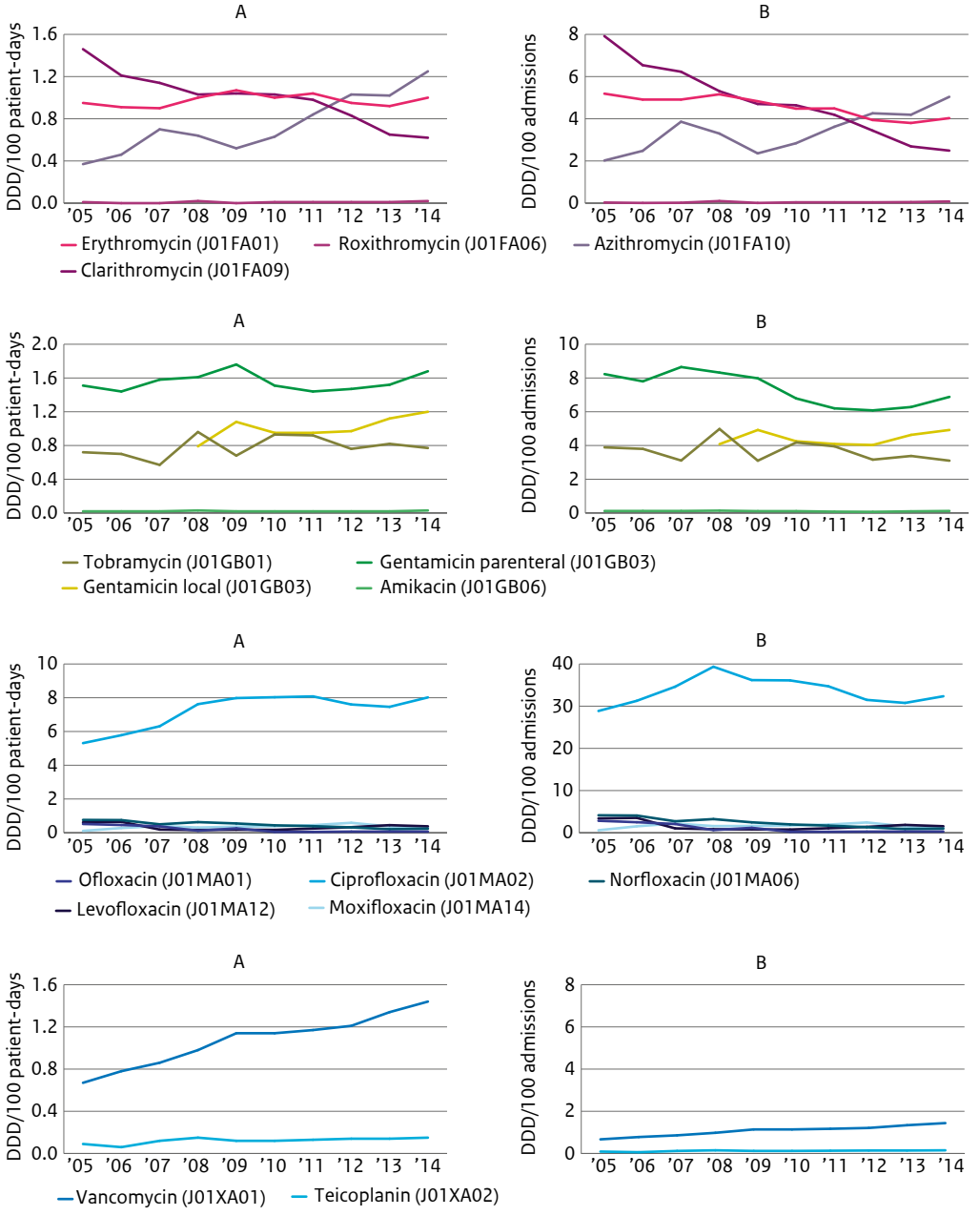


Table 3.3 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1000 inhabitant-days), 2005-2014 (Source: SWAB).

ATC Group	Therapeutic group	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	0,027	0,027	0,025	0,023	0,025	0,027	0,026	0,024	0,022	0,023
J01CA	Penicillins with extended spectrum	0,106	0,113	0,110	0,101	0,111	0,110	0,103	0,100	0,099	0,101
J01CE	Beta-lactamase sensitive penicillins	0,021	0,022	0,020	0,019	0,023	0,023	0,020	0,023	0,023	0,028
J01CF	Beta-lactamase resistant penicillins	0,089	0,091	0,087	0,086	0,093	0,097	0,089	0,093	0,100	0,105
J01CR	Penicillins + beta-lactamase-inhibitors	0,231	0,239	0,233	0,229	0,241	0,256	0,223	0,211	0,199	0,187
J01DB-DE	Cefalosporins	0,121	0,127	0,124	0,118	0,137	0,147	0,145	0,158	0,164	0,176
J01DF	Monobactams	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
J01DH	Carbapenems	0,008	0,009	0,010	0,011	0,014	0,015	0,018	0,019	0,020	0,019
J01EA	Trimethoprim and derivatives	0,009	0,009	0,009	0,007	0,007	0,009	0,006	0,005	0,004	0,003
J01EC	Intermediate-acting sulphonamides	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,001	0,000	0,000
J01EE	Sulphonamides + trimethoprim	0,035	0,034	0,033	0,029	0,030	0,030	0,026	0,024	0,024	0,022
J01FA	Macrolides	0,042	0,040	0,040	0,037	0,039	0,041	0,037	0,038	0,034	0,034
J01FF	Lincosamides	0,030	0,031	0,031	0,029	0,033	0,035	0,032	0,031	0,032	0,028
J01GB	Aminoglycosides	0,038	0,039	0,041	0,048	0,055	0,058	0,054	0,044	0,045	0,044
J01MA	Fluoroquinolones	0,115	0,121	0,124	0,139	0,129	0,138	0,127	0,124	0,116	0,112
J01MB	Other quinolones	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,000	0,000	0,000
J01XA	Glycopeptide antibacterials	0,010	0,011	0,011	0,012	0,015	0,016	0,017	0,017	0,018	0,018
J01XB	Polymyxins	0,005	0,005	0,006	0,008	0,009	0,006	0,003	0,002	0,003	0,002
J01XD	Imidazole derivatives	0,024	0,027	0,027	0,025	0,026	0,030	0,027	0,029	0,030	0,030
J01XE	Nitrofuran derivatives	0,017	0,016	0,018	0,016	0,017	0,018	0,015	0,018	0,016	0,018
J01XX08	Linezolid	0,001	0,001	0,000	0,001	0,001	0,001	0,001	0,001	0,001	0,001
	other antibiotics	0,001	0,001	0,001	0,001	0,002	0,001	0,001	0,001	0,001	0,001
J01	**Antibiotics for systemic use (total)**	**0,931**	**0,965**	**0,952**	**0,941**	**1,008**	**1,061**	**0,971**	**0,963**	**0,951**	**0,954**

Figure 3.5 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, 2005-2014 (Source: SWAB)

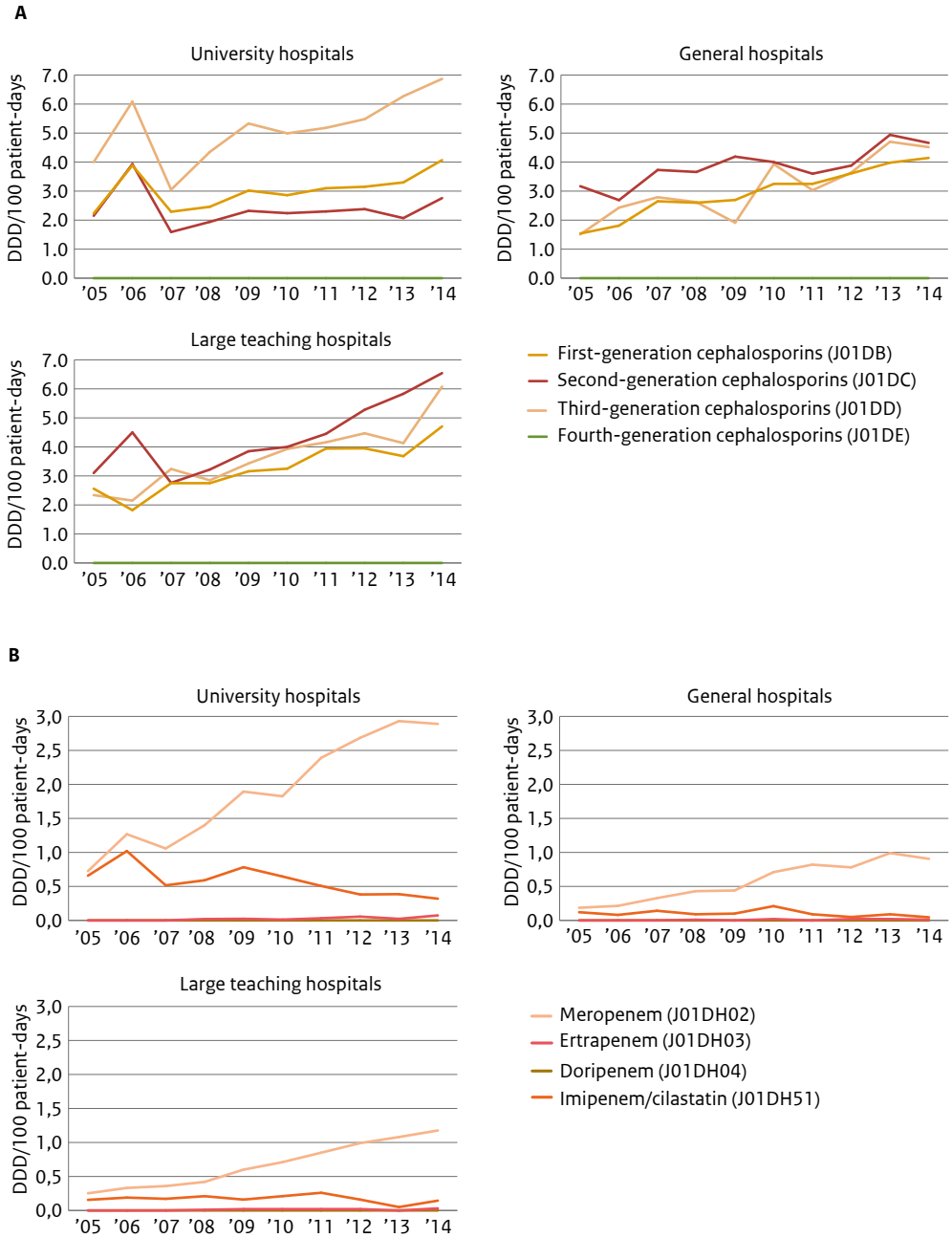
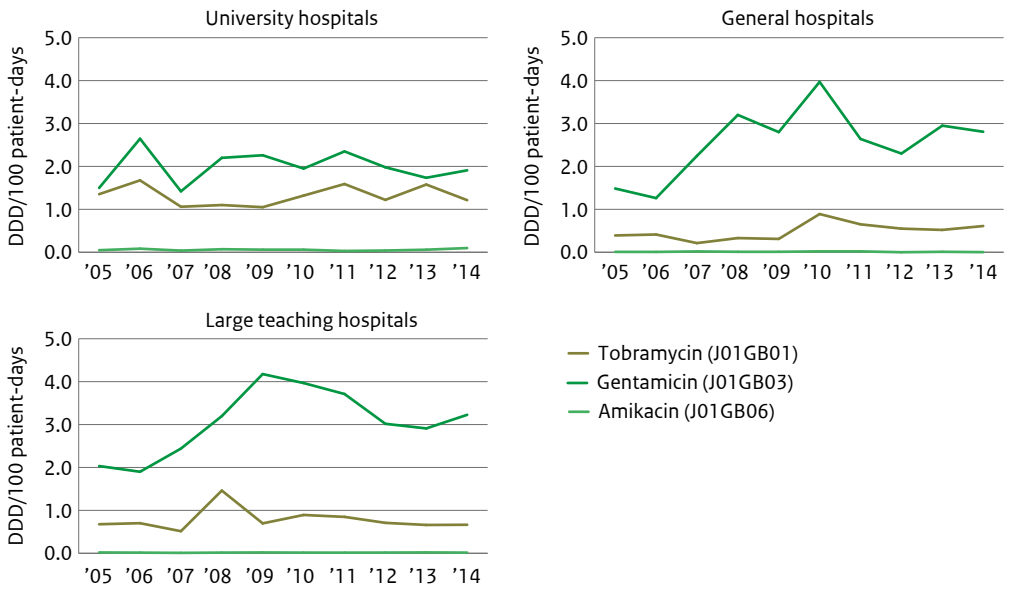


Figure 3.5 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2005-2014 (Source: SWAB)

C



D

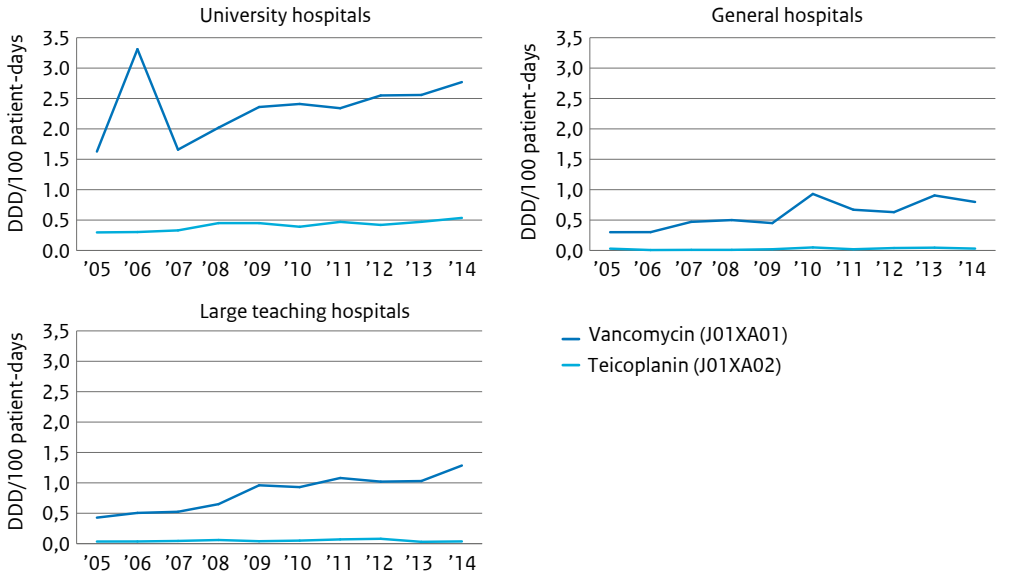
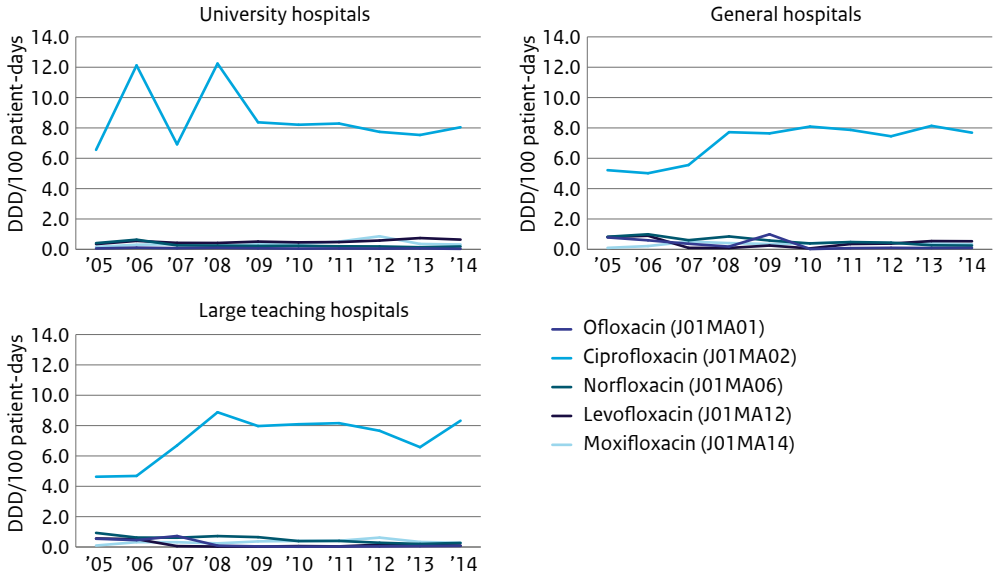


Figure 3.5 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2005-2014 (Source: SWAB)

E



Discussion

In 2014, we observed a further intensification of antibiotic use in hospitals. Overall, antibiotic use increased by almost 5% when measured in DDD/100 patient-days and 4% when expressed in DDD/100 admissions, compared to 2013. There are significant shifts between different subgroups of antibiotics. Mainly, the use of cephalosporins continues to rise. In more detail, university hospitals tend to use more third-generation cephalosporins, whereas large teaching hospitals, use more second-generation cephalosporins every year. The increase in use of third-generation cephalosporins might be explained by the use of cefotaxim for selective decontamination of the digestive tract, a procedure commonly used in the Netherlands on intensive care units. That the use of meropenem did not increase further we consider hopeful. More worrying is that after a decrease in use in the last two years, the use of quinolones started to rise again in 2014.

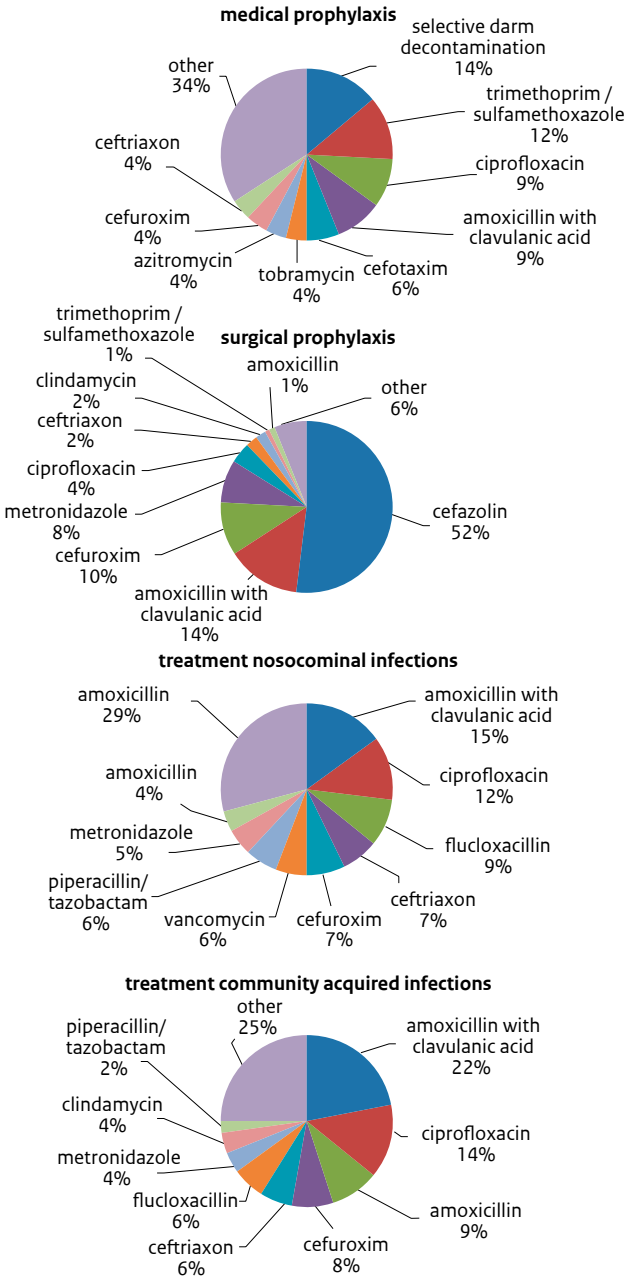
On the other hand, use of ciprofloxacin as medical prophylaxis diminished from 13 to 9% of the cases.

Table 3.4. Use of antimycotics. antimycobacterials and antivirals for systemic use (J02. J04. J05) in university hospitals (DDD/100 patient-days). 2007-2014 (Source: SWAB).

ATC Group*	Therapeutic group	2007	2008	2009	2010	2011	2012	2013	2014
J02AA01	Antibiotics (amphotericin B)	4.44	1.12	1.35	1.65	1.77	2.43	3.01	3.46
J02AB02	Imidazole derivatives (ketoconazole)	0.12	0.11	0.08	0.15	0.09	0.10	0.06	0.24
J02AC	Triazole derivatives	5.18	6.36	6.72	6.31	5.83	6.25	6.29	7.15
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.19	0.40	0.61	0.56	0.57	0.55	0.71	0.61
J02	**Antimycotics for systemic use (total)**	**9.93**	**7.98**	**8.77**	**8.66**	**8.26**	**9.33**	**10.06**	**11.47**
J04AA	Aminosalicylic acid and derivatives	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
J04AC	Hydrazides (mainly isoniazide)	0.39	0.29	0.40	0.34	0.30	0.40	0.57	0.56
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
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
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.04
J04BA	Drug for treatment of leprosy (dapson)	0.53	0.39	0.33	0.45	0.49	0.62	0.70	0.60
J04	**Antimycobacterials for systemic use (total)**	**2.74**	**2.33**	**2.35**	**2.58**	**2.62**	**2.57**	**2.88**	**2.87**
J05AB	Nucleosides excl. Reverse transcriptase inhibitors (J05AB)	1.72	2.00	2.22	2.02	2.18	2.24	2.33	2.71
J05AD	Phosphonic acid derivatives (J05AD)	0.06	0.11	0.13	0.10	0.10	0.15	0.12	0.16
J05AE	Protease inhibitors (J05AE)	0.70	0.92	0.75	0.78	0.55	0.81	0.63	0.40
J05AF	Nucleoside reverse transcriptase inhibitors (J05AF)	0.83	0.74	0.64	0.67	0.63	0.69	0.54	0.59
J05AG	Non-nucleoside reverse transcriptase inhibitors (J05AG)	0.20	0.25	0.23	0.22	0.14	0.18	0.16	0.18
J05AH	Neuraminidase inhibitors (J05AH)	0.02	0.05	n.a.#	0.21	0.42	0.19	0.49	0.16
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
J05AX	Other antivirals (J05AX)	0.00	0.06	0.06	0.15	0.17	0.24	0.29	0.22
J05	**Antivirals for systemic use (total)**	**3.86**	**4.65**	**4.59**	**4.91**	**4.89**	**5.41**	**5.47**	**5.37**

** from the 2014 edition of the Anatomical Therapeutic Chemical (ATC) classification system*
 # Total use not to be assesed because of alternative distribution during the pandemic

Figure 3.6 Distribution of the use of antibiotics for systemic use (J01) ; results of the point-prevalence studies 2015 (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 the same method as described in the intramural methods.

Results

Over 2014, data from 38 nursing homes were received. The size of these homes varied from 35 to 1150 residents per home, with a mean of 346 residents. In total, the antibiotic use of 8722 residents was included. The use of antibiotics varied hugely with a minimum of 17 and a maximum of 121 DDD/1000 residents/day. The mean use was 57.3 DDD/1000 residents/day. Combinations of penicillins (mainly amoxicillin with clavulanic acid), with 17.7 DDD/1000 residents/day, nitrofurantoin derivates (10.6 DDD/1000 residents/day) and fluoroquinolones (8.6 DDD/1000 residents/day) were most frequently used (Table 3.5).

Figure 3.7 depicts antibiotics used in the prevalence study in 60 nursing homes in 2015.

A total of 6989 residents were participating, of which 333 patients on antibiotics, with a total of 353 prescriptions Nitrofurantoin is the antibiotic used the most (27% of the total antibiotic use), followed by amoxicillin with clavulanic acid and ciprofloxacin with 17% and 13% respectively.

Discussion

Compared with previous years, by and large the same pattern of usage is seen. The most frequently used antibiotic is amoxicillin with clavulanic acid (31 %), followed by nitrofurantoin (19%) and fluoroquinolones (15%).

Notable is the relatively lower use of tetracyclines (8%) compared to the use in outpatients. 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 the SWAB surveillance data, with nitrofurantoin as most frequently prescribed antibiotic. SNIV data are based on prescriptions on an index day, whereas overall use is based on DDD's collected over 365 days.

Figure 3.7 Distribution of the use of antibiotics for systemic use (J01) in nursing homes; results of the point-prevalence studies 2015 (Source: SNIV)

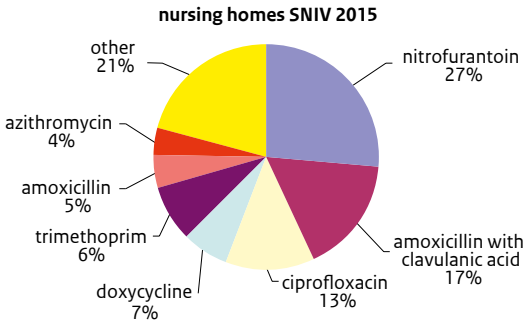
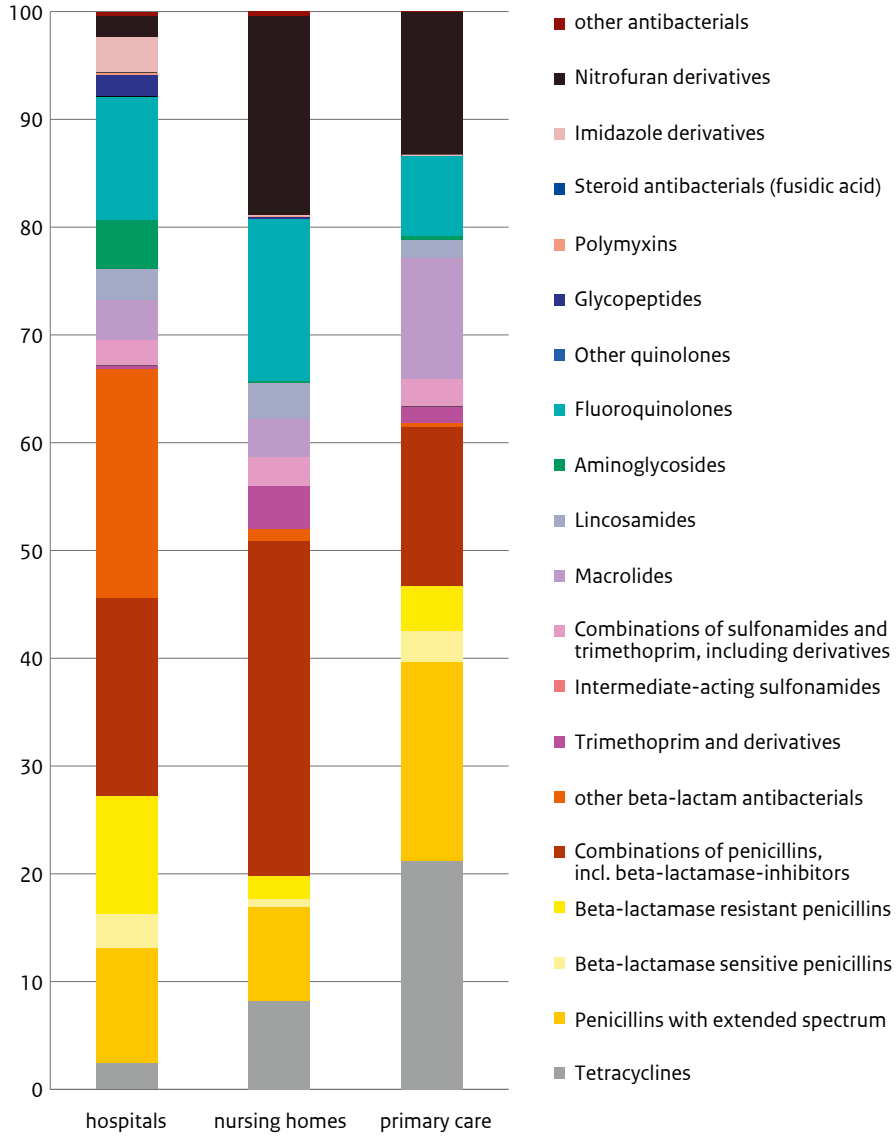


Table 3.5. Distribution of the use of antibiotics (J01) in nursing homes. expressed as DDD/1000 residents/day. 2011-2014 (Source: SWAB).

ATC Group*	Therapeutic group	2011	2012	2013	2014
J01AA	Tetracyclines	5.4	6.8	7.2	4.7
J01CA	Penicillins with extended spectrum	4.9	6.6	5.0	5.0
J01CE	Beta-lactamase sensitive penicillins	0.3	0.2	0.4	0.4
J01CF	Beta-lactamase resistant penicillins	2.5	3.7	1.6	1.3
J01CR	Combinations of penicillins. incl. beta-lactamase-inhibitors	18.6	18.1	18.9	17.7
J01DB -DE	Cephalosporins	0.7	1.3	1.1	0.7
J01DF	Monobactams	0.0	0.0	0.0	0.0
J01DH	Carbapenems	0.1	0.0	0.0	0.0
J01EA	Trimethoprim and derivatives	2.3	2.0	2.7	2.2
J01EC	Intermediate-acting sulfonamides	0.1	0.1	0.0	0.0
J01EE	Combinations of sulfonamides and trimethoprim. including derivatives	3.5	2.7	1.3	1.5
J01FA	Macrolides	2.1	2.4	2.4	2.1
J01FF	Lincosamides	3.7	4.5	2.2	1.9
J01GB	Aminoglycosides	0.1	0.1	0.0	0.1
J01MA	Fluoroquinolones	10.5	11.2	7.9	8.6
J01MB	Other quinolones	0.2	0.0	0.0	0.0
J01XA	Glycopeptides	0.1	0.1	0.1	0.1
J01XB	Polymyxins	0.4	0.4	0.0	0.0
J01XC	Steroid antibacterials (fusidic acid)	0.0	0.0	0.0	0.0
J01XD	Imidazole derivatives	0.1	0.1	0.0	0.1
J01XE	Nitrofuran derivatives	10.8	12.8	13.7	10.6
J01XX	other antibacterials	0.5	0.7	0.4	0.2
J01	**Antibiotics for systemic use (total)**	**67.0**	**73.8**	**65.0**	**57.2**

Figure 3.8 Comparison of the distribution of antibiotic usage (J01) in primary care, hospital care and care in nursing homes in 2013.



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; 67: 2283-2288

4 Surveillance of resistance

4.1 Methods and description of ISIS-AR data

4.1.1 Methods

Infectious Disease Surveillance Information System for Antimicrobial Resistance (ISIS-AR)
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 Disease Surveillance Information System for Antimicrobial Resistance (ISIS-AR). This surveillance system is a combined initiative of the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM), and is coordinated by the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) in Bilthoven. In 2015, ISIS-AR received data from 37 laboratories of which 23 laboratories had complete data over the five most recent years (2011 to 2015). Three of these laboratories served university hospitals, 19 laboratories served non-university hospitals and general practitioners and one laboratory only served general practitioners. To avoid bias in time trends due to incomplete data we used data from these 23 laboratories only for most analyses in the current report. We calculated resistance percentages and linear time trends over the five most recent years (2011 to 2015) 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 cattharalis*) we used data from 26 laboratories for which we had at least complete data in 2015 and that were known to use EUCAST recommendations (3 serving university hospitals, 22 serving non-university hospitals and general practitioners and 1 serving general practitioners only). For *Escherichia coli* isolates from general practitioner's patients an extra analysis was conducted to calculate resistance to a selection of antibiotics in 2015 by NUTS3-region. For this analysis we used data from a separate set of 23 non-university laboratories for which we had complete data in 2015.

Selection of isolates

Resistance levels and time trends were calculated as the percentage resistant isolates by site; i.e. general practice (GP), outpatient departments (OPD), inpatient departments (excl. intensive care units), intensive care units, and urology departments. For GP (chapter 4.2) we selected urinary isolates for analysis of resistance in Enterobacteriaceae, and wound/pus for analysis of resistance in *S. aureus*. For urology departments (chapter 4.3.5) we selected only urinary isolates. For the OPD (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 patients in inpatient hospital departments (incl. intensive care units, chapter 4.3.4). Finally, for the analysis on respiratory pathogens (*Haemophilus influenzae, Streptococcus pneumoniae*, and *Moraxella catarrhalis*) we selected isolates from blood, cerebrospinal fluid, higher respiratory tract, and lower respiratory tract (chapter 4.3.6).
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 isolates that were cultured for screening and inventory purposes. Furthermore, to avoid bias due to selective testing, for each pathogen-agent combination we included only data from laboratories in which at least 50% of isolates was tested for that specific agent. Finally, for representativeness of the results, the resistance level and time trend of each pathogen-agent combination is only shown 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 the variance in the 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 2015 breakpoints. In the tables presenting resistance levels we mentioned whether EUCAST indicates that breakpoints apply to specific diagnoses or methods of administration. However, for co-amoxiclav the MIC breakpoint for uncomplicated urinary tract infection could not be used to re-interpret the data because the maximum testvalue 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 used the co-amoxiclav breakpoint for non-uncomplicated urinary tract infections only.
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 2015 clinical breakpoints. Reasons that no reinterpretation could be achieved were a lack of raw data or a value that was not compatible with current breakpoints. For *Enterococcus faecium, Enterococcus faecalis, Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis* less than 75% of the MICs could be reinterpreted. Therefore the S/I/R interpretations, as reported by laboratories for which it was known that they used EUCAST recommendations in 2015, were used for calculating the percentage of resistant isolates.
Because testvalues of inducible clindamycin resistance tests were not available in ISIS-AR, clindamycin resistance in *S. aureus* was calculated both using reinterpreted MIC-values, which do not show inducible resistance, as well as 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/oxacillin results based on cefoxitin screening methods, resistance to flucloxacillin in *S. aureus* was estimated based on interpretation from the laboratories for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin.

In some tables, resistance levels are presented for a combination of agents against which comparable resistance mechanisms exist, namely amoxicillin/ampicillin, cefotaxime/ceftriaxone, meropenem/imipenem, and doxycycline/tetracycline. For these combinations, we calculated the resistance percentage against 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* 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 *S. aureus*.
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 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 agents per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/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 GP and urology outpatient departments, multidrug resistance in Enterobacteriaceae was calculated, defined as resistance to all of the following oral agents: co-trimoxazole, co-amoxiclav and ciprofloxacin.

Calculation of time trends

In addition to resistance levels in 2015, we calculated time trends over the five most recent years (2011 to 2015), 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 MICs were interpretable using EUCAST clinical breakpoints (i.e. *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus cloacae, Pseudomonas aeruginosa, Acinetobacter* spp. and *Staphylococcus aureus* and coagulase-negative staphylococci including *Staphylococcus epidermidis*). Because in *S. aureus* clindamycin resistance including inducible resistance is based on laboratory S/I/R interpretation adoption of EUCAST guidelines instead of CLSI guidelines by the laboratories could cause false time trends. We avoided this by changing the interpretation from intermediate to resistant if the MIC-value was >0.5 mg/l. Both CLSI and EUCAST did not change breakpoints for clindamycin since 2011, and use of different versions of the recommendations will therefore not cause a false time trend. With regard to flucloxacilline/oxacillin resistance, both CLSI and EUCAST did not change breakpoints for cefoxitin and oxacillin since 2011, and use of different versions of the recommendations will therefore not cause a false time trend.

Two sided p-values <0.05 were considered statistically significant. For estimation of clinical relevance the predicted resistance levels from the logistic model were used. If resistance in 2015 was below 10%, a change of ≥2.5% in the last 5 years was considered clinically relevant. If resistance in 2015 was above 10%, a change of ≥5% was considered clinically relevant. Statistically significant increasing trends that are considered clinically relevant are shown in the tables as a red coloured font, whereas decreasing trends that met 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 2011 to 2015 are shown in graphs.

4.1.2 Description of the ISIS-AR data

In the current 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 data could not be included in the current report although they were connected to the ISIS-AR surveillance system (see methods section for inclusion criteria). Therefore, laboratories included or excluded from analyses in the current report are given separate colours. In figure 4.1.2.2 the percentage of residents for whom at least one isolate was included in the analyses for the current report 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 table 4.1.2.1 some important descriptive characteristics are compared between laboratories for which data could be included in the analyses for the current report, and those for which data could not be included. 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, by institution type, is presented in figure 4.1.2.4.

Figure 4.1.2.1 Distribution of laboratories over the country by connection status

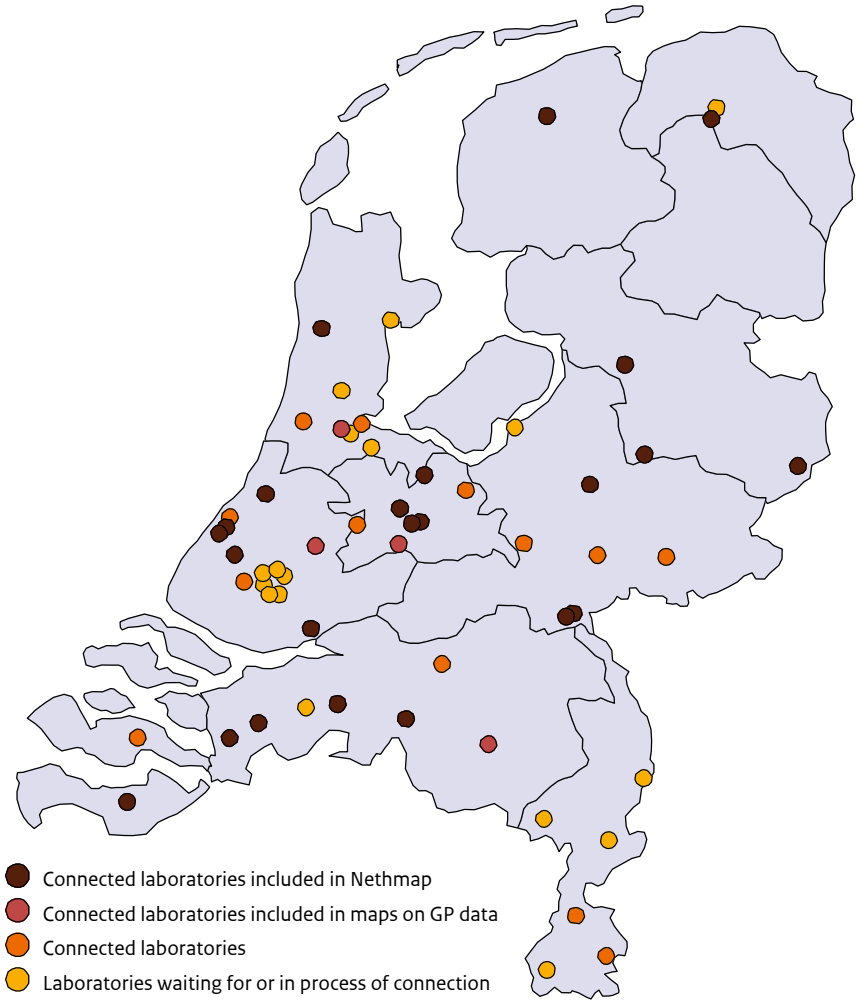


Figure 4.1.2.2 Percentage of residents (%) for whom at least one isolate was included in the analyses for the current report, by 4-digit postal code area

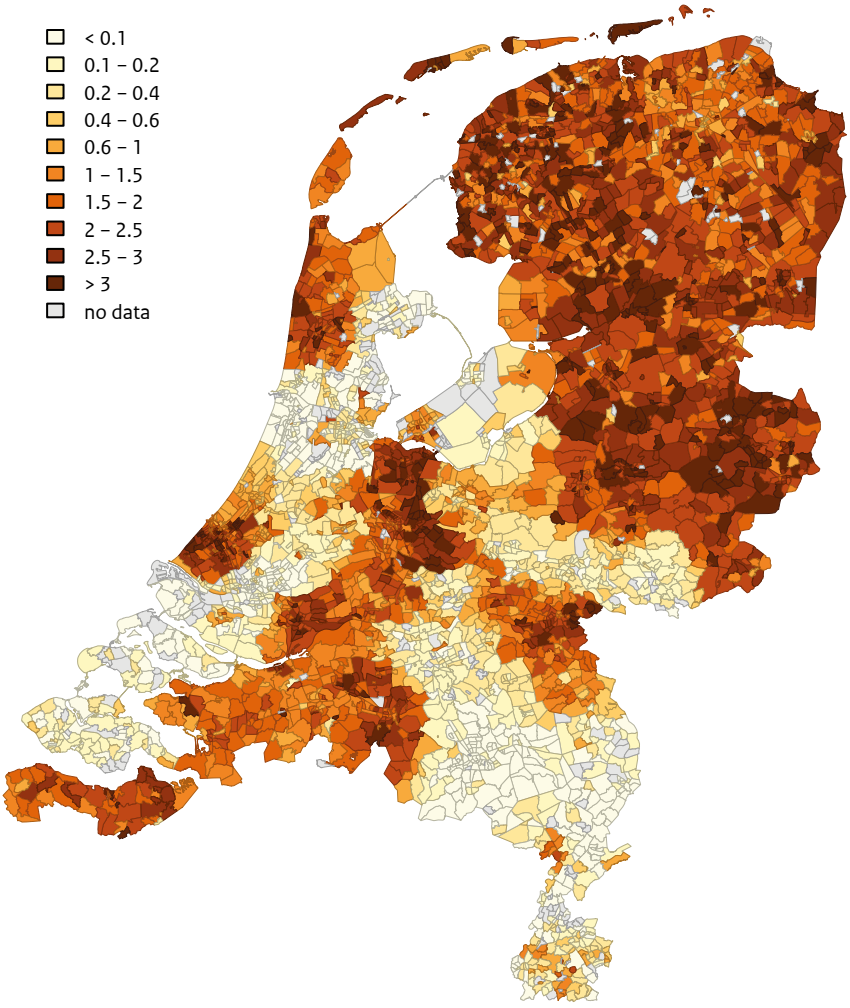


Figure 4.1.2.3 Percentage of residents (%) for whom at least one isolate from a general practitioner's patient was included in the analyses for the current report, by 4-digit postal code area

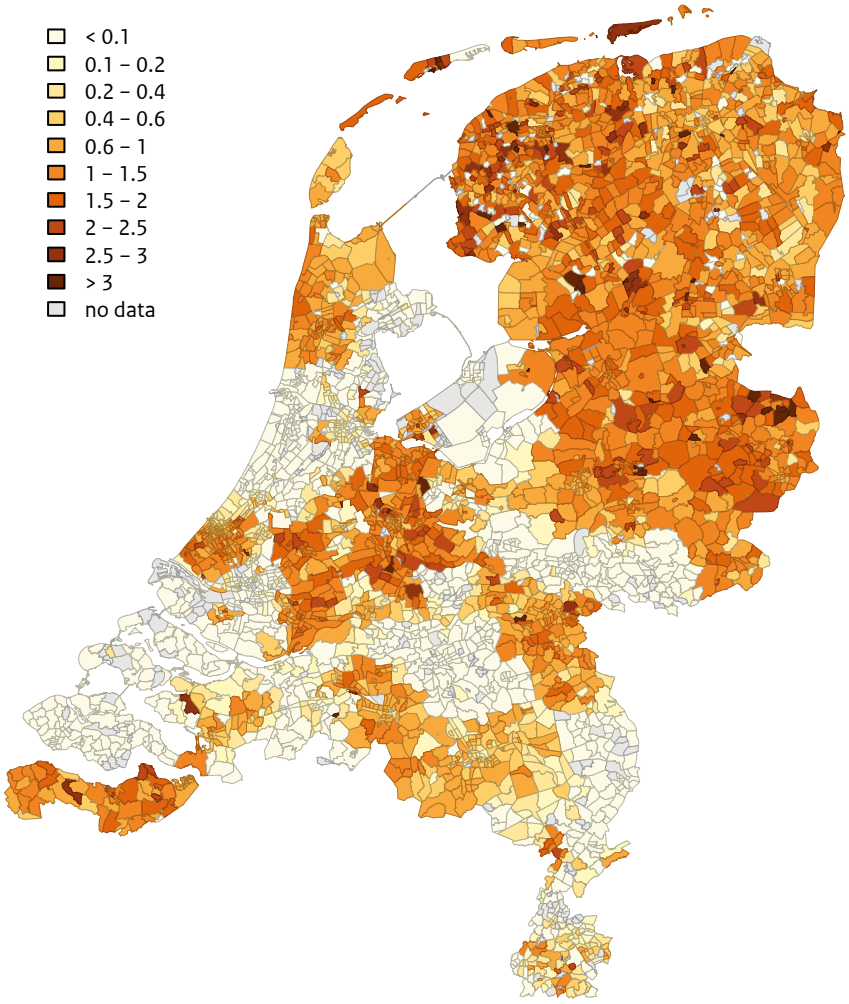


Table 4.1.2.1 Characteristics of isolates in 2015 from 23 laboratories included in the time trend analyses (laboratories that continuously reported to the ISIS-AR database from 2011 to 2015) and 14 laboratories excluded from the time trend analyses (laboratories that started reporting later than 2011, or that did not continuously report until 2015)

	Included	Excluded
Total number of isolates	298449	102934
Mean number of isolates per laboratory	12976	7352
Pathogen		
E. coli	35	33
K. pneumoniae	5	5
E. cloacae	2	2
P. mirabilis	4	4
P. aeruginosa	5	5
Acinetobacter spp.	1	1
E. faecalis	6	6
E. faecium	1	1
S. aureus	11	12
CNS	5	6
S. pneumoniae	1	2
H. influenzae	3	3
M. catarrhalis	1	1
Other Enterobacteriaceae*	8	7
Other non-fermenters**	1	1
Other gram-positives	10	11
Sex of patient		
Male	40	41
Female	60	59
Type of care		
GP	43	34
Outpatient departments	25	28
Inpatient departments (excl. Intensive Care Units)	28	34
Intensive Care Units	5	4
Age category of patient (y)		
0-4	4	3
5-18	5	4
19-64	38	36
>65	53	56

Table 4.1.2.1 (continued) Characteristics of isolates in 2015 from 23 laboratories included in the time trend analyses (laboratories that continuously reported to the ISIS-AR database from 2011 to 2015) and 14 laboratories excluded from the time trend analyses (laboratories that started reporting later than 2011, or that did not continuously report until 2015)

	Included	Excluded
Isolate source		
Blood	5	8
Lower respiratory tract	8	9
Urine	58	54
Wound/Pus	15	14
Other sterile	13	15
Type of hospital		
Not applicable (GP) or missing data	43	35
Non-university hospital	49	64
University hospital	9	0

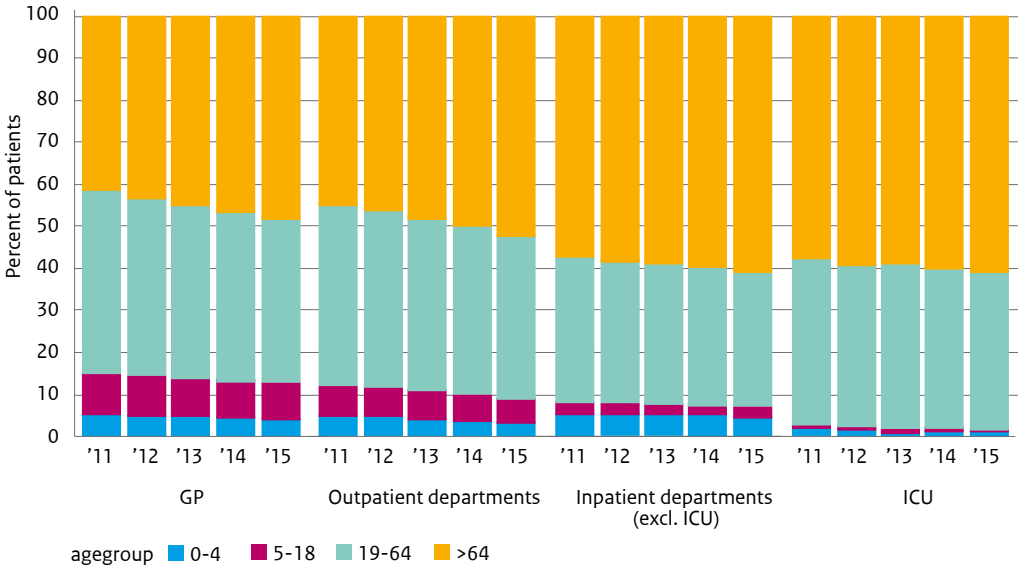
Values are percentages of the total number of isolates unless indicated otherwise
Only the first clinical isolate per patient was included
* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae).*
** *Pseudomonas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

Table 4.1.2.2 Characteristics of 241794 isolates in ISIS-AR in 2015, 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	105512	15379	6391	13239	13596	3211	16609	3714	34202	16138	4040	7590	2173
Sex of patient													
Male	27	33	53	40	52	49	50	50	52	50	54	53	52
Female	73	67	47	60	47	51	50	50	48	50	46	47	48
Type of care													
GP	60	48	31	49	32	51	38	8	25	19	10	13	13
Outpatient departments	16	21	25	23	32	26	26	11	42	15	28	41	39
Inpatient departments (excl. Intensive Care Units)	21	27	36	25	30	19	33	58	29	55	53	39	42
Intensive Care Units	3	4	8	3	5	4	4	23	4	11	9	7	6
Age category of patient (y)													
0-4	4	2	4	3	3	4	4	1	5	4	8	8	9
5-18	7	2	3	2	6	5	3	1	8	6	4	4	3
19-64	37	30	32	24	30	34	31	30	44	43	37	37	32
>65	52	66	61	70	60	57	62	68	43	47	51	51	57
Isolate source													
Blood	3	3	3	1	2	2	3	10	4	37	25	1	0
Lower respiratory tract	2	6	12	3	17	7	1	4	10	0	57	83	87
Urine	86	78	49	76	39	53	80	49	12	32	1	0	0
Wound/Pus	4	6	24	12	20	24	13	28	43	22	7	4	4
Other sterile	5	6	12	7	22	14	4	9	32	9	11	11	9
Type of hospital													
Not applicable (GP) or missing data	61	48	31	49	33	51	38	8	25	19	10	13	13
Non-university hospital	34	43	57	45	55	42	53	75	63	65	79	73	74
University hospital	5	8	13	6	13	7	9	18	12	16	10	14	12

Values are percentages of the total number of isolates unless indicated otherwise.

Figure 4.1.2.4 Age distribution of patients, by year and institution type



Key results

- Laboratories that were included for analyses in the current report are well distributed throughout the country, although the number of laboratories with complete data in Noord-Holland, and Limburg was relatively low (Figure 4.1.2.1).
- The laboratories included in Nethmap are reflected in the coverage data of Nethmap (Figure 4.1.2.2). The coverage is high in the northern part of the Netherlands, and low in Noord-Holland, Limburg and Zeeland. This pattern can also be found in figure 4.1.2.3 displaying coverage of isolates from general practitioner's patients, although in this figure also low coverage is seen in Noord-Brabant.
- Data were largely comparable between laboratories for which data could be included in the analyses and those for which that was not possible (table 4.1.2.1). However, because the one laboratory that only serves general practitioners, and that delivers data on a very large amount of isolates, was present in the included group, the percentage of isolates from general practitioners was higher among the included laboratories than among the excluded laboratories (43% versus 34%). Furthermore, because all laboratories connected to ISIS-AR that serve university hospitals were included, the percentage of isolates from that type of hospitals was larger in the included group (10 vs. 0%). However, due to large numbers in our database we do not expect that the overall resistance percentages will be substantially different with this type of laboratories included.
- Most pathogens were isolated from patients older than 65 years (41-70%, depending on the pathogen, table 4.1.2.2).
- Mean age of general practitioner's patients and patients in outpatient departments is somewhat lower than in hospital departments (figure 4.1.2.4). However, over the years the proportion of patients aged >65 years has increased (41% in 2011 to 48% in 2015 for GP, 44-52% in outpatient departments, 57-61% in inpatient departments excluding intensive care units, and 58-61% in intensive care units).
- Enterobacteriaceae were more often isolated from female patients (e.g. 73% of *E. coli* and 67% of *K. pneumoniae* in women), likely because women are more prone to urinary tract infections (table 4.1.2.2). Isolates of the other pathogens were more evenly distributed over men and women.
- The percentage of women was relatively large in GP populations (~74%), whereas in ICU departments the percentage of men was relatively high (~60%). However, the distributions have remained stable over time (data not shown).
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *S. aureus* were more often isolated from patients from general practitioners and outpatient departments (56-77%, depending on the pathogen, table 4.1.2.2), whereas the main part of *E. faecium* and coagulase negative Staphylococci was sampled in the hospital (81% and 66% respectively).
- Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *E. faecium*, and *E. faecalis* were mainly isolated from urine (39-86%, depending on the pathogen), whereas *S. aureus* was mainly isolated from wound or pus (43%), and *H. influenzae*, *S. pneumoniae*, *M. catarrhalis* from the respiratory tract (57-87%, 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* in GP patients, only wound and pus isolates were included. 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 GP patients'.

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 these isolates in 2015 are presented in table 4.2.3 and table 4.2.4. Five-year trends in resistance are shown in figure 4.2.1 and figure 4.2.2 for the respective pathogens. These 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 for a selection of antibiotics.

Table 4.2.1 Distribution of isolated pathogens N (%) in clinical urinary isolates from selected general practitioner's patients, presented by age category, ISIS-AR 2015

Pathogen	Age≤12 N (%)	Age>12 N (%)
E. coli	6632 (70)	57158 (56)
K. pneumoniae	125 (1)	7103 (7)
P. mirabilis	470 (5)	5702 (6)
P. aeruginosa	162 (2)	2457 (2)
S. aureus	112 (1)	1941 (2)
Other Enterobacteriaceae*	379 (4)	8569 (8)
Other non-fermenters**	148 (2)	1904 (2)
Enterococcus spp.	922 (10)	8697 (8)
Other gram-positives	542 (6)	9230 (9)

* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), and Klebsiella spp. (non-pneumoniae).*

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

Table 4.2.2 Distribution of isolated pathogens N (%) in clinical wound and pus isolates from selected general practitioner's patients, ISIS-AR 2015

Pathogen	N (%)
S. aureus	2842 (51)
Other gram-positives	829 (15)
Enterobacteriaceae*	1237 (22)
Other non-fermenters**	711 (13)

* *Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp., Morganella spp., Citrobacter spp., Serratia spp., Providencia spp.*

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

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, presented by age category, ISIS-AR 2015

	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	65	4	73	3	74	4	79
Antibiotic								
amoxicillin/ampicillin	36	40	-	-	16	21	-	-
co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	12	16	6	8	7	8	-	-
cefuroxime	3	7	7	13	1	1	-	-
cefotaxime	2	3	0	4	1	1	-	-
ceftazidime*	1	2	1	3	0	0	0	2
ciprofloxacin	3	10	1	4	4	8	1	7
norfloxacin	7	15	5	22	6	11	-	-
gentamicin	3	4	0	1	5	5	1	2
tobramycin	2	4	1	3	3	3	2	1
fosfomycin	1	1	11	31	12	16	-	-
trimethoprim - *according to breakpoint for uncomplicated urinary tract infection*	22	26	13	21	25	35	-	-
co-trimoxazole	21	24	10	11	21	28	-	-
nitrofurantoin - *according to breakpoint for uncomplicated urinary tract infection*	1	2	-	-	-	-	-	-
Multi-drug resistance								
HRMO**	2	5	2	5	2	3	-	-
multidrug-resistance*** - *for co-amoxiclav according to breakpoint for non-uncomplicated urinary tract infection*	1	3	3	2	0	1	-	-

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted

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

- *Resistance not calculated.*
* *For P. aeruginosa the breakpoints used to calculate resistance to ceftazidime relate to high dose therapy.*
** *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for Enterobacteriaceae as resistant to cefotaxim/ceftriaxone or ceftazidim as indicator compounds for the production of Extended-spectrum beta-lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides.*
*** *MultiDrug Resistance (MDR), defined as resistance to all of the following oral agents: co-trimoxazole, co-amoxiclav and ciprofloxacin.*

Table 4.2.4 Resistance levels (%) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients, ISIS-AR 2015

	S. aureus
Antibiotic	
oxacillin/flucloxacillin*	3
ciprofloxacin** *- according to breakpoint for high dose therapy*	6
erythromycin	10
clindamycin	2
clindamycin including inducible resistance***	8
doxycycline/tetracycline	5
fusidic acid	14
co-trimoxazole	4

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(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 flucloxacillin/oxacillin (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 4.2.1 Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from selected general practitioner's patients in ISIS-AR, presented by age category.

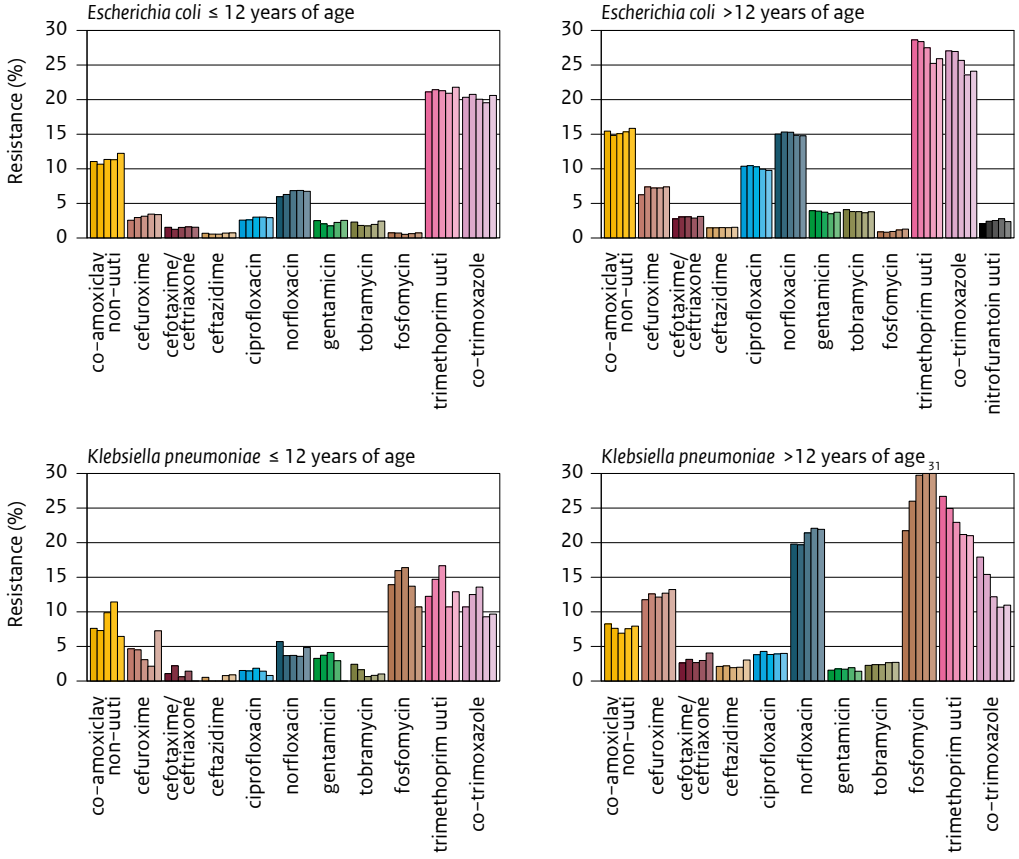
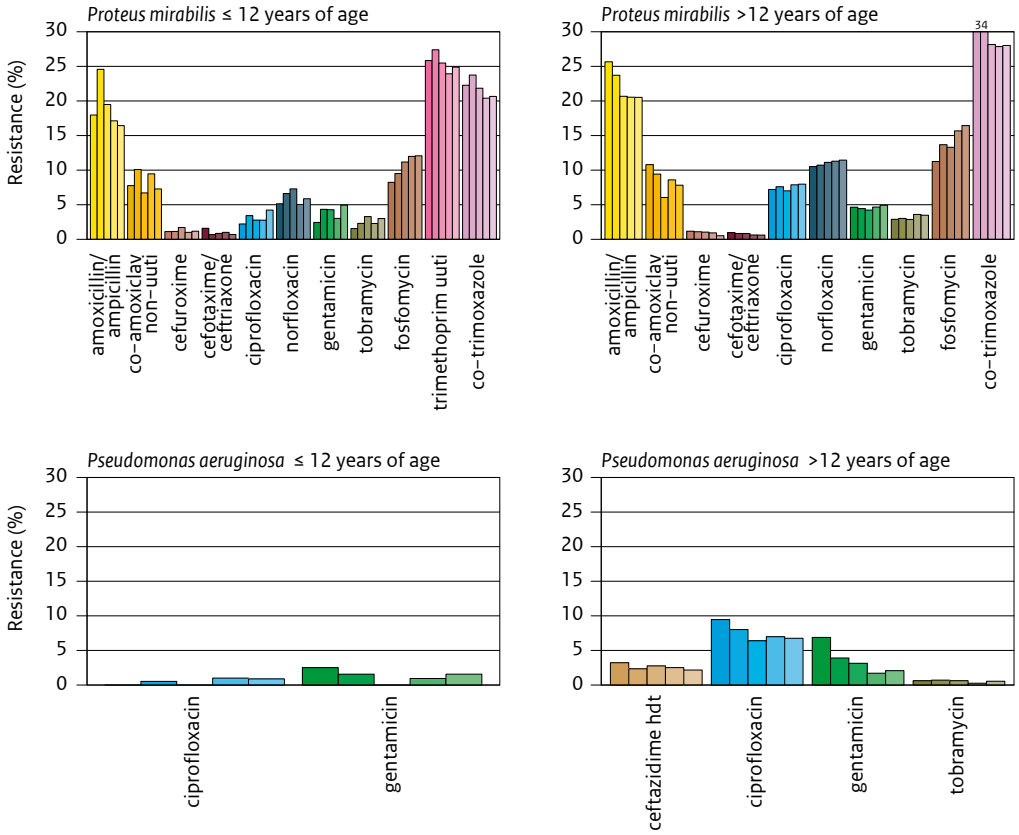
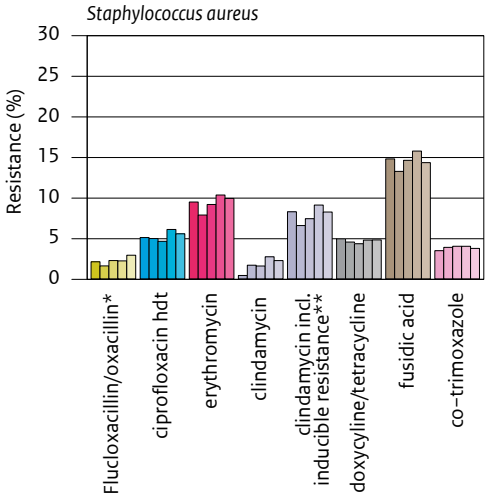


Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from selected general practitioner's patients in ISIS-AR, presented by age category.



uuti=according to breakpoint for uncomplicated urinary tract infection,
non-uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy

Figure 4.2.2 Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients in ISIS-AR.

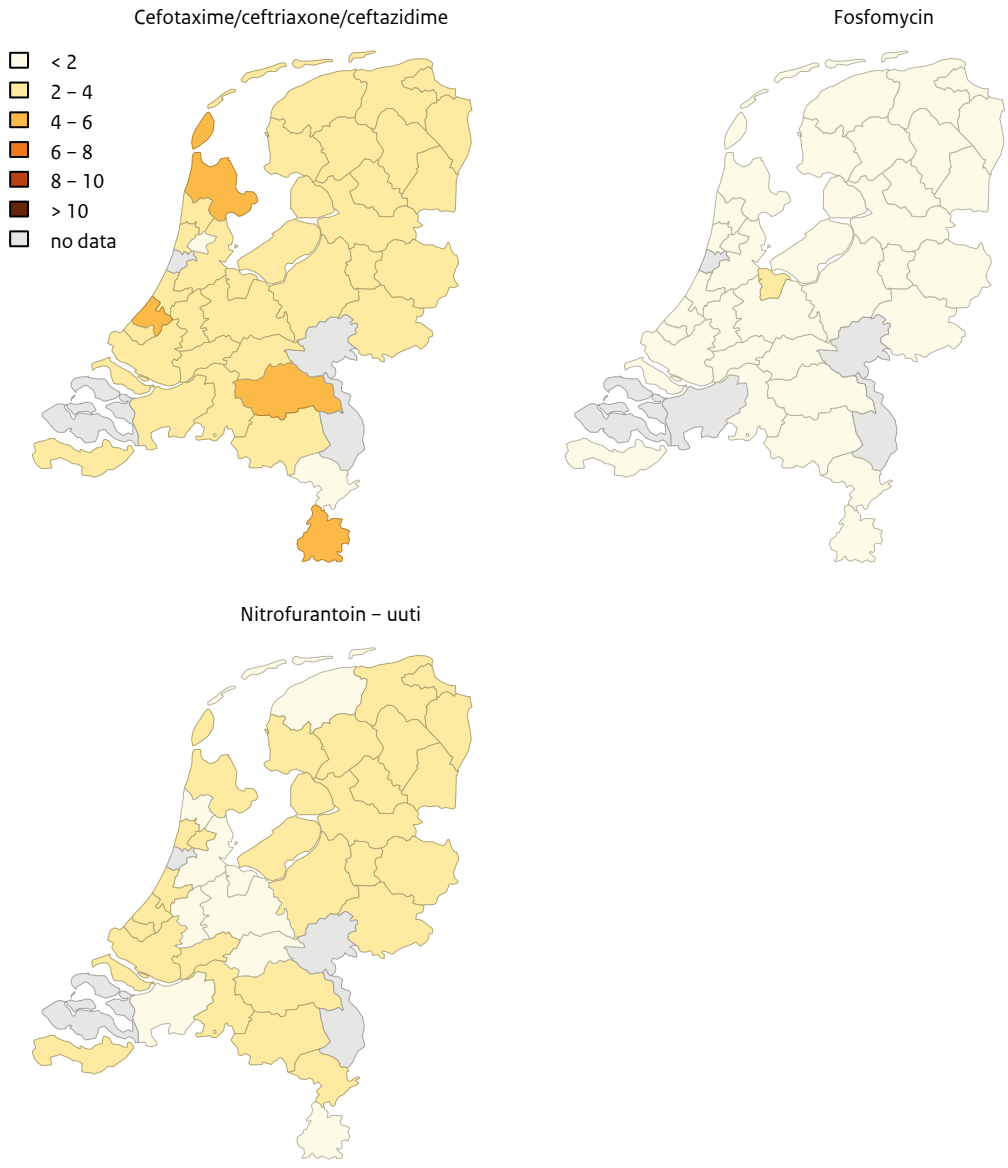


hdt=according to breakpoints for high dose therapy

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

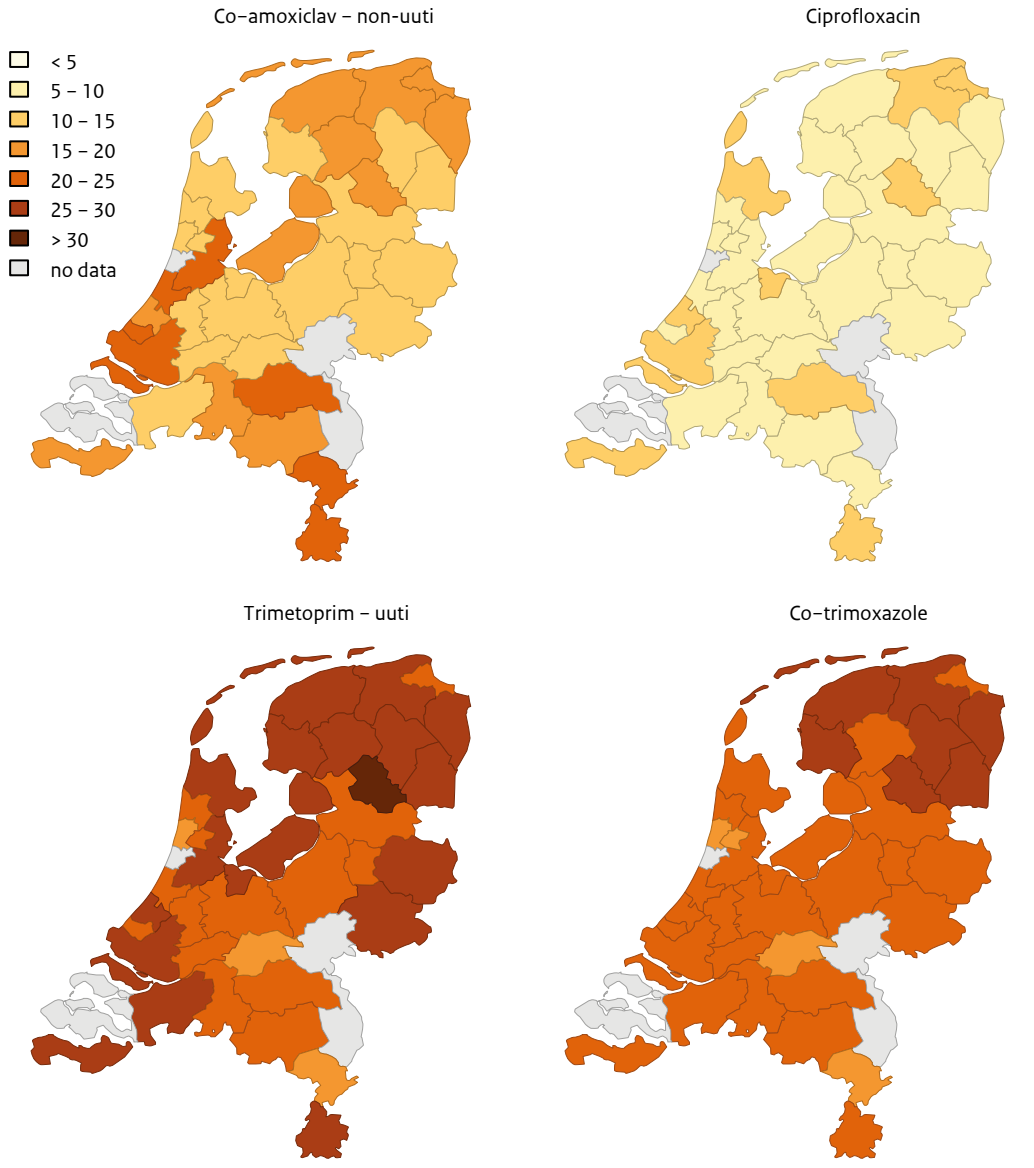
** *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see methods section for more detailed information)*

Figure 4.2.3 Resistance levels for 3rd generation cephalosporins, fosfomycin, and nitrofurantoin among clinical urinary isolates of *E. coli* from selected general practitioner's patients in ISIS-AR, presented by NUTS3-region



uuti=according to breakpoint for uncomplicated urinary tract infection

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, presented by NUTS3-region



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

Key results

- In general, resistance levels in selected GP patients aged >12 years were higher than in patients aged ≤12 years, in particular for fluoroquinolones.

Enterobacteriaceae

- For all Enterobacteriaceae resistance levels for cefuroxime, cefotaxime/ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, and tobramycin were below 8%, except for ciprofloxacin in *E. coli* in patients aged >12 (10%) and cefuroxime in *K. pneumoniae* in patients aged >12 (13%). For nitrofurantoin (<3%) and fosfomycin (1%) resistance levels were only low in both age categories in *E. coli*.
- With regard to isolates from those aged ≤12 years high levels of resistance were found for amoxicillin/ampicillin in *E. coli* (36%), for trimethoprim in *E. coli* (22%), and *P. mirabilis* (25%), and for co-trimoxazol in *E. coli*(21%) and *P. mirabilis* (21%). With regard to isolates from those aged >12 years resistance levels were high for amoxicillin/ampicillin in *E. coli* (40%) and *P. mirabilis* (21%), for norfloxacin in *K. pneumoniae* (22%), for trimethoprim in *E. coli* (26%), *K. pneumoniae* (21%), and *P. mirabilis* (35%), and for co-trimoxazol in *E. coli*(24%) 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, although resistance was still high in 2015 (from 26% in 2011 to 21% in 2015). Also resistance of *P. mirabilis* to co-trimoxazol decreased in this group of patients, from 34% in 2011 to 28% in 2015. In *K. pneumoniae*, although remaining high, trimethoprim and co-trimoxazole resistance levels of decreased significantly and to a clinically relevant extent in patients aged >12 years (from 25% in 2011 to 21% in 2015 for trimethoprim and from 18% in 2011 to 11% in 2015 for co-trimoxazole). A statistically significant and clinically relevant increase from 22% in 2011 to 31% in 2015 was seen for fosfomycin resistance in *K. pneumoniae* in patients aged >12 years.
- The percentage of highly resistant microorganisms (HRMO) and multidrug-resistance (resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin combined) remained relatively low over the last five years in all Enterobacteriaceae (≤5%).
- Resistance levels for *E. coli* were comparable between geographical regions for 3rd generation cephalosporins, fosfomycin, nitrofurantoin, ciprofloxacin, and trimethoprim. For co-trimoxazole and co-amoxiclav, there was some geographical variation in resistance levels, ranging from 15 to 28% for co-trimoxazole with highest resistance percentages found in the northern part of the Netherlands, and from 11 to 23% for co-amoxiclav with highest resistance levels found in the western and southern part of the Netherlands. However, because in several regions of the southern part the number of isolates was low (<300), there is a possibility that the higher resistance percentages for co-amoxiclav are due to chance.

P. aeruginosa

- Resistance levels for all agents were low (≤2%), except for ciprofloxacin in patients aged >12 years (7%).
- A decrease in resistance was seen for gentamicin from 7% in 2011 to 2% in 2015 in patients aged >12 years.

S. aureus

- Resistance levels for each of the selected agents were ≤10% in patients aged ≤12 years, except for fusidic acid (35%).
- In patients aged >12 years, resistance was 10% for erythromycin and 11% for fusidic acid, but ≤8% for all other selected agents.

4.3 Hospital departments

In the analyses for outpatient departments and inpatient departments (including intensive care units), the antimicrobial susceptibility results were based on 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 (chapter 4.3.4), and 2) for urinary isolates from patients in urology departments (outpatient and inpatient departments, 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 2015 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. 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 (N (%)) in clinical specimens from patients attending outpatient departments, ISIS-AR 2015

Pathogen	Lower respiratory tract N (%)	Urine N (%)	Wound or Pus N (%)
E. coli	513 (9)	16676 (44)	1382 (7)
K. pneumoniae	230 (4)	2930 (8)	273 (1)
P. mirabilis	154 (3)	2060 (5)	827 (4)
P. aeruginosa	1065 (19)	1399 (4)	1296 (7)
E. faecalis	1 (0)	3802 (10)	643 (3)
S. aureus	1281 (23)	1286 (3)	7670 (41)
Other Enterobacteriaceae*	785 (14)	4048 (11)	2062 (11)
Other non-fermenters**	480 (9)	567 (1)	520 (3)
Other *Enterococcus* spp.	3 (0)	643 (2)	166 (1)
Other gram-positives	1027 (19)	4738 (12)	3714 (20)

* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), and Klebsiella spp. (non-pneumoniae).*

** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas 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 2015

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	46	-	23	-
co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	20	10	10	-
piperacillin-tazobactam*	5	4	0	6
cefuroxime	12	13	1	-
cefotaxime/ceftriaxone	5	7	1	-
ceftazidime*	3	5	1	3
meropenem/imipenem*	0	0	0	3
ciprofloxacin	16	6	10	7
norfloxacin	23	20	15	-
gentamicin	6	3	6	3
tobramycin	6	4	4	1
trimethoprim - *according to breakpoint for uncomplicated urinary tract infection*	30	21	37	-
co-trimoxazole	28	14	30	-
nitrofurantoin - *according to breakpoint for uncomplicated urinary tract infection*	3	-	-	-
Empiric therapy combinations				
gentamicin + amoxicillin/ampicillin	5	-	5	-
gentamicin + co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	3	2	2	-
gentamicin + cefuroxime	2	2	0	-
gentamicin + cefotaxime/ceftriaxone	1	2	0	-
gentamicin + ceftazidime	1	1	0	0

Table 4.3.1.2 (continued)

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Multi-drug resistance				
HRMO**	8	8	4	1
multidrug-resistance*** *- for co-amoxiclav according to breakpoint for non-uncomplicated urinary tract infection*	5	3	2	-

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

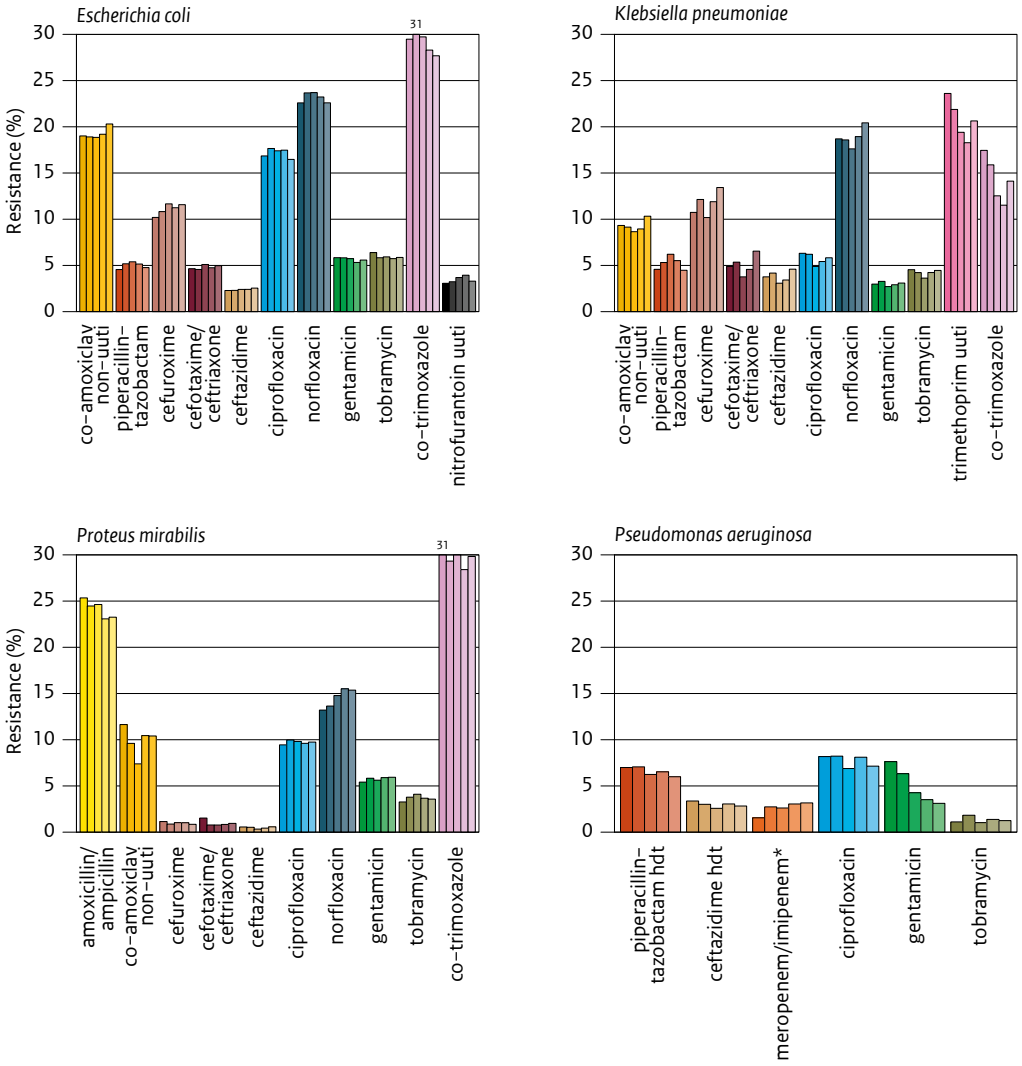
- *Resistance not calculated.*
  Fosfomycin resistance levels are not presented because less than half of the included laboratories tested at least 50% of the isolates for this agent (see methods section for more details on inclusion criteria).

* *For P. aeruginosa the breakpoints used to calculate resistance to piperacillin-tazobactam, ceftazidime, and imipenem relate to high dose therapy.*

** *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for Enterobacteriaceae as resistant to cefotaxim/ceftriaxone 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 ≥ 3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

*** *MultiDrug Resistance (MDR), 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 2011 to 2015) among clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from patients attending outpatient departments in ISIS-AR



uuti=according to breakpoint for uncomplicated urinary tract infection,
non-uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy
* *For P. aeruginosa the breakpoints used to calculate resistance to imipenem relate to high dose therapy*

Table 4.3.1.3 Resistance levels (%) among clinical isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2015

	S. aureus
Antibiotic	
oxacillin/flucloxacillin*	2
ciprofloxacin** *- according to breakpoint for high dose therapy*	9
gentamicin	1
erythromycin	12
clindamycin	4
clindamycin including inducible resistance***	11
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0
co-trimoxazole	3
rifampicin	0

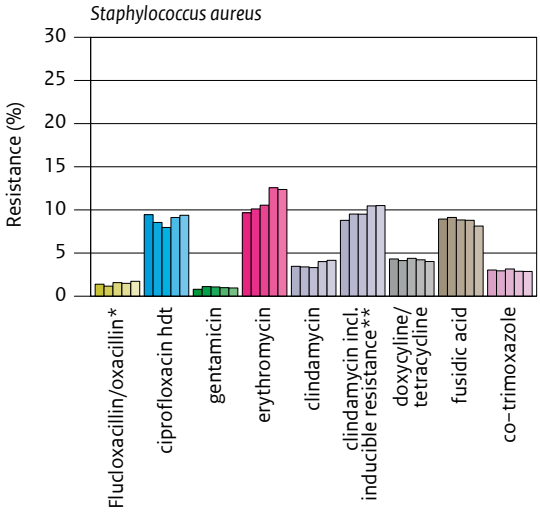
10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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 2011 to 2015) among clinical isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR



hdt=according to breakpoints for high dose therapy

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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 for piperacillin/tazobactam (≤5%), cefotaxime/ceftriaxone (≤7%), ceftazidime (≤5%), meropenem/imipenem (0%) and gentamicin and tobramycin (both ≤6%) were ≤7% in all Enterobacteriaceae in 2015. Furthermore, nitrofurantoin resistance was 3% in *E. coli*, ciprofloxacin resistance was 6% in *K. pneumoniae*, and resistance to cefuroxime was 1% in *P. mirabilis*.
- Resistance to amoxicillin/ampicillin and trimethoprim was higher than 20% for all Enterobacteriaceae. Additionally, norfloxacin resistance was high in *E. coli* (23%) and *K. pneumoniae* (20%). Co-trimoxazole resistance was high in *E. coli* (28%) and *P. mirabilis* (30%). Last, in *E. coli*, resistance to co-amoxiclav (20%) was high as well.
- For empiric therapy combinations, resistance was ≤5%.
- The percentage of HRMO was ≤8%, and the proportion of multidrug resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin combined, was ≤5%.
- Statistically significant and clinically relevant decreasing or increasing trends between 2011 and 2015 were not observed for any of the selected pathogen-antimicrobial combinations.

P. aeruginosa

- Resistance to each of the selected agents was ≤7%.
- Gentamicin resistance decreased significantly, from 8% in 2011 to 3% in 2015, which was also considered clinically relevant.

S. aureus

- Resistance to each of the selected agents except clindamycin (including inducible resistance, 11%) and erythromycin (12%) was lower than 10% and remained stable over the last five years.

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 2015 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*). 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*). 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 culturing is expected to be limited or non-existing.

Table 4.3.2.1 Distribution of isolated pathogens (N (%)) in clinical specimens from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2015

Pathogen	Blood or Cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or Pus N (%)
E. coli	3626 (25)	1143 (14)	15775 (43)	3460 (15)
K. pneumoniae	600 (4)	474 (6)	2695 (7)	663 (3)
P. mirabilis	246 (2)	235 (3)	2432 (7)	755 (3)
E. cloacae	194 (1)	420 (5)	869 (2)	868 (4)
P. aeruginosa	312 (2)	1254 (15)	1806 (5)	1316 (6)
Acinetobacter spp.	54 (0)	79 (1)	178 (0)	240 (1)
E. faecalis	431 (3)	41 (0)	3915 (11)	1511 (6)
E. faecium	281 (2)	20 (0)	1217 (3)	827 (4)
S. aureus	1465 (10)	1681 (20)	1213 (3)	5979 (25)
CNS	4622 (31)	12 (0)	1107 (3)	2413 (10)
Other Enterobacteriaceae*	602 (4)	1249 (15)	2999 (8)	2014 (9)
Other non-fermenters**	40 (0)	442 (5)	141 (0)	236 (1)
Other gram-positives	2287 (15)	1336 (16)	2250 (6)	3278 (14)

* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp. (non cloacae), Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae).*

** *Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas 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 2015

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter spp.*
Antibiotic						
amoxicillin/ampicillin	47	-	-	24	-	-
co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	21	11	-	11	-	-
piperacillin-tazobactam*	5	5	-	1	7	-
cefuroxime	12	13	-	1	-	-
cefotaxime/ceftriaxone	5	7	-	1	-	-
ceftazidime*	3	5	-	1	4	-
meropenem/imipenem*	0	0	0	0	3	1
ciprofloxacin	13	6	3	9	7	6
gentamicin	5	3	3	6	3	3
tobramycin	5	5	4	3	1	4
co-trimoxazole	25	13	7	27	-	3
nitrofurantoin - *according to breakpoint for uncomplicated urinary tract infection*	2	-	-	-	-	-
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	5	-	-	4	-	-
gentamicin + co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	3	2	-	2	-	-
gentamicin + piperacillin-tazobactam	1	1	-	0	1	-
gentamicin + cefuroxime	2	2	-	0	-	-
gentamicin + cefotaxime/ceftriaxone	1	2	-	0	-	-
gentamicin + ceftazidime	1	1	-	0	0	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	1	-
Multi-drug resistance						
HRMO**	8	8	1	4	1	3

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

\- *Resistance not calculated*

* *For P. aeruginosa and Acinetobacter spp., the breakpoints used to calculate resistance to piperacillin-tazobactam, ceftazidime, and imipenem relate to high dose therapy.*

** *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 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 ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/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 2011 to 2015) 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

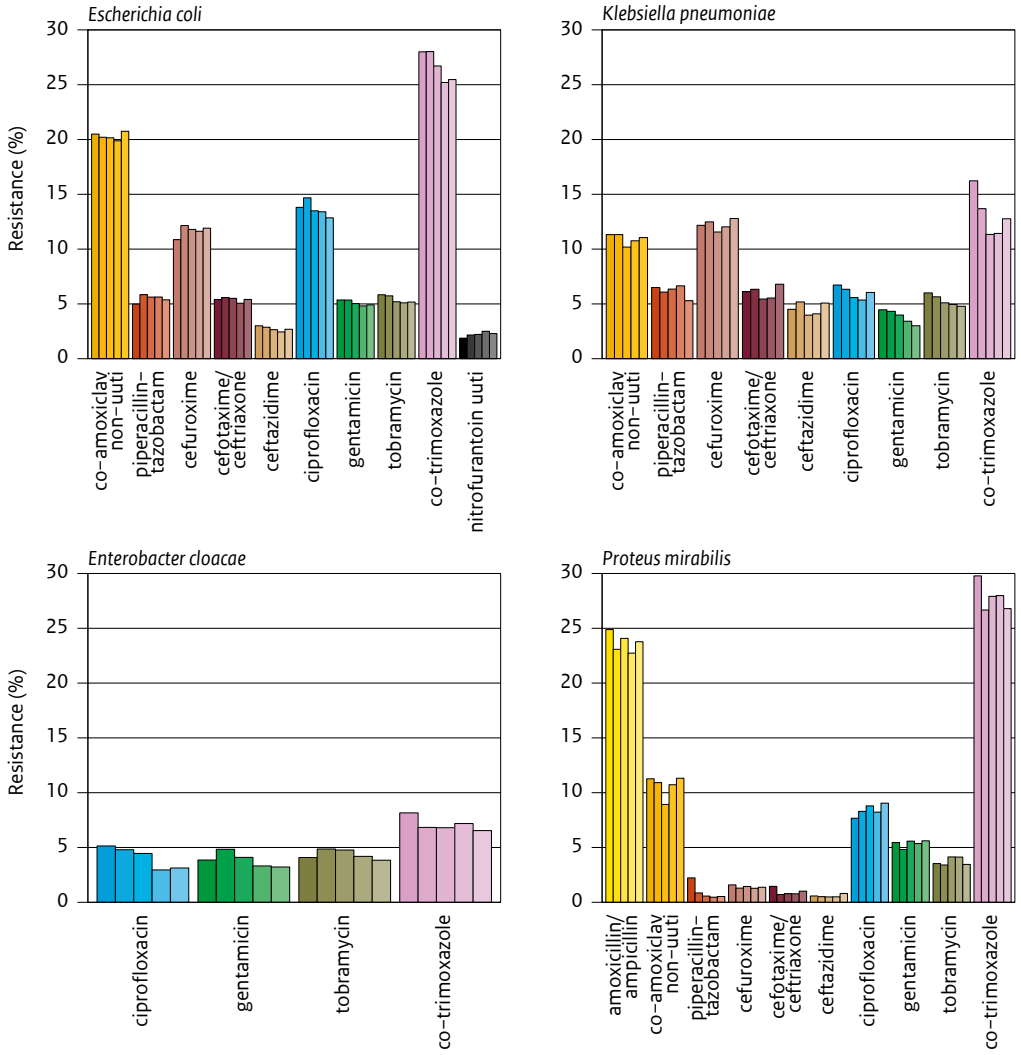
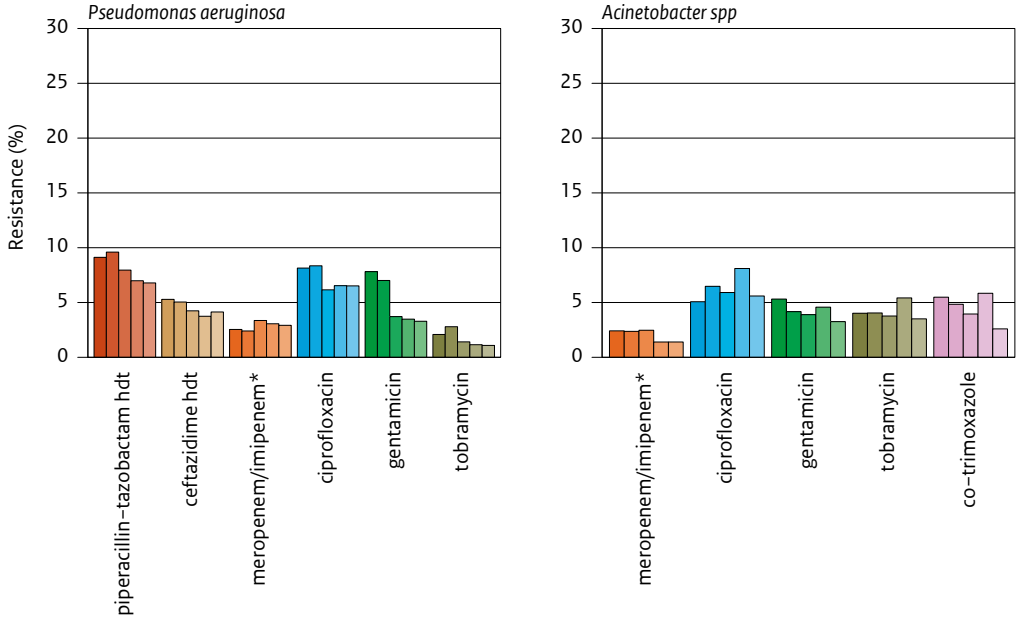


Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



uuti=according to breakpoint for uncomplicated urinary tract infection,
non-uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy
* *For P. aeruginosa and Acinetobacter spp. the breakpoints used to calculate resistance to imipenem relate to high dose therapy*

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 2015

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

- *Resistance not calculated*

Table 4.3.2.4 Resistance levels (%) among clinical isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2015

	S. aureus
Antibiotic	
oxacillin/flucloxacillin*	2
ciprofloxacin** *- according to breakpoint for high dose therapy*	10
gentamicin	1
erythromycin	12
clindamycin	4
clindamycin including inducible resistance***	10
doxycycline/tetracycline	4
fusidic acid	6
linezolid	0
co-trimoxazole	3
rifampicin	0

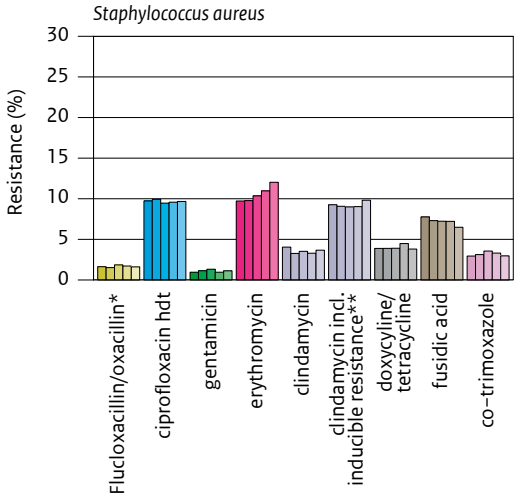
10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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 2011 to 2015) among clinical isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



hdt=according to breakpoints for high dose therapy

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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

- Overall, resistance to piperacillin/tazobactam (≤5%), cefotaxime/ceftriaxone (≤7%), ceftazidime (≤5%), meropenem/imipenem (0%), ciprofloxacin (≤9% except for E.coli; 13%), gentamicin (≤6%), and tobramycin (≤5%), was below 10%. Resistance levels lower than 10% were also found for nitrofurantoin in *E. coli* (2%), co-trimoxazole in *E. cloacae* (7%), and cefuroxime in *P. mirabilis* (1%).
- Resistance to amoxicillin/ampicillin (≥24%) and co-trimoxazole (≥25%) was high in *E. coli* and *P. mirabilis*. Furthermore, co-amoxiclav resistance was high in *E. coli* (21%).
- For empiric therapy combinations, resistance was ≤5%.
- The percentage of HRMO was 8% (*E. coli*, *K. pneumoniae*) or lower.
- Statistically significant and clinically relevant decreasing or increasing trends between 2011 and 2015 were not observed for any of the selected pathogen-antimicrobial combinations.

P. aeruginosa

- Resistance to each of the selected agents, empiric therapy combinations, and the percentage HRMO, was ≤7% in 2015.
- A significant and clinically relevant decrease in resistance was observed for piperacillin-tazobactam, especially in the last four years (from 10% in 2012 to 7% in 2015), and for gentamicin (from 8% in 2011 to 3% in 2015).

Acinetobacter spp.

- Resistance to each of the selected agents, and the percentage HRMO, was ≤6%.

E. faecalis and E. faecium

- In *E. faecalis* (0%) and *E. faecium* (1%), vancomycin resistance was rare.

S. aureus

- Except for erythromycin (12%), resistance to each of the selected agents was below 10% and was rather stable over the last five years.

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 2015 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*), 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*, and *P. aeruginosa*) 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 culturing is therefore unlikely.

Table 4.3.3.1 Distribution of isolated pathogens (N (%)) in clinical specimens from patients admitted to intensive care units, ISIS-AR 2015

	Blood or Cerebrospinal fluid	Lower respiratory tract	Urine	Wound or Pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	351 (13)	483 (13)	821 (40)	549 (18)
K. pneumoniae	75 (3)	231 (6)	138 (7)	124 (4)
P. mirabilis	28 (1)	107 (3)	116 (6)	89 (3)
E. cloacae	30 (1)	223 (6)	37 (2)	127 (4)
P. aeruginosa	60 (2)	346 (9)	123 (6)	199 (6)
Acinetobacter spp.	8 (0)	37 (1)	10 (0)	20 (1)
E. faecalis	91 (3)	90 (2)	243 (12)	309 (10)
E. faecium	214 (8)	196 (5)	188 (9)	425 (14)
S. aureus	217 (8)	770 (20)	57 (3)	275 (9)
CNS	1221 (45)	55 (1)	67 (3)	343 (11)
Other Enterobacteriaceae*	107 (4)	688 (18)	173 (8)	303 (10)
Other non-fermenters**	12 (0)	223 (6)	7 (0)	39 (1)
Other gram-positives	278 (10)	384 (10)	65 (3)	269 (9)

* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp. (non cloacae), Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae).*

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

Table 4.3.3.2 Resistance levels (%) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to intensive care units, ISIS-AR 2015

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	48	-	-	24	-
co-amoxiclav *- according to breakpoint for non-uncomplicated urinary tract infection*	23	12	-	13	-
piperacillin-tazobactam*	6	6	-	0	12
cefuroxime	15	15	-	1	-
cefotaxime/ceftriaxone	8	8	-	2	-
ceftazidime*	4	6	-	1	8
meropenem/imipenem*	0	1	0	0	7
ciprofloxacin	13	7	3	11	8
gentamicin	5	4	6	6	6
tobramycin	5	7	7	4	4
co-trimoxazole	25	13	8	29	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	-	4	-
gentamicin + co-amoxiclav *- according to breakpoint for non-uncomplicated urinary tract infection*	3	3	-	3	-
gentamicin + piperacillin-tazobactam*	1	2	-	0	3
gentamicin + cefuroxime	3	4	-	1	-
gentamicin + cefotaxime/ceftriaxone	2	4	-	1	-
gentamicin + ceftazidime	1	3	-	1	0
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	3
Multi-drug resistance					
HRMO**	10	9	1	4	3

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

- *Resistance not calculated*
* *For P. aeruginosa the breakpoints used to calculate resistance to piperacillin-tazobactam, ceftazidime, and imipenem relate to high dose therapy.*
** *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. cloacaeas resistant to cefotaxim/ceftriaxone 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 ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to intensive care units in ISIS-AR

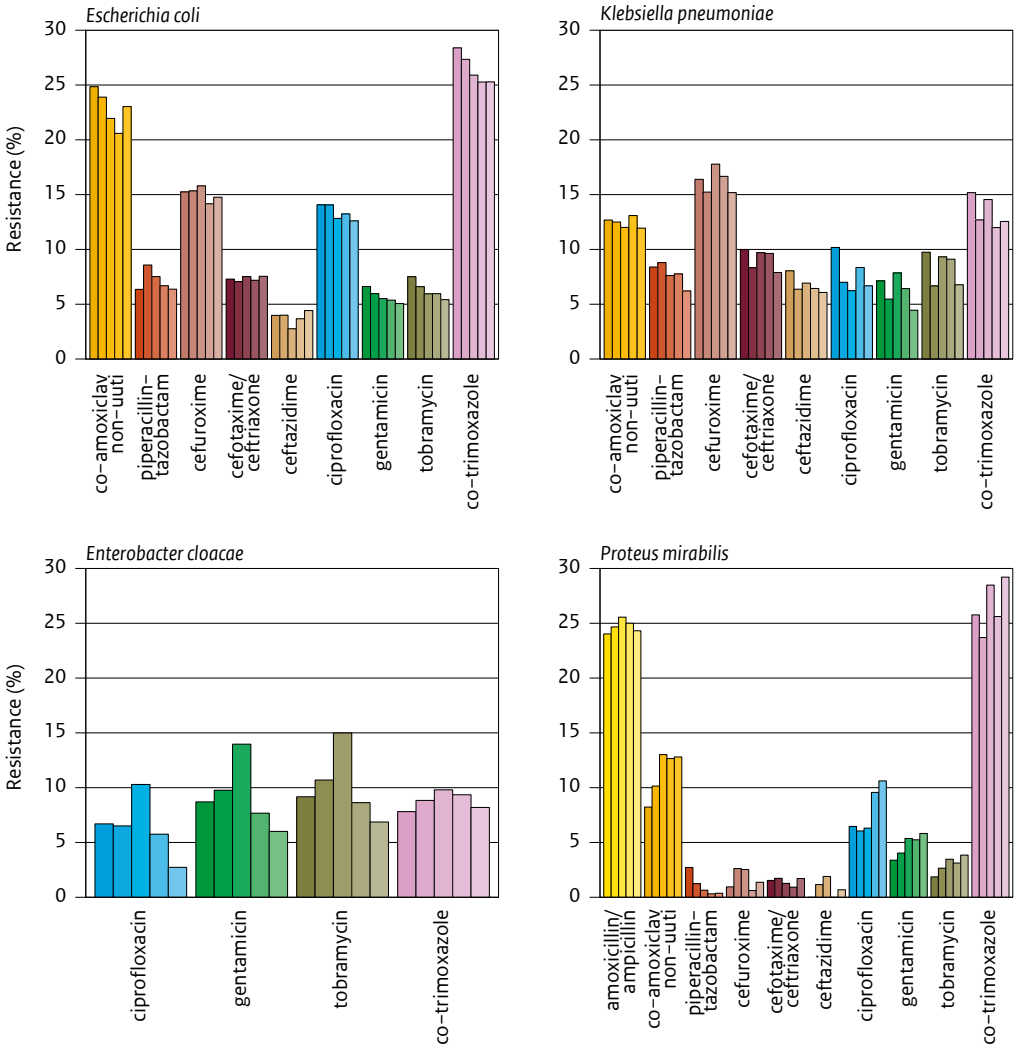
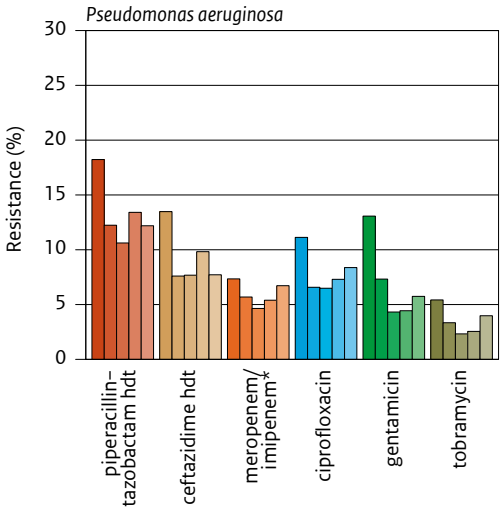


Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to intensive care units in ISIS-AR



non-uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy
* *For P. aeruginosa the breakpoints used to calculate resistance to imipenem relate to high dose therapy*

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 2015

	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 2015

	S. aureus	CNS
Antibiotic		
oxacillin/flucloxacillin*	2	71
ciprofloxacin** *- according to breakpoint for high dose therapy*	6	57
gentamicin	1	52
erythromycin	11	65
clindamycin	3	44
clindamycin including inducible resistance***	9	57
doxycycline/tetracycline	5	26
linezolid	0	1
co-trimoxazole	2	43
rifampicin	0	8

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted

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

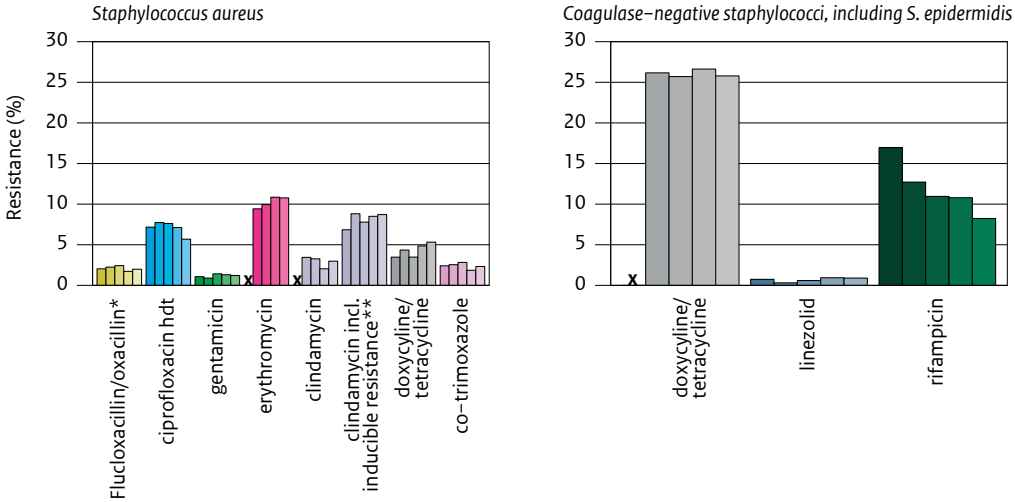
* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (see methods section for more detailed information).*

** *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).*

CNS=Coagulase-negative staphylococci, including S. epidermidis

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical isolates of *S. aureus* and coagulase negative staphylococci from patients admitted to intensive care units in ISIS-AR



An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 75%.
hdt=according to breakpoints for high dose therapy

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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

- Overall, resistance to piperacillin/tazobactam (≤6%), cefotaxime/ceftriaxone (≤8%), ceftazidime (≤6%), meropenem/imipenem (≤1%), gentamicin (≤6%), tobramycin (≤7%) was below 10%. Resistance levels lower than 10% were also found for cefuroxime in *P. mirabilis* (1%), ciprofloxacin in *K. pneumoniae* (7%) and *E. cloacae* (3%), and co-trimoxazole in *E. cloacae* (8%).
- Resistance to the empiric therapy combinations (≤5%), and the percentage HRMO (except for *E. coli*; 10%) were below 10% as well.
- Resistance to amoxicillin/ampicillin and co-trimoxazole was high in *E. coli* and *P. mirabilis* (≥24%). Furthermore, co-amoxiclav resistance was high in *E. coli* (23%).
- In *E. cloacae*, there was a significant and clinically relevant decrease in ciprofloxacin resistance from 7% in 2011 to 3% in 2015. Furthermore, the percentage of HRMO decreased significantly and to a clinically relevant extent, from 4% in 2011 to 1% in 2015.
- In *P. mirabilis*, a significant and clinically relevant increase was seen in resistance to co-amoxiclav (from 8% to 13%) and ciprofloxacin (from 6% to 11%) between 2011 and 2015.

P. aeruginosa

- Resistance to each of the selected agents (except piperacillin-tazobactam, 12%), the empiric therapy combinations, and the percentage HRMO, were ≤9%.
- A statistically significant and clinically relevant decrease in resistance levels was seen for ceftazidime (from 13% to 8%) and gentamicin (13% to 6%) between 2011 and 2015.

E. faecalis and E. faecium

- Resistance to vancomycin was rare (0% in *E. faecalis*, 1% in *E. faecium*).

S. aureus

- Resistance to each of the selected agents, except for erythromycin (11%), was lower than 10%.

Coagulase-negative staphylococci

- Apart from linezolid (1%) and rifampicin (8%), resistance to each of the selected agents was high (≥26%).
- Resistance to ciprofloxacin (from 63% to 57% between 2012 and 2015), gentamicin (from 56% to 52% between 2012 and 2015), erythromycin (from 69% in 2013 to 65% in 2015) and rifampicin (from 17% to 8% between 2011 and 2015) decreased in the last three to five years, whereas co-trimoxazole resistance increased from 33% in 2011 to 43% in 2015.

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

The distribution of pathogens isolated from blood of patients admitted to inpatient departments (incl. intensive care units) is presented in table 4.3.4.1. The resistance levels for blood isolates in 2015 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*), 4.4.4.3 (*E. faecalis* and *E. faecium*.), and 4.3.4.4 (*S. aureus*).
Five-year trends in resistance are presented in figures 4.3.4.1 and 4.3.4.2 for each pathogen except *E. faecalis* en *E. faecium*. In most hospitals blood specimens are cultured from all patients with a body temperature of >38.5 °C. Bias of the results presented below due to selective sampling is therefore unlikely.

Table 4.3.4.1 Distribution of pathogens N (%) in clinical blood isolates from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2015

Pathogen	Blood N (%)
E. coli	3923 (23)
K. pneumoniae	659 (4)
P. mirabilis	272 (2)
E. cloacae	217 (1)
P. aeruginosa	367 (2)
Acinetobacter spp.	57 (0)
E. faecalis	503 (3)
E. faecium	480 (3)
S. aureus	1604 (9)
CNS	5649 (33)
Other Enterobacteriaceae*	686 (4)
Other non-fermenters**	51 (0)
Other gram-positives	2497 (15)

* *Morganella spp., Citrobacter spp., Serratia spp, Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae).*

** *Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas 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 2015

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	47	-	-	22	-
co-amoxiclav *- according to breakpoint for non-uncomplicated urinary tract infection*	21	8	-	6	-
piperacillin-tazobactam*	5	3	-	0	7
cefuroxime	12	12	-	1	-
cefotaxime/ceftriaxone	5	7	-	1	-
ceftazidime*	3	5	-	1	5
meropenem/imipenem*	0	0	0	0	4
ciprofloxacin	13	6	4	9	5
gentamicin	5	2	5	5	3
tobramycin	5	5	5	4	2
co-trimoxazole	27	13	9	23	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	-	5	-
gentamicin + co-amoxiclav *- according to breakpoint for non-uncomplicated urinary tract infection*	3	2	-	1	-
gentamicin + piperacillin-tazobactam	1	1	-	0	2
gentamicin + cefuroxime	2	2	-	0	-
gentamicin + cefotaxime/ceftriaxone	2	2	-	1	-
gentamicin + ceftazidime	1	1	-	0	0
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	1
Multi-drug resistance					
HRMO**	8	9	2	3	2

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

- *Resistance not calculated*
* *For P. aeruginosa the breakpoints used to calculate resistance to piperacillin-tazobactam, ceftazidime, and imipenem relate to high dose therapy.*
** *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/ceftriaxoneor 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 ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2011 to 2015) 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.

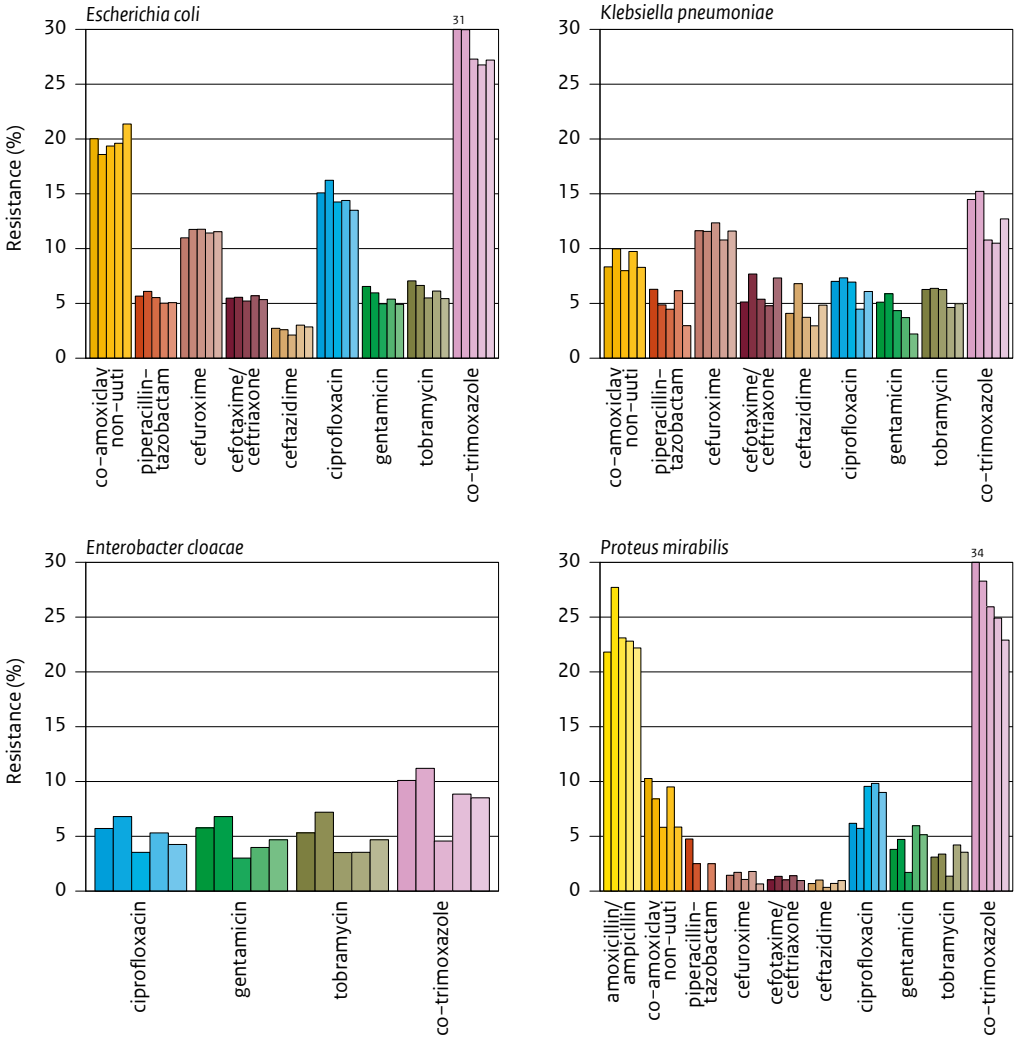
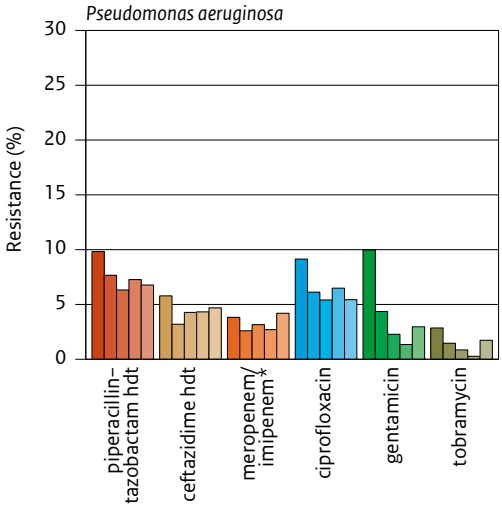


Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



non- uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy
* *For P. aeruginosa the breakpoints used to calculate resistance to imipenem relate to high dose therapy*

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 2015

	E. faecalis	*E. faecium*	
Antibiotic			
amoxicillin/ampicillin	-	90	
vancomycin	0	1	

- *Resistance not calculated*

Table 4.3.4.4 Resistance levels (%) among clinical blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2015

	S. aureus
Antibiotic	
oxacillin/flucloxacillin*	1
ciprofloxacin** - *according to breakpoint for high dose therapy*	9
gentamicin	1
erythromycin	10
clindamycin	3
clindamycin including inducible resistance***	8
doxycycline/tetracycline	3
linezolid	0
co-trimoxazole	3
rifampicin	0

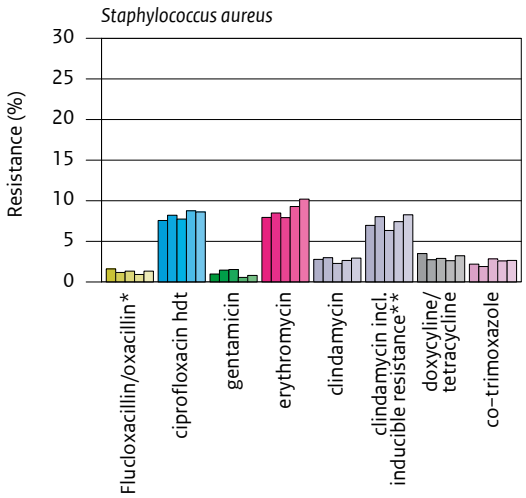
10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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 2011 to 2015) among clinical blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR.



hdt=according to breakpoints for high dose therapy

* *Resistance against flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for flucloxacillin/oxacillin (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

- Resistance levels were similar to resistance levels in all specimens combined, which are described in chapter 4.3.2 (inpatient departments excl. ICU) and 4.3.3 (ICU).
- Resistance to gentamicin in *K. pneumoniae* had decreased significantly and clinically relevant from 5% in 2011 to 2% in 2015. Significant and relevant decreasing five-year trends were also seen for combined resistance to gentamicin + co-amoxiclav in *K. pneumoniae* (from 4% to 2%), for co-trimoxazole resistance in *P. mirabilis* (from 34% to 23%) and gentamicin resistance in *P. aeruginosa* (from 10% to 3%).
- Combined resistance to gentamicin + amoxicillin/ampicillin in *P. mirabilis* increased from 2% in 2011 to 5% in 2015, which was clinically relevant as well.
- The percentage of HRMO remained stable over time.

E. faecalis, E. faecium, and S. aureus

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

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 2015 are presented in tables 4.3.5.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*) and 4.3.5.3 (*E. faecalis*). 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 N (%) in clinical urinary isolates from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2015

Pathogen	OPD N (%)	IPD N (%)	
E. coli	8331 (41)	1446 (34)	
K. pneumoniae	1599 (8)	300 (7)	
P. mirabilis	1061 (5)	240 (6)	
P. aeruginosa	705 (3)	229 (5)	
E. faecalis	2210 (11)	501 (12)	
Other Enterobacteriaceae*	2457 (12)	628 (15)	
Other non-fermenters**	389 (2)	103 (2)	
Other *Enterococcus* spp.	322 (2)	149 (4)	
Other gram-positives	3341 (16)	660 (16)	

* *Morganella spp., Citrobacter spp., Serratia spp., Providencia spp., Enterobacter spp., Proteus spp. (non-mirabilis), Klebsiella spp. (non-pneumoniae).*

** *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), and Stenotrophomonas 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 2015

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	48	52	-	-	24	26	-	-
co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	20	22	10	15	11	14	-	-
piperacillin-tazobactam*	5	6	4	6	-	0	6	5
cefuroxime	12	16	16	17	1	4	-	-
cefotaxime/ceftriaxone	5	9	7	13	1	4	-	-
ceftazidime*	3	4	5	9	1	2	2	0
meropenem/imipenem*	0	0	0	0	0	0	3	1
ciprofloxacin	20	26	7	9	12	15	9	10
gentamicin	6	8	3	6	8	9	2	4
tobramycin	7	9	5	8	4	4	1	0
co-trimoxazole	30	33	15	19	32	35	-	-
nitrofurantoin - *according to breakpoint for uncomplicated urinary tract infection*	4	3	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ampicillin	6	7	-	-	6	8	-	-
gentamicin + co-amoxiclav - *according to breakpoint for non-uncomplicated urinary tract infection*	3	4	2	4	3	5	-	-
gentamicin + piperacillin-tazobactam*	-	1	-	2	-	0	1	1
gentamicin + cefuroxime	2	5	2	3	0	3	-	-
gentamicin + cefotaxime/ceftriaxone	2	3	2	3	0	3	-	-
gentamicin + ceftazidime	1	2	1	2	0	2	0	0
tobramycin + ceftazidime	-	-	-	-	-	-	0	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	1	0

Table 4.3.5.2 (continued) 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 2015

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Multi-drug resistance								
HRMO**	9	13	8	15	5	6	1	1
multidrug-resistance*** - *for co-amoxiclav according to breakpoint for non-uncomplicated urinary tract infection*	6	-	3	-	2	-	-	-

10	Significant and clinically relevant increasing trend since 2011
10	Significant and clinically relevant decreasing trend since 2011
10	No significant or clinically relevant time trend or no test for trend conducted
	(For the definition of a clinically relevant trend see the methods section)

- *Resistance not calculated*
* *For P. aeruginosa the breakpoints used to calculate resistance to piperacillin-tazobactam, ceftazidime, and imipenem relate to high dose therapy.*
** *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for Enterobacteriaceae as resistant to cefotaxim/ceftriaxone 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 ≥ 3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*
*** *MultiDrug Resistance (MDR), 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 2011 to 2015) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.

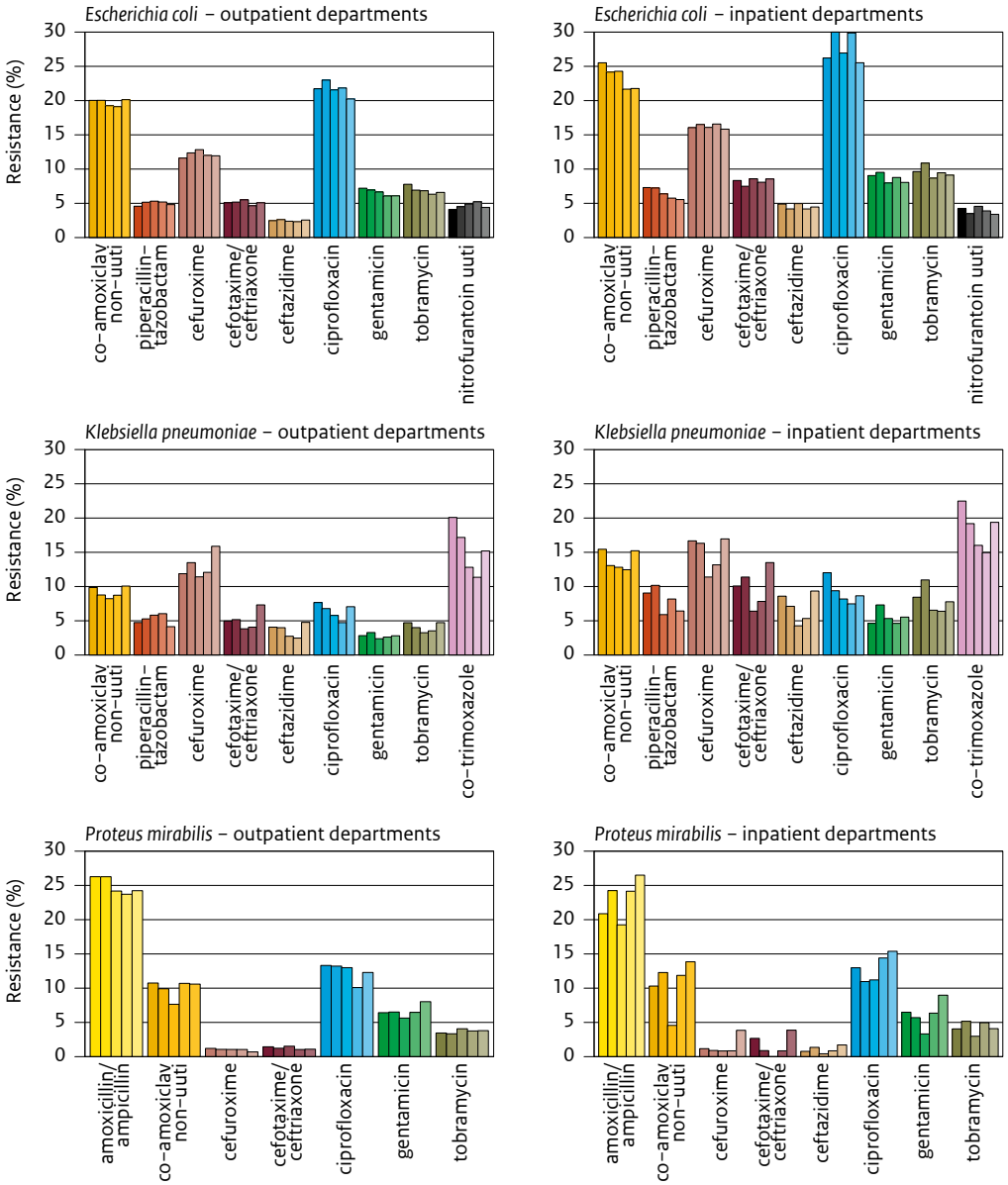
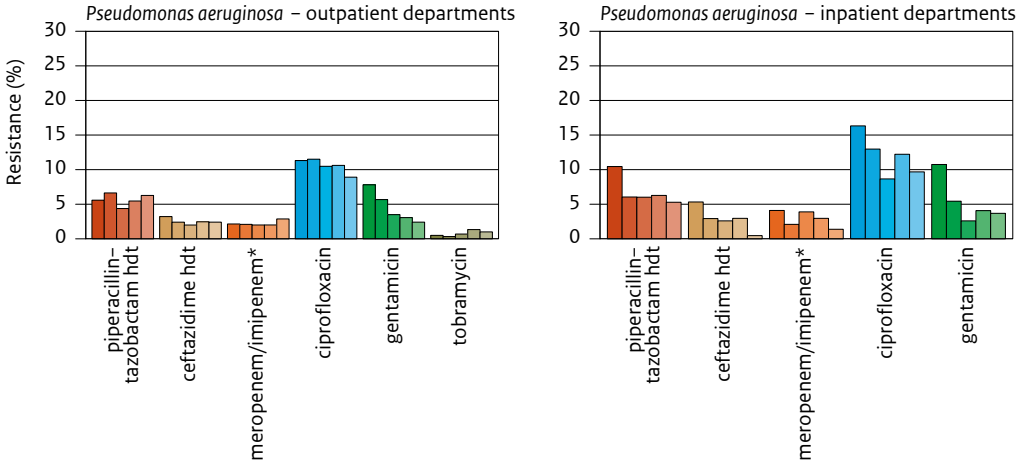


Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2011 to 2015) among clinical urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR.



uuti=according to breakpoint for uncomplicated urinary tract infection,
non-uuti=according to breakpoint for non-uncomplicated urinary tract infection,
hdt=according to breakpoints for high dose therapy
* *For P. aeruginosa the breakpoints used to calculate resistance to imipenem relate to high dose therapy*

Table 4.3.5.3 Resistance levels (%) among clinical urinary isolates of *E. faecalis* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2015

	E. faecalis		
	OPD	IPD	
Antibiotic			
vancomycin	0	0	
nitrofurantoin	1	1	

Key results

Enterobacteriaceae

- In general, resistance levels were higher in urology inpatient departments than in urology outpatient departments.
- Low resistance levels were found for meropenem/imipenem (0%) in all Enterobacteriaceae. Low resistance was also found for nitrofurantoin and ceftazidime in *E. coli* (≤4%), for cefuroxime, cefotaxime/ceftriaxone, ceftazidime, and tobramycin (≤4%) in *P. mirabilis*.
- High levels of resistance were found for amoxicillin/ampicillin (≥24%) in all Enterobacteriaceae, for co-trimoxazol in *E. coli* and *P. mirabilis* and for co-amoxiclav (≥20%) and ciprofloxacin (≥20%) in *E. coli*.
- Resistance levels for co-trimoxazole decreased significantly and to a clinically relevant extent in *E. coli* isolates from inpatient urology departments (from 37% in 2011 to 33% in 2015), and in *K. pneumoniae* isolates from outpatient urology departments(20% to 15%).
- With regard to empirical therapy combinations, significant and relevant increasing five-year trends in resistance were found for gentamicin + amoxicillin/ampicillin (3% to 8%) and for gentamicin + co-amoxiclav (1% to 5%) in *P. mirabilis* isolates from inpatient departments.
- Multidrug resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin combined (≤6%) and HRMO levels (5-15% depending on the pathogen) remained stable throughout the years.

P. aeruginosa

- Resistance to each of the selected agents was ≤6%, except for ciprofloxacin (9% in outpatient departments and 10% in inpatient departments).
- Resistance to ceftazidime (from 5% to 0%), ciprofloxacin (from 16% to 10%) and gentamicin (from 11% to 4%) decreased significantly and to a clinically relevant extent in the last five years in inpatient departments, whereas for outpatient departments this was only the case for gentamicin (from 8% to 2%).
- The percentage HRMO remained low (1%).

E. faecalis

- Resistance to vancomycin (0%) and nitrofurantoin (1%) were both rare.

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 more resistant 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 this type of 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 (N (%)) in clinical specimens from general practitioner's patients, ISIS-AR 2015

	Lower respiratory tract	Upper respiratory tract
Pathogen	**N (%)**	**N (%)**
S. pneumoniae	187 (18)	37 (39)
H. influenzae	624 (61)	42 (44)
M. catarrhalis	207 (20)	16 (17)

Table 4.3.6.2 Resistance levels (%) among clinical isolates of respiratory pathogens from general practitioner's patients, ISIS-AR 2015

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)	1	-	-
(benzyl)penicillin (I+R)	6	-	-
amoxicillin/ampicillin	-	20	-
co-amoxiclav	-	7	1
erythromycin	14	-	9
doxycycline/tetracycline	11	0	3
co-trimoxazole	8	16	11

\- *Resistance not calculated*

Table 4.3.6.3 Distribution of isolated respiratory pathogens (N (%)) in clinical specimens from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2015

	Blood or Cerebrospinal fluid	Lower respiratory tract
Pathogen	**N (%)**	**N (%)**
S. pneumoniae	1225 (90)	2686 (23)
H. influenzae	133 (10)	7150 (60)
M. catarrhalis	9 (1)	2096 (18)

Table 4.3.6.4 Resistance levels (%) among clinical isolates of respiratory pathogens from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2015

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (R)	0	-	-
(benzyl)penicillin (I+R)	4	-	-
amoxicillin/ampicillin	-	20	-
co-amoxiclav	-	6	2
erythromycin	10	-	7
doxycycline/tetracycline	9	1	2
co-trimoxazole	7	19	9

\- *Resistance not calculated*

Key results

S. pneumoniae

- Resistance to (benzyl)penicillin (≤1%), co-trimoxazole (≤8%), and doxycycline/tetracycline (hospital patients only; 9%) was below 10%.

H. influenzae

- Resistance to co-amoxiclav (≤7%) and doxycycline/tetracycline (≤1%) was below 10%.
- Resistance to amoxicillin/ampicillin was high (20%).

M. cattharalis

- Except for co-trimoxazole resistance in GP patients (11%), resistance to each of the selected agents was below 10%.

4.4 Highly resistant microorganisms

4.4.1 Carbapenem-resistant Enterobacteriaceae

Introduction

Importance of carbapenem-resistance

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, particularly those caused by extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria, 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].

Resistance mechanisms

In Gram-negative bacteria resistance against carbapenems can be caused by several mechanisms. First, due to alterations in the genes coding for porins, structures that allow in- and export of nutrients and other compounds. As a result, porins may be lost or changed in permeability causing the bacteria to become less accessible for antibiotics. Second, changes in regulatory regions of genes coding for efflux pumps, structures that pump out compounds from the bacterial cell, may result in enhanced export of antibiotics that have entered the bacteria. Finally, bacteria may also possess genes that code for enzymes that actively break down the carbapenem antibiotics. These enzymes are designated as carbapenemases.

The genes involved in the expression of porins and efflux pumps are located on the chromosome of the bacterium. Therefore, this type of resistance cannot be transferred between bacteria and spread of resistance relies on spread of the bacterial strain with altered porines or efflux pumps. In contrast, the genes coding for the carbapenemases are mostly located on plasmids or other mobile elements, such as integrons. As a consequence, this type of resistance is easily transferred between bacterial strains, even if these strains are of different bacterial species. Therefore, surveillance of carbapenemase-producing bacteria is more important than that of carbapenem-resistance.

Carbapenemase-coding genes

Many different carbapenemase-coding genes and allelic variants thereof have been identified thus far. The most important carbapenemases are classified into two major molecular families based on their active sites: serine-carbapenemases with main representatives KPC and OXA-48, and metallo-carbapenemases, of which NDM, VIM, and IMP are the most commonly detected members.

Prevalence of CRE in The Netherlands

The ISIS-AR database (year 2015) 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.

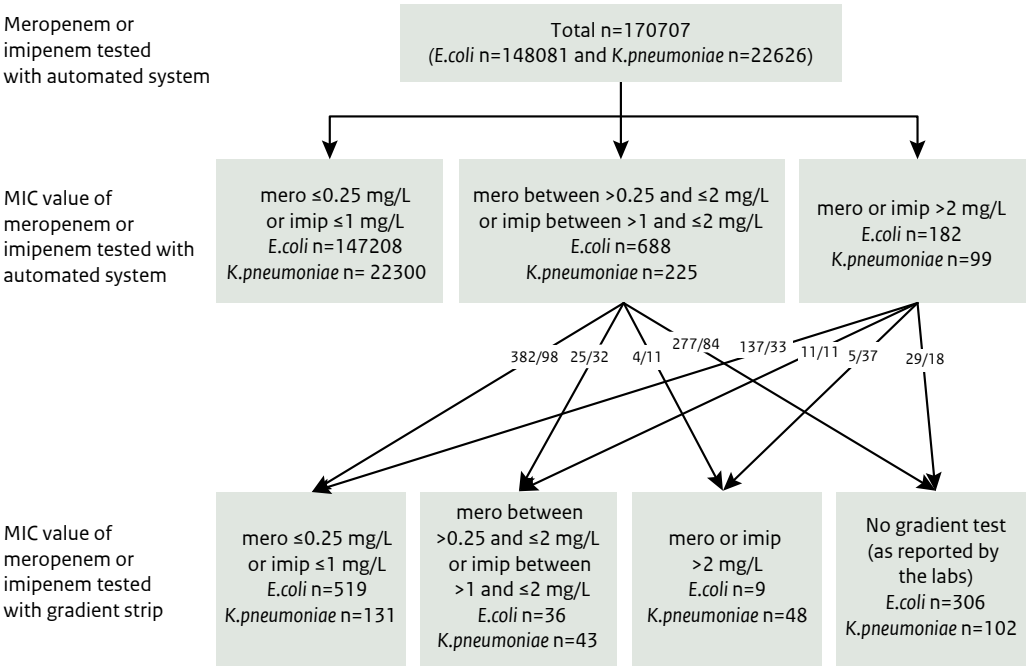
Results of sequential testing of carbapenem susceptibility and genotypic/phenotypic testing of carbapenemase production, as prescribed by the NVMM, are presented in figure 4.4.1.1. Of a total number of 170,707 isolates (148,081 *E. coli* and 22,626 *K. pneumoniae*), an elevated meropenem and/or imipenem MIC on automated testing was found in 0.7% of isolates (compared to 0.7% in 2013-2014). Confirmation of these elevated carbapenem MIC values using gradient testing was performed in 65.8% of eligible isolates, while in 2013-2014, 59.8% of eligible isolates underwent further testing. A gradient test strip was performed more often in isolates found non-susceptible on automated testing (83.3% of eligible isolates, compared to 82.1% in 2013-2014) than in isolates found screen positive on automated testing (60.5% of eligible isolates, compared to 55.0% in 2013-2014).
Confirmatory testing in eligible isolates using a gradient strip method confirmed elevated carbapenem MIC values in 8% of *E. coli* (identical to 2013-2014) and 41.0% of *K. pneumoniae* (compared to 32% in 2013-2014). This means that the overall yield of further testing was low: in the remaining 92% of *E. coli* and 59% of *K. pneumoniae* isolates, gradient strip testing showed MIC values below the screening breakpoint. Even in isolates non-susceptible on automated testing, 90% of *E. coli* and 41% of *K. pneumoniae* had MIC values below the screening breakpoint on gradient strip testing (87% and 39% in 2013-2014 respectively).
In total, 9 carbapenem resistant *E. coli* isolates and 48 carbapenem resistant *K. pneumoniae* isolates were found in 56 patients. One patient was carrying both an OXA-48 *E. coli* as well as an OXA-48 *K. pneumoniae*. The overall proportion on confirmed non-susceptible *E. coli* and *K. pneumoniae* was 0.01% and 0.21% respectively (compared to 0.01% and 0.16% in 2013-2014).

In conclusion, the proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values on automated testing remains stable over the past 3 years. Confirmatory testing of elevated MIC values with a gradient strip method has increased, especially in screen positive isolates. Of the *K. pneumoniae* isolates with elevated MIC values on automated testing, the proportion with confirmed elevated MIC values (by gradient testing) increased from 32% in 2013-2014 to 41.0% in 2015. An increase in carbapenem non-susceptibility in *K. pneumoniae* from 0.16% to 0.21% of all isolates was observed.

Molecular epidemiology

In 2015, 317 Enterobacteriaceae isolates obtained from 280 persons were submitted to the RIVM by 44 MMLs, for which species and minimal inhibitory concentration for meropenem were confirmed by the RIVM.
Carbapenemase production was measured by the carbapenemase inactivation method (CIM) and the presence of carbapenemase-coding genes were assessed by PCR (carba-PCR). Isolates were classified based on the combination of species and carbapenemase-coding gene. Only the first unique species-gene combination per person per year was used. This resulted in 130 unique carbapenemase-producing Enterobacteriaceae isolates submitted in 2015.
In 111 of the 124 cases, a single carbapenemase-producing species was found and in 13 cases multiple unique carbapenemase-producing species (19 isolates) were isolated from the same person (table 4.4.1.1). There was a high concordance between carbapenemase production (CIM) and detection of the

Figure 4.4.1.1 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.



carbapenemase-coding gene by PCR. The most frequently identified genes were bla_{OXA-48} (39%), bla_{NDM} (35%) and bla_{KPC} (15%). The nine carbapenemase-producing isolates (7%) that did not yield a PCR product in the carba-PCR were all Enterobacter species. In 2015 an outbreak of NDM producing *K. pneumoniae* occurred and 46% of the NDM-positive isolates presented here were isolated at that outbreak location.

Risk groups

In 2015, of 57 patients with confirmed CPE isolates, additional epidemiological data were available, collected through a risk questionnaire in the OSIRIS system. Of those, 25 (44%) had a history of admission to a foreign hospital longer than 24 hours within the previous two months. Seven patients (12%) were related to a foreign hospital in a different way than mentioned before and 17 patients (30%) were admitted to a health care facility with a known outbreak of CPE at the same time. For eight patients (14%), no known risk factors could be identified.

Table 4.4.1.1 Carbapenemase encoding genes in Enterobacteriaceae isolates submitted in 2015 as detected by PCR, based on first isolate per patient per year.

Single carbapenemase-producing isolate per person	No gene detected	*bla*$_{NDM}$	*bla*$_{OXA-48}$	*bla*$_{KPC}$	*bla*$_{VIM}$	*bla*$_{OXA-23}$	Persons (N)
Klebsiella pneumoniae		25	25	14			64
Escherichia coli		9	13	1	1		24
Enterobacter spp.	9		2	2	3		16
Other species		1	3	1	1	1	7
Total	**9**	**35**	**43**	**18**	**5**	**1**	**111**

Multiple different carbapenemase-producing isolates per person	No gene detected	*bla*$_{NDM}$	*bla*$_{OXA-48}$	*bla*$_{KPC}$	*bla*$_{VIM}$	*bla*$_{OXA-23}$	Persons (N)
Two species carrying the same gene		5	3	1			9
Two species each carrying a different gene		2	2				2
Three species two different genes		3	3				2
Total		**10**	**8**	**1**			**13**

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4.4.2 Vancomycin-resistant Enterococci in Dutch hospitals

Epidemiology

In 2015 VRE outbreaks were reported in 16 Dutch hospitals through the Signaling Consultation of Hospital acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR, see section 4.4.6). In total, since the start of SO-ZI/AMR in April 2012, 44 hospital outbreaks with VRE have been reported in the Netherlands, 9 in 2012, 10 in 2013, 13 in 2014, and as indicated above, 16 in 2015. Since the UMC Utrecht started to offer molecular diagnostics on clinical VRE-isolates, which started in May 2012, 42 hospitals and laboratory have sent 709 VRE to the UMC Utrecht (status of March 23rd 2016). These represented 363 isolates carrying the *vanA* gene cluster, 340 the *vanB* gene cluster, four isolates carried both the *vanA* and the *vanB* gene cluster and two isolates carried the *vanD* gene cluster. Of these 709 VRE, 623 were typed by Multi Locus Sequence Typing (MLST). This revealed a total of 43 different Sequence Types, suggesting that at least 43 VRE clones circulated in Dutch hospitals. The sudden increase of VRE in Dutch hospitals can therefore not be attributed to spread of a single clone. On the other hand, 17 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 (27 hospitals), ST203 (22 hospitals), ST18 (15 hospitals), ST80 (12 hospitals).

It is known that MLST does not provide optimal resolution to track transmission of VRE clones. To enhance the resolution of the current *E. faecium* MLST scheme, the UMCU has developed and evaluated a standardized core genome allele-based typing scheme, or core genome MLST (cgMLST) scheme. In this *E. faecium* cgMLST scheme the allelic variation in 1423 core genes is indexed, which is an important extension of the number of analyzed genes from seven, in classical MLST, to the entire core genome of the species. (1) In the study by de Been *et al*., an analysis of all pairwise allelic differences revealed that 92% of all likely epidemiologically related pairs of isolates differed by less than 20 alleles. Therefore, this threshold of 20 alleles difference was used for designating cluster types (CTs), i.e. for identifying clonally related *E. faecium*.

To investigate the molecular epidemiology of VRE in Dutch hospitals using the published cgMLST scheme, whole genome sequencing of 587 VRE (297 vanA-VRE, 288 vanB-VRE, and 2 vanD-VRE), from 38 hospitals, collected between 2005 and 2015 was performed. This revealed 86 CTs, suggesting that 86 different VRE clones have spread in Dutch hospitals. Furthermore, in 28 hospitals more than one CT was found, ranging from 2-19 CTs. This further illustrates the polyclonal nature of the epidemic rise of VRE in Dutch hospitals. On the other hand, of the 86 CTs, 13 CTs were represented by more than one isolate and found in only one hospital, thus representing cases of clonal transmission unique for one hospital. Also, 27 CTs were found among more than one hospital (ranging from 2-14 hospitals) suggesting an epidemiological link between hospitals that share VRE with the same CT. The most widespread CTs were CT-20 (14 hospitals), CT-24 (12 hospitals) and CT-103 (12 hospitals). This may represent clonal transmission between two or more hospitals or acquisition of the same clone by multiple hospitals from a yet unknown source.

Future

Currently, researchers at the UMC Utrecht and RIVM are trying to link whole genome based epidemiological data to patient referral data to assess whether patient referrals between hospitals can explain the presumed epidemiological linkage of particular hospitals. In addition, plasmid assemblies and reconstructions will be performed from whole genome sequence data with the aim to fully assemble and subsequently study the epidemiology of plasmids containing vancomycin-resistance genes.

Table 4.4.2.1 Incidence of VRE in various hospital departments in the Netherlands in 2015 based on ISIS-AR

Type of department	Number of isolates tested for all relevant antibiotic classes	Absolute number of VRE*
GP	293	2 (0.7)
Outpatient departments	435	4 (0.9)
Inpatient departments excluding Intensive Care Units	2,171	22 (1)
Intensive Care Units	761	5 (0.7)

* *VRE is defined as resistant to amoxicillin/ampicillin and vancomycin, based on S-I-R interpretation of the laboratories. Numbers are based on data from a selection of 26 laboratories within ISIS-AR The first clinical E. faecium isolate per patient was selected*

References

[1] de Been M, Pinholt M, Top J, Bletz S, Mellmann A, van Schaik W, et al. Core Genome Multilocus Sequence Typing Scheme for High- Resolution Typing of *Enterococcus faecium*. Journal of clinical microbiology. 2015;53(12):3788-97.

4.4.3 Methicillin-resistant Staphylococcus aureus

Introduction

In the Netherlands, a low MRSA prevalence country, enhanced MRSA surveillance started in 1989 by the National Institute for Public Health and the Environment. Typing of the MRSA isolates has been performed using successively phage typing, pulsed-field gel electrophoresis and Staphylococcal protein A (*spa*)-typing. In 2008, multiple-locus variable number of tandem repeat analysis (MLVA) was introduced for *S. aureus* for the Dutch MRSA surveillance. MLVA is a typing technique based on the composition of 8 genomic loci containing tandem repeats and is based on accurate band sizing using an automated DNA sequencer. Between 2008 and 2014 more than 30,000 MRSA isolates have been characterized by both *spa* and MLVA. However, MLVA turned out to have a considerably higher discriminatory power than *spa*-typing. Furthermore, performing both methods did not increase typing resolution making MLVA superior to *spa*-typing. Therefore, all MRSA isolates were typed using MLVA only starting in 2015.

Prevalence

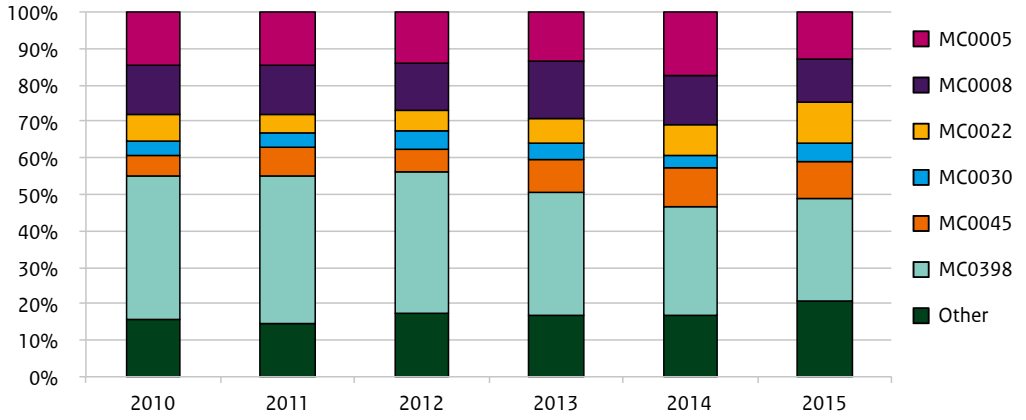
In the ISIS-AR database, *Staphylococcus aureus* isolates and MRSA isolates were identified for unique patients in 2015. Numbers are based on data from 28 laboratories that continuously reported to the ISIS-AR database during the whole year in 2015. The first *S. aureus* isolate per patient was selected. The proportion of *S. aureus* that was MRSA positive in clinical isolates (including blood samples) was 1.7% (463/27,961), ranging from 1.4% (165/11,386) in outpatient departments to 2.2% (117/5403) in general practices (table 4.4.3.1). Potentially, screening samples could be misclassified as clinical samples, thereby falsely increasing the proportion of MRSA in clinical isolates. Furthermore, these numbers could be biased because clinical samples may only be taken in case of therapy failure or recurrent infections, leading to more infections with an increased risk of MRSA being included. In blood isolates, expected to be unbiased in that respect, the MRSA prevalence was 1.0% (22/2173).

Enhanced MRSA surveillance

For the national enhanced MRSA surveillance medical microbiology laboratories (MMLs) submit all MRSA for molecular typing, with the restriction that they only send the MRSA that was first isolated from a person. Nevertheless, the RIVM occasionally receives consecutive isolates from the same person. The data used here are based on the first isolate of the same person in 2015 only. It is assumed that the collection represents more than 85% of all persons found to be MRSA-positive by the MMLs. In 2015, the RIVM received 3774 MRSA isolates for which a person ID was known and these isolates were obtained from 3496 persons.

Based on culture methods and origin of the samples, 59% (2065/3496) of the isolates were identified as screening samples (mainly swabs of nose, throat and perineum). A total of 709 samples were identified as infection-related, with a majority being wound material or pus (474/709, 69%) and only 28 blood samples (4%).

Figure 4.4.3.1 Distribution of the major MLVA-complexes among MRSA isolates received in the Dutch MRSA surveillance in the years 2010-2015.



Only the first MRSA isolate per year per person was used.
MC398 represents LA-MRSA and MC-other represents the total of MLVA-complexes not belonging to the major MLVA-complexes specified in the figure.

Molecular epidemiology

The genotypic structure of the MRSA population can be visualized by performing molecular typing. Using MLVA typing data the MRSA population is at the moment divided into 25 MLVA-complexes (MCs). Among the isolates collected in the Dutch MRSA surveillance, MC398, representing livestock MRSA (LA-MRSA), is predominant (Figure 4.4.3.1). Since its first identification by Voss *et al*. there has been a steady increase of LA-MRSA with a peak in 2009 (43%). After 2009, fewer LA-MRSA were submitted to the RIVM each year and in 2015, 25% of all isolates belonged to this MRSA variant. In contrast, the annual number of MRSA received has increased since 2009. The major reason is that isolates of a number of MCs have been rapidly increasing since 2009. This increase is almost completely attributable to MLVA-types MT1352 (MC45), MT4239 and MT0491 (both MC22). The group of MT1352 isolates is predominantly found in Dutch nursing homes. The first MT1352 MRSA were found in 2007 in the province Noord-Holland from which they seem to spread from nursing to nursing home in eastern direction. The MT4239 isolates first emerged in 2013 in the province Zuid-Holland. In 2015, MT4239 isolates were the third most frequently found non-LA-MRSA type in the Netherlands and were predominantly found in the province of Noord-Holland. MT0491 isolates were found in the entire Netherlands.
Of those isolates from patients having an MRSA-associated infection, 18.6% (132/709) were LA-MRSA subtypes, in contrast to the MRSA-positive screening samples, of which more than one third (34.0%) harbored the livestock-associated MLVA-complex (table 4.4.3.2).

Table 4.4.3.1 Percentage of clinical isolates of MRSA in the Netherlands in 2015

Type of department	No. of *S. aureus*	No. of MRSA, (% of MRSA)
GP	5403	117 (2.2)
Outpatient departments	11386	165 (1.4)
Inpatient departments excluding Intensive Care Units	10033	157 (1.6)
Intensive Care Units	1139	24 (2.1)
Total	**27961**	**463 (1.7)**

* *The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2) or, if these tests were lacking, resistance to flucloxacillin, methicillin, oxacillin, or cefoxitin screentest, Based on re-interpretation according to EUCAST 2015*
Numbers are based on data from a selection of 28 laboratories within ISIS-AR
The first clinical S. aureus isolate per patient was selected

Table 4.4.3.2 Percentage of LA-MRSA and non-LA-MRSA among infection-related and screening isolates of MRSA in the enhanced MRSA surveillance

	No. of LA-MRSA (%)	No. of non-LA-MRSA (%)	No. of isolates (% of total)
Infection-related*	132 (18.6)	577 (81.4)	709 (20.3)
Screening*	703 (34.0)	1362 (66.0)	2065 (59.1)
Unknown*	143 (19.8)	579 (80.2)	722 (20.7)
Total	**978 (28.0)**	**2518 (72.0)**	**3496**

* *Based on culture methods and origin of the samples*
Numbers based on the first isolate of one person in 2015 only, submitted to the enhanced MRSA surveillance
LA-MRSA was represented by MLVA-complex MC398

Risk groups

For the enhanced surveillance, an MRSA risk factor questionnaire, according to the WIP guidelines[1], could be completed for the submitted isolates. Around a quarter (903/3496) of the isolates with molecular typing data mentioned above could be matched with the questionnaires. Completed data on risk categories were available for 747 (83%) of these isolates.
The majority of these patients (42%, 312/747) had a high risk of being MRSA-positive, identified by a WIP risk-category 2, with almost half of them (151/312) having work-related exposure to livestock pigs, cattle or broiler chickens. 77/747 (10%) were already known to be MRSA-positive previously (WIP risk-category 1). For 37% (279/747) of the patients with available data, no risk factors for MRSA carriage had been detected.
Those MRSA-positives who were known to have work-related contacts with pigs, cattle or broiler chickens, were almost all (96%) carriers of LA-MRSA, being MLVA-complex MC398.

Future prospects

The currently used molecular typing technique MLVA is well suited for surveillance purposes for the non-LA-MRSA isolates and in many cases, MLVA will be sufficient to support epidemiological data that transmission of MRSA has occurred. For surveillance of LA-MRSA, MLVA has insufficient discriminatory power. The use of next-generation sequencing (NGS) to characterize LA-MRSA, but also non-LA-MRSA, will most likely solve this problem. However, the time currently required to obtain and analyze the NGS-data will limit its applicability for regular MRSA surveillance and transmission studies. The most likely approach in the near future will be, screening with a fast, high throughput typing method and if needed perform NGS.

References

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

4.4.4 Carbapenem-resistant *Pseudomonas aeruginosa* and other non-fermenters

Introduction

Pseudomonas aeruginosa is one of the most common nosocomial pathogens. *P. aeruginosa* is intrinsically resistant to various antibiotics, but may also acquire additional resistance either by chromosomal mutations or by horizontal gene transfer. The intrinsic resistance is caused by a concerted action of multidrug 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 through horizontal gene transfer. The emergence of these MDR *P. aeruginosa* is a problem of global concern. 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 sustained, 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.

There are several other bacterial species that, like *P. aeruginosa*, belong to the non-fermenter group of bacteria and may cause health-care related infections. Of latter group, the most frequently found species associated with hospital infections worldwide is *Acinetobacter baumannii*. However, the number of infections due to MDR-*Pseudomonas* spp. and MDR-*Acinetobacter* spp. in the Netherlands is not known yet.

Prevalence

Table 4.4.4.1 and 4.4.4.2 show the number of multi-drug resistant *P. aeruginosa* and *Acinetobacter* spp., as defined by the working group of infection prevention (WIP) in their guideline "Highly resistant microorganisms (HRMO)" in 2015 in the Netherlands, based on ISIS-AR. The highest percentages of multi-drug resistance in both microorganisms were found in Intensive Care Units.

Molecular epidemiology

Since 2010 the RIVM performs surveillance of carbapenemase-producing *Enterobacteriaceae* (CPE). Although this surveillance is aimed at collecting *Enterobacteriaceae*, the majority of the submitted isolates are non-fermenters. For this reason, these data cannot be used to infer prevalence or accurate distribution of carbapenemase-producing non-fermenters in the Netherlands.
In 2015, the medical microbiology laboratories (MMLs) sent 782 isolates for genotypic confirmation in the CPE-surveillance to the RIVM. Species and minimal inhibitory concentration for meropenem were confirmed by the RIVM and this revealed that in 2015, 449 non-fermenter isolates obtained from 385 persons were submitted to the RIVM by 37 MMLs. Carbapenemase production was measured by the carbapenemase inactivation method (CIM)[1] and the presence of carbapenemase-coding genes were assessed by PCR (carba-PCR). This in house developed carba-PCR is able to detect bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA23}, bla_{OXA24}, bla_{OXA-58} and $bla_{OXA-134}$. Isolates were classified based on the

combination of species and carbapenemase-coding gene. Only the first unique species-gene combination per person per year was used. This resulted in 77 unique carbapenemase-producing non-fermenter isolates submitted in 2015.
In 76 of the 77 cases, a single carbapenemase-producing species was found and in one case two different carbapenemase-producing non-fermenter species were isolated from the same person (table 4.4.4.3). Approximately 26% of the carbapenemase-producing (CIM) isolates did not yield a PCR product in the carba-PCR. In addition, a single carbapenemase-negative *Acinetobacter* baumannii isolate yielded a product in the carba-PCR for bla_{OXA-51}. The most frequently identified genes found among the 77 cases were bla_{VIM} (34%), only found in Pseudomonas spp. and a combination of bla_{OXA-23} and bla_{OXA-51} (21%), only found in *Acinetobacter* spp. The majority (78%) of the *P. aeruginosa* isolates carried the bla_{VIM} gene, but in none of the other carbapenemase-producing *Pseudomonas* spp. a carbapenemase-coding could be identified. A combination of bla_{OXA-23} and bla_{OXA-51} genes was found in 64% of the carbapenemase-producing *A. baumannii* isolates.

Risk groups

Risk groups for MDR *Pseudomonas* spp. were recently defined in a systematic review[2]. The meta-analyses showed that carbapenem use (odds ratio [OR]7.09; 95% confidence interval [CI]5.43 to 9.25) and medical devices ([OR]5.11; 95% [CI]3.55 to 7.37) generated the highest pooled estimates. For *Acinetobacter* spp such a systematic review is not available yet.

Prognosis and discussion

Prognosis of the incidence of MDR Pseudomonas and probably also from *Acinetobacter* spp. will highly depend on the defined risk factors for acquisition. Most of these factors will not decrease in near future. In addition, it is expected that, similar to ESBL's, an increasing incidence will be found in travelers. It is therefore likely that the prevalence of these MDR microorganisms will increase.

Table 4.4.4.1 Percentage of multidrug resistant *P. aeruginosa* in the Netherlands in 2015 based on ISIS-AR

Type of departement	No. of isolates	No. of MDR *P. aeruginosa* (%)*	No. of MDR *P. aeruginosa* resistant to carbapenems (%)
GP	3270	9 (0.3)	6 (67)
Outpatient departments	3905	31 (0.8)	17 (55)
Inpatient departments excluding Intensive Care Units	4555	53 (1.2)	36 (68)
Intensive Care Units	647	17 (2.6)	14 (82)

* *Multidrug resistant P. aeruginosa is defined as resistant to ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam, based on re-interpretation according to EUCAST 2015*
Numbers are based on data from a selection of 28 laboratories within ISIS-AR
The first clinical P. aeruginosa isolate per patient was selected

Table 4.4.4.2 Percentage of multidrug resistant *Acinetobacter* spp. in the Netherlands in 2015 based on ISIS-AR

Type of department	No. of isolates	No. of MDR *Acinetobacter* spp.(%)*
GP	1452	1 (0.1)
Outpatient departments	740	1 (0.1)
Inpatient departments excluding Intensive Care Units	544	4 (0.7)
Intensive Care Units	66	5 (7.6)

* *Multidrug resistant Acinetobacter spp. is defined as resistant to meropenem/imipenem, ciprofloxacine, and at least one out of gentamycine and tobramycine, based on laboratory S-I-R interpretation Numbers are based on data from a selection of 26 laboratories within ISIS-AR The first clinical Acinetobacter isolate per patient was selected*

Table 4.4.4.3 Carbapenemase encoding genes in the non-fermenter isolates submitted in 2015 as detected by PCR, based on first isolate per patient per year.

	bla-type										
Carbapenemase producing isolates per person	**No gene detected**	VIM	IMP	NDM	OXA-51	OXA-58	OXA-23 & OXA-51	IMP & OXA-58	NDM & OXA-51	OXA-24 & OXA-51	**Persons (N)**
Pseudomonas aeruginosa	4	25	3								32
Pseudomonas spp.	11										11
Acinetobacter baumannii	1				3		14		2	2	22
Acinetobacter spp.	1			1		1	1	2			6
Stenotrophomonas maltophilia	4		1								5
Pseudomonas aeruginosa (bla_{VIM}) & *Acinetobacter baumannii* (bla_{OXA-23} & bla_{OXA-51})		1					1				1
Total	**21**	**26**	**4**	**1**	**3**	**1**	**16**	**2**	**2**	**2**	**77**

References

[1] The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. Kim van der Zwaluw, Angela de Haan, Gerlinde N. Pluister, Albert J. Neeling, Leo M. Schouls. PloS one. 2015;10(3):e0123690).

[2] A Systematic Review and Meta-Analyses Show that Carbapenem Use and Medical Devices Are the Leading Risk Factors for Carbapenem-Resistant *Pseudomonas aeruginosa*. Anne F. Voor in 't holt, Juliëtte A. Severin, Emmanuel M. E. H. Lesaffre, Margreet C. Vos. Antimicrobial Agents and Chemotherapy p. 2626–2637, May 2014, Volume 58, Number 5

4.4.5 Extended spectrum beta-lactamase producing bacteria

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, and as concluded in NethMap 2015, overall prevalence of ESBL in either screening or diagnostic samples did not exceed 10%. Over the last year these findings were confirmed in several studies. In Amsterdam, representative samples of the general population were taken in five general practices in 2011. ESBL-E were found in 145 of 1695 samples (8.6%). Most of these were either CTX-M15 (n=59) or CTX-M1 (n=25)[1]. In another study, trends of ESBL carriage were determined over a 5 year period (2010-2014) in a large Dutch teaching hospital. Out of 2,695 patients, 135 (5.0%) were tested ESBL-E positive. The overall ESBL-E prevalence was stable over the years[2]. In a study in day care centres, the overall prevalence of ESBL- *E. coli* was 4.5%, and it was 8% in <1-year-old attendees[3]. In a study in a university hospital, it was shown that instant typing of *Klebsiella pneumoniae* and taking immediate measures could help in reducing further spread of ESBLs in the hospital setting[4].
The prevalence of ESBLs in The Netherland was also estimated using the ISIS-AR database (Table 4.4.5.1) using EUCAST breakpoint criteria for third generation cephalosporins. The prevalence is slightly lower as compared to the ones in prospective studies and there is a clear increase correlated with the complexity of care.

In summary, the overall prevalence of ESBLs at present appears to be well below 10% and there is no clear signal that this number is increasing significantly.

Table 4.4.5.1 Percentage of ESBL in the Netherlands in 2015 based on ISIS-AR

Type of department	No. of isolates	No. of ESBL (%)*
GP	89071	2532 (2.8)
Outpatient departments	31104	1348 (4.3)
Inpatient departments excluding Intensive Care Units	37817	2063 (5.5)
Intensive Care Units	3505	272 (7.8)

* *ESBL is estimated by resistance to cefotaxime and/or ceftriaxone and/or ceftazidime, based on re-interpretation of test values according to EUCAST 2015 in all Enterobacteriaceae except Enterobacter spp. Numbers are based on data from a selection of 28 laboratories within ISIS-AR The first clinical isolate per organism per patient was selected*

References

[1] Reuland EA, Al Naiemi N, Kaiser AM, Heck M, Kluytmans JA, Savelkoul PH, Elders PJ, Vandenbroucke-Grauls CM. Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. J Antimicrob Chemother. 2016 Apr;71(4):1076-82.

[2] Willemsen I, Oome S, Verhulst C, Pettersson A, Verduin K, Kluytmans J (2015) Trends in Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae and ESBL Genes in a Dutch Teaching Hospital, Measured in 5 Yearly Point Prevalence Surveys (2010-2014). PLoS ONE 10(11): e0141765.

[3] Koningstein M, Leenen MA, Mughini-Gras L, Scholts RM, van Huisstede-Vlaanderen KW, Enserink R, Zuidema R, Kooistra-Smid MA, Veldman K, Mevius D, van Pelt W. Prevalence and Risk Factors for Colonization With Extended-Spectrum Cephalosporin-Resistant *Escherichia coli* in Children Attending Daycare Centers: A Cohort Study in the Netherlands. J Pediatric Infect Dis Soc. 2015 Dec;4(4):e93-9.

[4] Voor in 't Holt AF, Severin JA, Goessens WH, Te Witt R, Vos MC. Instant Typing Is Essential to Detect Transmission of Extended-Spectrum Beta-Lactamase-Producing Klebsiella Species. PLoS One. 2015 Aug 28;10(8):e0136135.

4.4.6 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 phase 1-4. Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to the SO-ZI/AMR.

In 2015, a total of 62 new outbreaks were reported by 42 healthcare institutions (6 nursing homes and 36 hospitals, see Table 4.4.6.1). None of the outbreaks were considered uncontrollable or a direct threat to public health. Most of these outbreaks (n=57) ended in 2015, which means that the causative bacteria and the source were identified, and that transmission to other patients was stopped. The main reason for reporting an outbreak was the potential closure of (a part of) the healthcare institution (n=54). The median duration of the outbreaks was 48 days, with a range of 1 days to 194 days.

Most outbreaks were related to methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), norovirus and multiresistant *Pseudomonas aeruginosa* (defined as resistant to at least 3 of the following categories or agents: fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam). Outbreaks of other bacteria or viruses were notified sporadically. There were many small outbreaks, only eleven outbreaks included >10 patients. An outbreak that lasts more than 2 months progresses from phase 1 to phase 2, automatically. If a possible threat to the community exists, it will be classified as phase 3. Only 4 outbreaks were classified as phase 2 (n=2 both VRE) or 3 (n=2 VRE and MRSA). The median (range) number of patients that were involved was 5 (1-126).

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 21 (0-358) days. Seven outbreaks had a large interval (>3 months). Of these, 2 were outbreaks in nursing homes that were initially not reported. In 5 outbreaks the outbreak was detected not long before reporting, but some related patients were identified retrospectively over a longer period. Five institutions requested help for outbreaks. These were outbreaks with VIM-producing *Pseudomonas aeruginosa* in the Intensive care unit, ESBL-producing *Klebsiella pneumoniae*, carbapenemase producing *Klebsiella pneumoniae* and VRE (2 outbreaks).

Conclusions

1. Similarly to previous years, 3-4 outbreaks are reported to the SO-ZI/AMR each month.
2. Most outbreaks are reported within a month after detection
3. VRE and MRSA remain the most common outbreak microorganisms.
4. Most outbreaks are controlled quickly (<2 months), outbreaks that take >3 months to control are rare.
5. The median number of patients involved in an outbreak was 5

Table 4.4.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2015.

	2015 n=62 n (%)
Microorganism (resistance mechanism)*	
Staphylococcus aureus (MRSA)	22 (36)
Enterococcus faecium (VRE)	16 (26)
Klebsiella pneumoniae (ESBL)	2 (3)
Klebsiella pneumoniae (CPE)	1 (2)
Escherichia coli	1 (2)
Citrobacter freundii	1 (2)
Serratia marcescens	1 (2)
Pseudomonas aeruginosa	4 (7)
Clostridium difficile	2 (3)
Streptococcus pneumoniae (PRP)	1 (2)
Bordetella pertussis	1 (2)
Sarcoptes scabiei	1 (2)
Respiratoy syncytial virus	1 (2)
Norovirus	7 (11)
Endophthalmitis	1 (2)
Reason of reporting	
(threatened) closure	54 (87)
ongoing transmission	4 (6.5)
unknown	4 (6.5)
Highest level phase	
phase 1	58 (94)
phase 2	2 (3)
phase 3	2 (3)
phase 4	0 (0)
phase 5	0 (0)
Median number of patients: (range)	5 (1-126)
Median duration outbreak in days from reporting date until end of the outbreak (phase 0): (range)	48 (1-194)
Duration in days between detection of the first patient and day of reporting to the SO-ZI/AMR: (range)	21 (0-358)
Request for help	5 (8)

* *MRSA=methicillin resistant Staphylococcus aureus; VRE=vancomycin resistant Enterococcus faecium; ESBL=extended-spectrum beta-lactamase; CPE=carbapenemase producing Enterobacteriaceae; PRP=penicillin resistant Streptococcus pneumoniae*

4.5 Resistance in specific pathogens

4.5.1 *Neisseria meningitidis*

From 1995-2015 a total of 3989 strains from cerebrospinal fluid (CSF) and 2786 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 E-test and the EUCAST criteria for resistance were applied (susceptible: MIC ≤ 0.06 mg/l; resistant: MIC > 0.25 mg/l).
Table 4.5.1.1 and 4.5.1.2 show penicillin susceptibility and resistance percentages of *N. meningitidis* isolated from CSF or CSF and blood, and blood only respectively for 2009-2015. Penicillin resistance was occasionally found until 2006, in 2013 in one strain from CSF and one from blood. In 2014 and 2015 no penicillin-resistant isolates were received. The number of strains moderately susceptible to penicillin (MIC 0.064-0.25 mg/l) was 1-5% until 2009, increased to 33% for blood isolates and 39% for CSF isolates in 2012, and decreased subsequently to 12% (6/52) and 3% (1/32) respectively in 2015. No resistance to ceftriaxone or rifampicin was found in 2015.

In 2015, of 7 moderately susceptible strains from blood and/or CSF, 6 belonged to serogroup B and one to serogroup Y.

Alterations in the *penA* gene, associated with non-susceptibility to penicillin, were detected in 8 (10%) of the 84 isolates (one from CSF and 7 blood strains; one *penA* gene was associated with penicillin resistance, seven were associated with moderately susceptibility). Of these 8 isolates, one was phenotypically susceptible and seven were moderately susceptible by E-test (table 4.5.1.3).

The interpretation of the phenotypic susceptibility testing might not be fully reliable, because the susceptible/moderately susceptible breakpoint is exactly at the peak of the susceptibility distribution (0.064 mg/l). As E-test, like most assays, is not 100% reproducible, this can give rise to a considerable number of minor and major interpretation errors.

Table 4.5.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2009-2015

	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

* *MIC values in mg/l*

In 2015 apparently, E-test with EUCAST criteria yields less strains (8%) non-susceptible to penicillin than *penA* genotyping does (10%) and both methods do not agree completely. With both E-test and *penA* sequencing one moderately susceptible CSF isolate was found. With *penA* sequencing six moderately susceptible blood isolates were found, of which five were moderately susceptible and one was susceptible according to E-test. One isolate was resistant to penicillin according to *penA* sequencing, but moderately susceptible according to E-test. One or more of the following reasons may be involved: 1) other factors than *penA* gene alterations also confer non-susceptibility to penicillin; 2) a considerable number of minor interpretation errors occurs because the susceptible/moderately susceptible breakpoint lies at the peak of the susceptibility distribution; 3) this EUCAST breakpoint is too low and should be repositioned at 0.25 mg/l.

Conclusions

1. Penicillin resistance is sporadic (two strains in 2013, none in 2014, none in 2015).
2. Increase of strains moderately susceptible to penicillin is observed with a peak in 2012; the clinical relevance of this observation is matter of discussion.
3. Alterations in the penA gene are present in about 10%.
4. Resistance to ceftriaxone is not found; resistance to rifampicin sporadic (one strain in 2013).

Table 4.5.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2015

	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

* *MIC values in mg/l*

Table 4.5.1.3 Alterations in the penA gene penicillin susceptibility in *Neisseria meningitidis*

	Number of strains with penicillin MIC:			
Alterations penA gene*	**MIC ≤ 0.06 sensitive**	**0.064< MIC ≤ 0.25**	**0.25< MIC ≤ 1.0**	**MIC >1.0**
Yes	1	7	0	0
No	76	0	0	0
Total	**77**	**7**	**0**	**0**

4.5.2 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a species of Gram-negative bacteria responsible for the sexually transmitted infection gonorrhoea. 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 is made by culture or PCR on patients' materials, with a decrease in percentages of cultures over time (Figure 4.5.2.1). Susceptibility testing for 11,940 isolates was performed by E-test 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. Last year, testing for spectinomycin was also made optional. In 2015, penicillin and tetracycline were removed from the panel. Resistance levels were calculated using the EUCAST breakpoints for resistance.
In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500 mg). This is in contrast with international guidelines from e.g. the American and European CDC, where combination therapy with azithromycin is advised, also in the absence of a co-infection with Chlamydia. However, in the Netherlands no clinical failure of ceftriaxone has been reported. Also, mathematical models show that when resistance to azithromycin is already present in a population, combination therapy does not slow down the development of resistance when compared to ceftriaxone monotherapy (1). Therefore, combination therapy is not advised at the moment in the Netherlands. Future challenges will probably include the increasing resistance to azithromycin, as most countries do use combination therapy.

Results

- Resistance to ciprofloxacin (27%) has decreased since 2009 and resistance to cefotaxime (2%) decreased somewhat since last year, while it appeared to stabilise during recent years. Resistance to azithromycin (11%) has increased since last year (Figure 4.5.2.2).
- No resistance was found for ceftriaxone (Figure 4.5.2.2).
- Cefotaxime resistance in 2015 was highest among heterosexual women (3%), patients who worked as commercial sex workers in the last 6 months (7%), and in patients from Turkish (14%) or Eastern European (8%) origin.
- Azithromycin resistance in 2015 was highest among MSM (14%) and in patients from Dutch Antilles (20%) or Turkish (17%) origin.
- MIC distributions of cefotaxime and ceftriaxone were both highly skewed to the right and showed a unimodal shape (Figure 4.5.2.3a&b), whilst the MIC distribution of azithromycin shows a more normal distribution (Figure 4.5.2.3c).

Conclusions

1. Increase in diagnoses by non-culture, a continuing decrease in the relative number of diagnoses by culture to 29% in 2015.
2. Continuing increase of resistance to azithromycin from 8% in 2014 to 11% in 2015.
3. No resistance to ceftriaxone.

Figure 4.5.2.1 Diagnoses of gonorrhoea in STI centres in the Netherlands since 2006

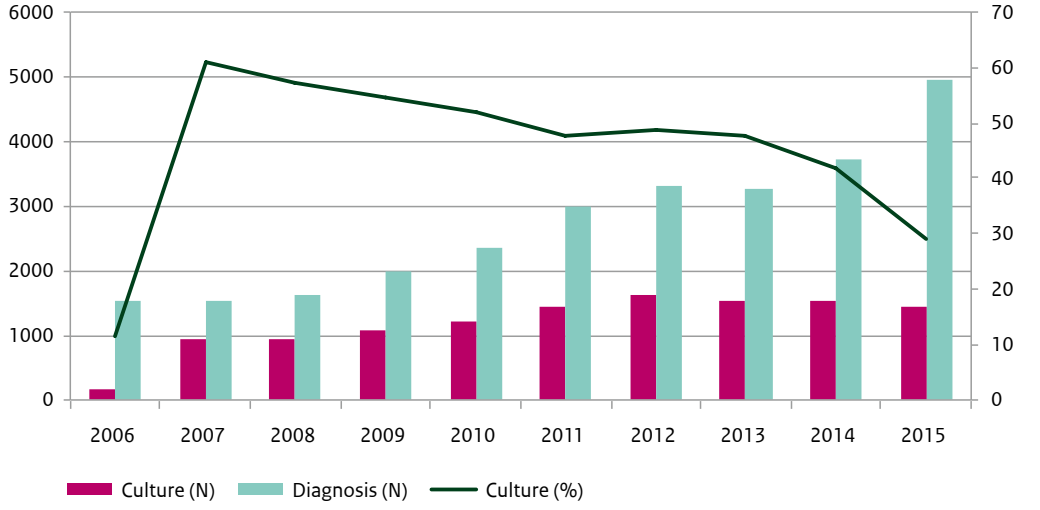
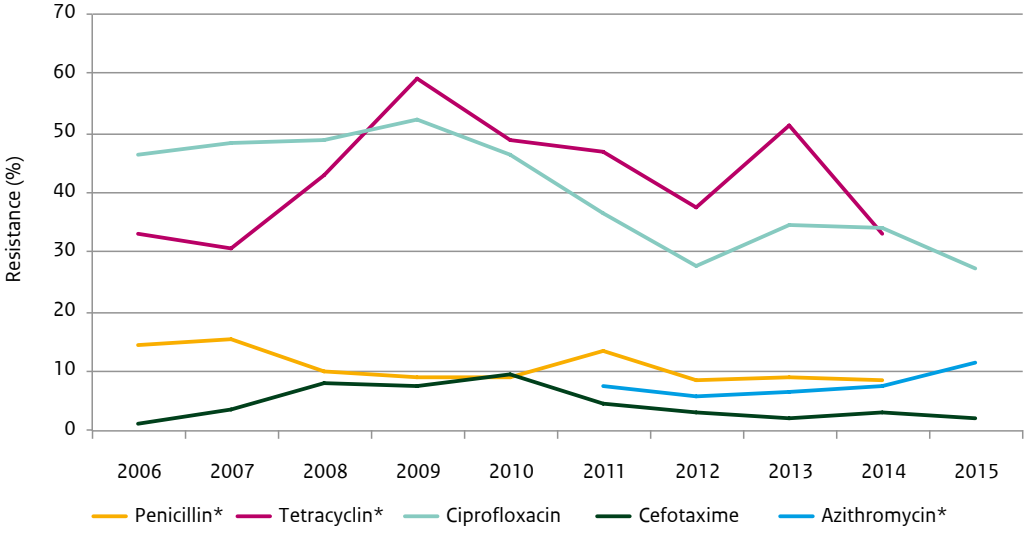


Figure 4.5.2.2 Trends in antibiotic resistance among *Neisseria gonorrhoeae* (N=11,940)



* *Ceftriaxone, Azithromycin and Spectinomycin were added to GRAS in 2011*
** *Penicillin and Tetracyclin were removed from GRAS in 2015*

Figure 4.5.2.3a MIC distributions of cefotaxime for *Neisseria gonorrhoeae*

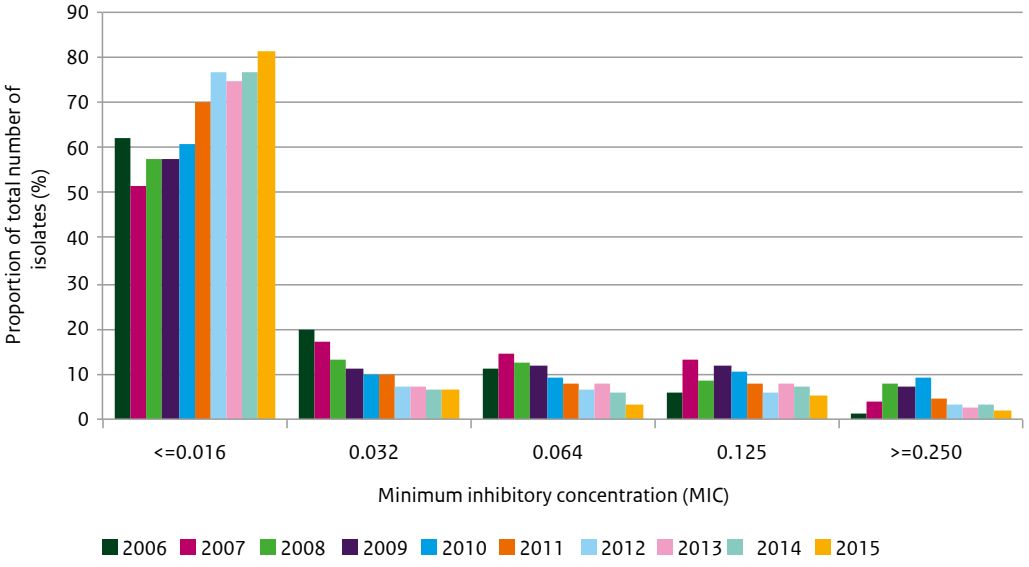


Figure 4.5.2.3b MIC distributions of ceftriaxone for *Neisseria gonorrhoeae*

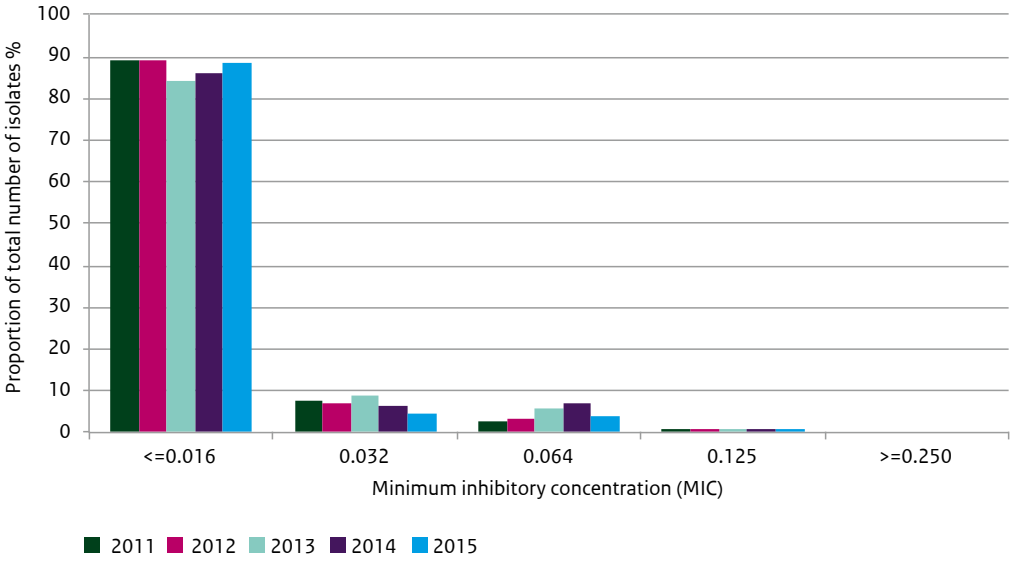
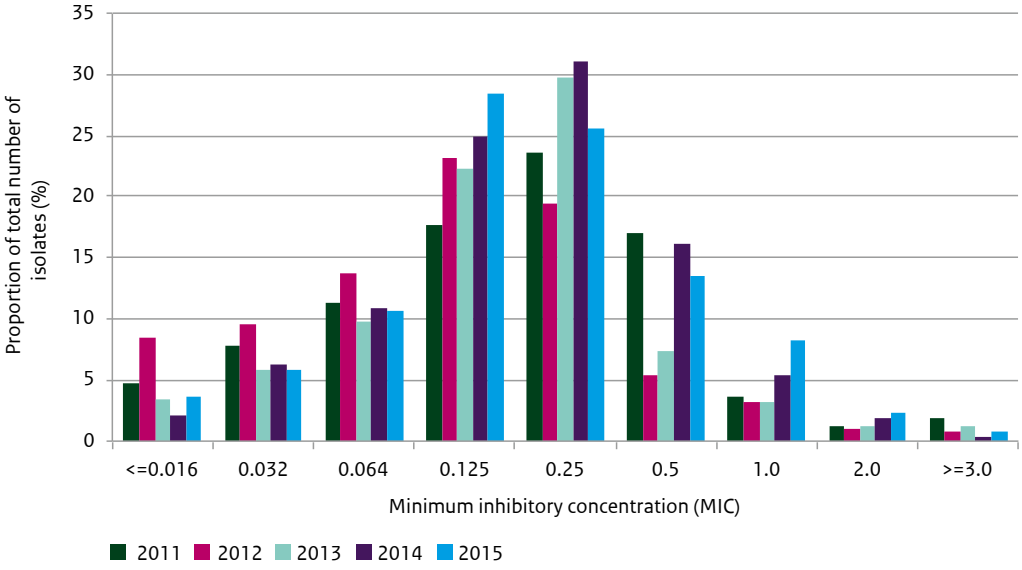


Figure 4.5.2.3c MIC distributions of azithromycin for *Neisseria gonorrhoeae*



References

[1] Xiridou M, Soetens LC, Koedijk FD, Van der Sande MA, Wallinga J. Public health measures to control the spread of antimicrobial resistance in Neisseria gonorrhoeae in men who have sex with men. Epidemiol Infect. 2015 Jun;143(8):1575-84.

4.5.3 *Mycobacterium tuberculosis*

The data presented is preliminary; not all cases from 2015 can be included at this moment, because mycobacteria grow very slowly; we still receive cultures from 2015.
Since 2011, not all drug susceptibility testing is performed at the RIVM, around 25% of these tests are done at peripheral laboratories. We assume that the results of the external drug susceptibility testing invariably represent sensitive tuberculosis, as otherwise we would have been requested to verify the results and test additional drugs.

Results

- Multidrug (MDR) resistant tuberculosis (MDR-TB), defined as at least resistant to isoniazid (INH) and rifampicin, was found in 2.8% of the isolates in 2013 and 1.1% of the isolates in 2014. In 2015, 1.7% of the isolates were MDR. Extensively drug-resistant (XDR-)TB was not diagnosed in 2015. (figure 4.5.3.1)
- In 2015, we received 594 *M. tuberculosis* complex isolates for epidemiological typing. Drug susceptibility testing at the RIVM was performed, on request, for 429 strains.
- Since 2010, the number of *M. tuberculosis* strains submitted per year decreased gradually from 784 in 2010 to 534 in 2014. For the first time in years the number of submitted *M. tuberculosis* isolates increased to 594 strains in 2015, probably due to the increased number of asylum seekers. This was in line with a national rise in notification of TB of 6%.
- Until 2010, INH resistance increased to 9.0%, but since 2011 it decreased yearly down to 6.6% in 2014. From 2012 to 2014, the INH resistance remained stable. In 2015, INH resistance decreased further to 5.4%. (figure 4.5.3.2)
- Rifampicin resistance decreased from 3.1% in 2013 to 1.3% in 2014. In 2015, rifampicin resistance slightly increased to 2.0%.
- Resistance to ethambutol remained low, fluctuating in the period 1998 to 2015 between 0.4% and 1.6%. In 2015, the ethambutol resistance amounted to 1.0%.

Conclusions

1. Overall, resistance to the antibiotics used for *Mycobacterium tuberculosis* strains remained stable over the last four years.
2. MDR-TB decreased from 2.8% in 2013 to 1.7% in 2015.
3. In 2015, for the first time in years, there was an increase in the number of *Mycobacterium tuberculosis* complex strains isolated, in line with an increase in notification of TB of 6%.

Figure 4.5.3.1 Trends in combined resistance TB

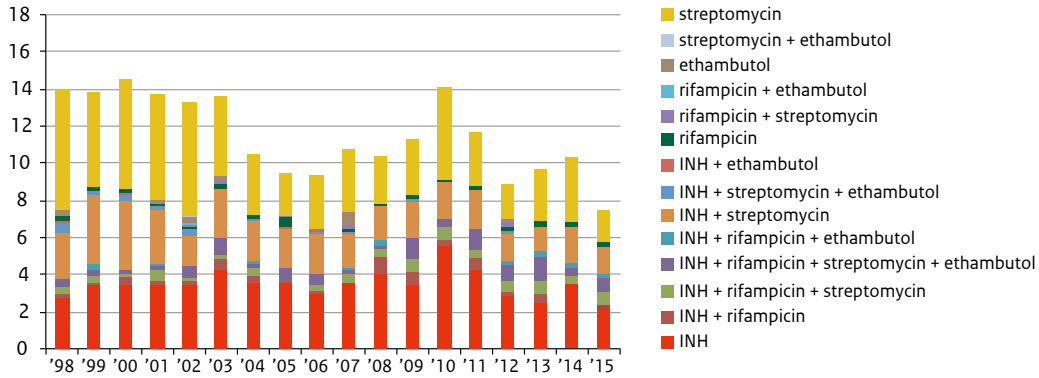
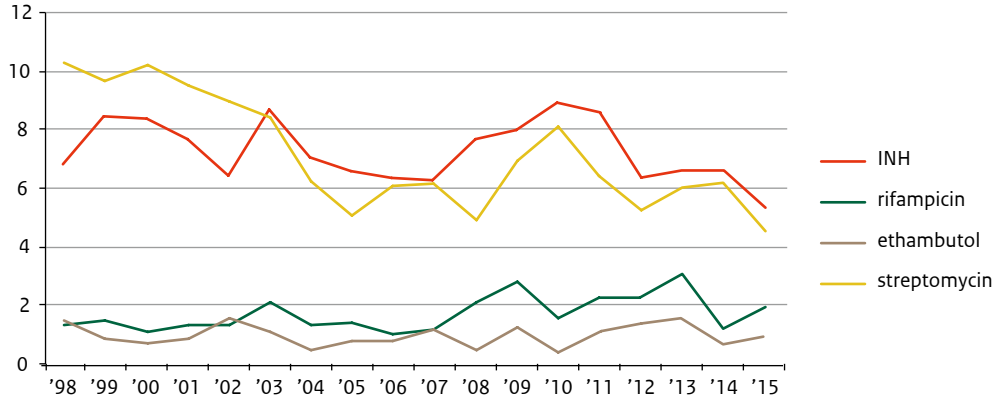


Figure 4.5.3.2 Trends in antibiotic resistance TB



4.5.4 Resistance to influenza antiviral drugs

Introduction

Of the three influenza virus types A, B and C that infect humans causing upper and lower respiratory tract infections, types A and B cause seasonal influenza epidemics impacting human public health with high morbidity and excess mortality every year. In the Netherlands, the susceptibility for 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 and being monitored since the 2005/2006 winter season. This monitoring is embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinel stations, 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) assay for known resistance markers for both the M2Bs and NAIs. For a subset of influenza viruses, the susceptibility to NAIs is determined using an enzyme inhibition assay, which generates a 50% inhibitory concentration of the drug (IC50). In the absence of known NAI resistance amino acid substitutions detected by genotypic assays, determination of the IC50 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.

Molecular epidemiology and its relation with resistance emergence

A single amino acid substitution is sufficient for reduced susceptibility for each of the antivirals. For M2B this is a major drawback as such substitutions do not affect the function of the ion channel. For NAI such substitutions affect functionality and reduce fitness of the virus and transmission and spread. Permissive amino acid substitutions elsewhere in the NA or the hemagglutinin can compensate for this loss of fitness and the same amino acid substitution might have different effects between types and subtypes of influenza virus and even sometimes within a subtype. The NA Y155H in former seasonal A(H1N1) results in reduced susceptibility for oseltamivir whereas in A(H1N1)pdm09 it has no effect. Despite carrying the same N1 subtype notification, genetically and on protein level they are distinct enough to have this effect.

Prevalence in the Netherlands

Table 4.5.4.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2005/2006 influenza season. Figure 4.5.4.1 shows the prescriptions for oseltamivir, zanamivir and amantadine. New findings since the 2014/2015 season not reported in the 2015 NethMap report are highlighted here. The NIC received an A(H1N1)pdm09 positive specimen that was collected from a patient in February 2015, which appeared to carry a mixture of wildtype NA 275H and NA 275Y oseltamivir 'highly reduced

Table 4.5.4.1 (Higly) reduced inhibition of influenza viruses by NAIs and M2Bs in the Netherlands, 2005/2006-2015/2016[1]

Season	A(H3N2)		A(H1N1) seasonal		A(H1N1)pdm09		B
	NAI	M2B	NAI	M2B	NAI	M2B	NAI
2005/2006	1/39 (3%)[2]	29/39 (74%)	NA	NA	NA	NA	2/48 (4%)[3]
2006/2007	0/50	38/51 (75%)	0/5	0/6	NA	NA	0/3
2007/2008	0/10	12/12 (100%)	47/172 (27%)[4]	0/49	NA	NA	1/81 (1%)[2]
2008/2009	5/74 (7%)[5]	8/8 (100%)	5/5 (100%)	ND	0/492	8/8 (100%)	0/19
2009/2010	ND	1/1 (100%)	NA	NA	20/627 (3%)[6]	54/54 (100%)	NA
2010/2011	0/2	2/2 (100%)	NA	NA	0/58	40/40 (100%)	0/64
2011/2012	0/257	34/34 (100%)	NA	NA	2/7 (29%)[7]	7/7 (100%)	0/10
2012/2013	0/156	15/15 (100%)	NA	NA	3/125 (2.4%)[8]	10/10 (100%)	0/8
2013/2014	2/220 (<1%)[9]	31/31 (100%)	NA	NA	1/150 (<1%)[10]	20/20 (100%)	0/4
2014/2015	0/727	50/50 (100%)	NA	NA	1/130 (<1%)[11]	9/9 (100%)	0/42
2015/2016[12]	0/10	3/3 (100%)	NA	NA	0/358	24/24 (100%)	0/2

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; NA = not applicable as there were no viruses of the given type or subtype tested; ND = viruses available, but analysis was not done.*

2 *The virus with reduced inhibition had an extreme outlier IC50 for oseltamivir and mild outlier IC50 for zanamivir.*

3 *Both viruses with reduced inhibition had outlier IC50 values for oseltamivir as well as zanamivir.*

4 *Viruses with highly reduced inhibition by oseltamivir only. Viruses were susceptible for zanamivir and M2Bs.*

5 *The 5 viruses had mild outlier IC50 values for oseltamivir but normal IC50 values for zanamivir.*

6 *Nineteen viruses had highly reduced inhibition by oseltamivir due to the H275Y amino acid substitution and normal inhibition by zanamivir; 18 from oseltamivir treated patients and one from an untreated patient, all epidemiological unlinked. One other virus had a 3-fold increased IC50 for oseltamivir and a 5-fold increased IC50 for zanamivir.*

7 *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.*

8 *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.*

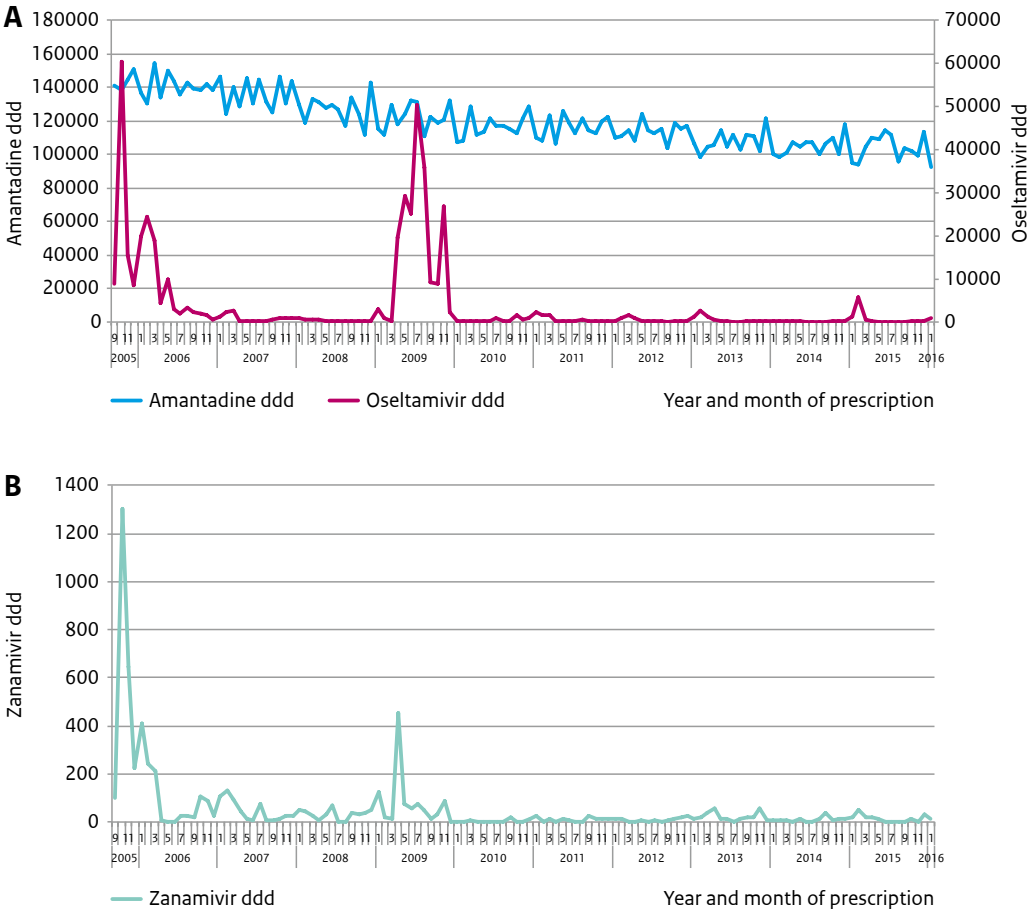
9 *Two clinical specimens from two patients with mixture of 292R and 292K amino acid composition; R292K is associated with highly reduced inhibition for oseltamivir and zanamivir. No patient characteristics or viral exposure data available.*

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

11 *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.*

12 *Preliminary data.*

Figure 4.5.4.1 Prescriptions of amantadine and oseltamivir (A) and zanamivir (B). Shown are the Defined Daily Doses (ddd) cumulated by month. Prescriptions of oseltamivir and zanamivir are linked to the seasonal epidemiology of influenza virus infections.



inhibition' amino acid substitution. The patient was treated with oseltamivir prior to specimen collection. None of the A(H1N1)pdm09, A(H3N2) and B influenza viruses analysed so far for the 2015/2016 season showed reduced or highly reduced inhibition by the neuraminidase inhibitors. All A(H1N1)pdm09 and A(H3N2) influenza viruses tested for M2B susceptibility showed since the 2008/2009 season the M2 S31N amino acid substitution associated with M2B resistance.

Risk groups

Specific risk groups for development of reduced susceptible influenza viruses are antiviral treated immunocompromised patients who typically experience prolonged shedding of virus and generate a more abundant range of quasispecies.

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, and most reduced susceptible viruses come from antiviral treated patients and do not spread. However, now and then there are clusters of NAI reduced susceptible viruses suggesting spread. Except for the emergence and sustained worldwide circulation of oseltamivir reduced susceptible former seasonal A(H1N1) in 2007/2008, these clusters did not result in sustained transmission of reduced susceptible virus. Nevertheless, these findings show that NAIs are still appropriate for prophylaxis and treatment and that it is important to monitor susceptibility of influenza viruses for the antivirals used.

4.5.5 Resistance among human anaerobic pathogens

An inventory was made of all anaerobic human pathogens isolated from patients at the University Medical Center Groningen. All strains were identified using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The MIC values for amoxicillin, amoxicillin-clavulanic acid (only for gram-negative anaerobes), clindamycin and metronidazole were determined using Etest. Resistance was assessed for strains belonging to the genera *Bacteroides*, *Bilophila*, *Fusobacterium*, *Parabacteroides*, *Porphyromonas*, *Prevotella*, *Veillonella*, *Actinomyces*, gram-positive anaerobic cocci (GPAC), *Clostridum*, *Eggerthella* and *Propionibacterium*, using breakpoints derived from EUCAST.

Gram-negative anaerobic bacteria

The susceptibility profiles for gram-negative anaerobic bacteria are summarized in Table 4.5.5.1. Resistance for amoxicillin was encountered in the genera *Bacteroides* (92%), *Bilophila* (78%), *Parabacteroides* (55%), *Prevotella* (41%), *Porphyromonas* (22%) and *Fusobacterium* (6%). Similar percentages of resistance were encountered in the previous years[1]. In most cases the resistance was due to the production of beta-lactamases. Several strains of *Fusobacterium* (6%), *Bacteroides* (0.6%) and *Parabacteroides* (17%) were resistant to both amoxicillin and amoxicillin-clavulanic acid, indicative for a mechanism of resistance other than beta-lactamase production.

Clindamycin resistance was encountered in the genera *Bacteroides* (21%), *Porphyromonas* (11%) and *Prevotella* (17%). None of the other genera of gram-negative anaerobic bacteria showed resistance to clindamycin. In the year 2014, 27% of the isolated *Parabacteroides* strains were resistant, in 2015 no resistance was encountered. From 2011-2014 the clindamycin resistance in *Prevotella* species varied from 8% to 11%1. In 2015, this amounted to 17%.

All tested strains were sensitive to metronidazole, except for one *Prevotella melaninogenica* strain. Last year we reported two resistant *Bacteroides fragilis* strains and in 2013 two metronidazole resistant *Prevotella* bivia strains. These findings necessitate alertness for metronidazole resistance in *Prevotella* isolates.

Gram-positive anaerobic bacteria

The susceptibility profiles for gram-positive anaerobic bacteria are summarized in Table 4.5.5.2. Amoxicillin resistance was only observed among species in the genus *Clostridium* (7%). This is slightly lower than observed in previous years (10%-14%)1. In all other tested genera amoxicillin resistance was not observed.

Clindamycin resistance was observed among *Actinomyces* sp. (7%), GPAC (13%) and *Clostridium* sp. (22%). Similar percentages of resistance were observed in the previous years1. It should be noted that the resistance to clindamycin among clostridia varies through the years, from 0% to 33%. No resistance for metronidazole was encountered.

Table 4.5.5.1 The range, MIC50 and percentage resistance of the last years observed for gram-negative anaerobic bacteria.

				% resistance				
	Antibiotic	**range (2015)**	**MIC50 (2015)**	**2015**	**2014**	**2013**	**2012**	**2011**
Bacteroides (n=163-166)[a]	amoxicillin	0,016 - >256	24	92	93	91	98	98
	amoxi-clav	0,016 - >256	0,5	1	2	0	0	1
	clindamycin	<0,016 - >256	1,5	21	20	20	27	27
	metronidazole	0,016 - 1	0,25	0	2	0	0	0
Parabacteroides (n=11-12)[a]	amoxicillin	1 - >256	4	55	55	60	n.a.[b]	n.a.[b]
	amoxi-clav	0,75 - 24	1,5	17	9	0	n.a.[b]	n.a.[b]
	clindamycin	0,016 - 4	1	0	27	60	n.a.[b]	n.a.[b]
	metronidazole	0,032 - 1	0,25	0	0	0	n.a.[b]	n.a.[b]
Prevotella sp. (n=58-59)[a]	amoxicillin	<0,016 - >256	1	41	51	60	33	42
	amoxi-clav	<0,016 - 1,5	0,064	0	0	0	0	0
	clindamycin	<0,016 - >256	0,016	17	11	4	10	8
	metronidazole	0,016 - >256	0,19	2	0	4	0	0
Fusobacterium (n=16)	amoxicillin	<0,016 - >256	0,032	6	0	16	9	22
	amoxi-clav	<0,016 - >256	0,032	6	0	5	0	0
	clindamycin	0,003 - 0,25	0,047	0	0	0	0	0
	metronidazole	<0,016 - 0,125	0,016	0	0	0	0	0
Porphyromonas (n=9)	amoxicillin	<0,016 - 24	0,016	22	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	amoxi-clav	<0,016 - 0,25	0,016	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	clindamycin	<0,016 - >256	0,016	11	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	metronidazole	<0,016 - 0,75	0,016	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
Bilophila sp. (n=9)	amoxicillin	0,125 - >256	12	78	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	amoxi-clav	0,032 - 2	1	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	clindamycin	0,125 - 0,75	0,38	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	metronidazole	<0,016 - 0,125	0,032	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
Veillonella sp. (n=13)	amoxicillin	0,047 - 2	0,5	0	22	0	0	n.a.[b]
	amoxi-clav	0,023 - 2	0,5	0	20	0	0	n.a.[b]
	clindamycin	0,015 - 1,5	0,064	0	0	0	0	n.a.[b]
	metronidazole	0,38 - 4	0,75	0	0	0	0	n.a.[b]

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

Table 4.5.5.2 The range, MIC50 and percentage resistance of the last years observed for gram-positive anaerobic bacteria..

	Antibiotic	range (2015)	MIC50 (2015)	% resistance 2015	2014	2013	2012	2011
Actinomyces sp. (n=99-102)[a]	amoxicillin	<0,016 - 2	0,125	0	0	0	0	0
	clindamycin	<0,016->256	0,12	7	11	0	0	8
	metronidazole	n.a.b	n.a.b	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
GPAC (n=143-149)[a]	amoxicillin	<0,016 - 3	0,064	0	0	0	0	0
	clindamycin	<0.016 - >256	0,25	13	18	10	6	14
	metronidazole	<0,016 - 3	0,125	0	0	1	0	0
Clostridium (n=41-46)[a]	amoxicillin	<0,016 - >256	0,064	7	14	0	10	0
	clindamycin	0,016 - >256	1,5	22	0	27	33	19
	metronidazole	<0,016 - 2	0,38	0	0	0	0	0
E. lenta (n=9-10)[a]	amoxicillin	0,19 - 4	0,5	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	clindamycin	0,094 - 1,5	0,25	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
	metronidazole	0,064 - 0,125	0,094	0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
Propionibacterium sp. (n=207-210)[a]	amoxicillin	<0,016 - 1	0,064	0	0	0	0	0
	clindamycin	<0,016 - >256	0,032	1	3	3	4	3
	metronidazole	n.a.b	n.a.b	n.a.b	n.a.b	n.a.b	n.a.b	n.a.b

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

References

[1] Veloo ACM, van Winkelhoff AJ. Antibiotic susceptibility profiles of anaerobic pathogens in The Netherlands. Anaerobe 2015 31:19-24.

4.5.6 *Clostridium difficile*

Introduction

The Dutch *C. difficile* Reference Laboratory operates since the recognition of PCR ribotype 027 outbreaks in the Netherlands in 2005. The transmission of ribotype 027 from Canada and the United States towards Europe was associated to fluoroquinolone resistance of two distinct ribotype 027 lineages.[1] The Netherlands succeeded to control ribotype 027 transmission during 2006.[2]
In 2009, the national *C. difficile* Infection (CDI) sentinel surveillance program was initiated. This program is currently implemented in twenty-three acute care hospitals. *C. difficile* isolates of all included patients were investigated by PCR ribotyping. Antibiotic resistance was determined for a selection of *C. difficile* sentinel surveillance isolates.

Epidemiology

Between May 2014 and May 2015, ribotype 027 was less prevalent (1%) amongst 931 submitted samples than in the preceding five years (2-4%). The most frequently isolated PCR ribotypes were 014/020 (16%), 078/126 (13%), and 002 (7%). The prevalence of ribotype 001 continued to decrease (from 21% in 2010-2011 to 6% in 2014-2015). No important new or emerging ribotypes were observed.[3] The Reference Laboratory also typed 133 *C. difficile* isolates from healthcare institutes that did not participate in the sentinel surveillance program. In these samples, ribotype 027 was most frequently isolated (14%), followed by ribotype 078/126 (13%). One 027 outbreak was observed in the North-Western part of the Netherlands, whereas five 027 outbreaks were reported in 2013-2014. Some 027 cases in surrounding nursing homes were detected as well. An outbreak management team was able to rapidly control the outbreak, and the local public health service was consulted to coordinate *C. difficile*-related measures in surrounding nursing homes.[3]

Resistance

Antibiotic resistance was determined for 50 randomly selected *C. difficile* sentinel surveillance isolates, collected between November 2014 and July 2015. None of the tested isolates was found to be resistant to the therapeutic drugs metronidazole and fidaxomicin, using CLSI/EUCAST breakpoints[4,5] (Table 4.5.6.1).

Conclusions

1. Ribotype 027 (associated to fluoroquinolone resistance) was less prevalent (1%) than in the in the preceding five years (2-4%).
2. No resistance of *C. difficile* to metronidazole and fidaxomicin was found in 2015.

Table 4.5.6.1 MIC_{50}, MIC_{90} and range (mg/L) of 50 C. *difficile* sentinel surveillance isolates. All isolates were tested in duplicate, results were summarized.

	MIC_{50}	MIC_{90}	Range
Ribotype 014 (n = 7)			
Fidaxomicin	0.06	0.25	0.06-0.25
Metronidazole	0.25	0.25	0.25-0.5
Clindamycin	8	32	4-32
Ribotype 078 (n = 7)			
Fidaxomicin	0.125	0.125	0.06-0.25
Metronidazole	0.25	0.25	0.125-0.25
Clindamycin	32	64	4-64
Other ribotypes (n = 36)			
Fidaxomicin	0.125	0.25	0.06-0.5
Metronidazole	0.25	0.25	0.06-0.5
Clindamycin	16	64	0.5-64

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4.5.7 Azole resistance in *Aspergillus fumigatus*

The saprophytic mold *Aspergillus fumigatus* is known to cause a spectrum of diseases, ranging from allergic aspergillosis to acute invasive disease. According to the most recent studies (azole-susceptible) invasive aspergillosis (IA) carries a mortality of approximately 30% in high-risk patient groups, when treated with azoles, such as voriconazole.[1]

Resistance to azoles has emerged as a clinical problem, and has now been reported in six continents: Asia, North America, South America, Europe and Australia.[2] Although resistance may develop during azole therapy, the main burden of resistance is through resistance selection in the environment. The use of azole fungicides might be an important factor in the selection of azole resistance in the environment. Although *A. fumigatus* is not a phytopathogen, many fungicides show activity against this fungus.[3] It is believed that *A. fumigatus* develops resistance to the azole fungicides and that the activity of medical azoles is lost due to similarity in molecule structure between azole fungicides and medical azoles.3 There are no clear patient risk factors for azole-resistant aspergillosis as previous surveillance studies indicated that two-thirds of patients with resistant disease have no previous history of azole therapy.[4,5] Case series show a mortality rate of azole-resistant IA between 50% and 100%.[4,5] The resistance is caused by a limited number of resistance mechanisms associated with the *Cyp51A*-gene, including TR34/L98H, TR53, and TR46/Y121F/T289A.

Azole resistance surveillance is performed using an agar-based screening plate on which *A. fumigatus* from primary culture is subcultered. If aspergillus is able to grow on azole-supplemented agar, the probability of resistance is very high. These isolates are further characterized in the Radboud University Medical Center. The total number of isolates that is screened is registered in the laboratory information systems of the participating centers and is used to calculate the resistance frequency.

In 2015 the resistance frequency was calculated for 4 UMCs, which screened unselected isolates, while in one center isolates obtained from ICU and hematology patients were screened (Table 4.5.7.1). Azole resistance frequency varied between 6.7% and 16.3% of patients with a positive *A. fumigatus* culture. The resistance frequency had increased in three centers, compared with 2014. The overall resistance frequency in 2015 was 10.7%, which is higher than observed in the two previous years.

In total 114 *A. fumigatus* isolates were analyzed for the presence of mutations in the *Cyp51A*-gene. Overall, in 78 isolates (68.4%) TR34/L98H was found, while 18 isolates (15.8%) harbored TR46/Y121F/T289A. Unlike previous years, which showed an increasing trend of TR46/Y121F/T289A, in 2015 the frequency of this mutation was much lower than in 2014: 15.8% versus 39.7%. There is no evident explanation for this shift in resistance mutations. In 14.9% of isolates point mutations were found in the *Cyp51A*-gene or no mutations at all. Overall, resistance mechanisms of environmental origin were found in 85.1% of azole-resistant *A. fumigatus* isolates, which is comparable with previous years. Overall, 87.7% of the isolates were resistant to itraconazole, 88.6% to voriconazole and 89.5% to posaconazole. In 2015 a new antifungal azole, isavuconazole, was approved for primary therapy of IA. Unfortunately, this azole shows cross-resistance with the currently available azoles, and 99.1% of the azole-resistant isolates were resistant to isavuconazole.

New resistance mutations

A new mutation was found in *A. fumigatus* cultured from compost (reported by Wageningen University). The isolate harbored three 46 bp repeats (TR463) and 4 mutations in the *Cyp51A*-gene: Y121F/M172I/T289A/G448S. As clinical azole-resistant isolates are screened using Y121F-mutation as marker, it was decided to re-analyze Y121F-positive isolates from 2010 and onwards as these may harbor the TR463 mutation. Indeed, three clinical *A. fumigatus* isolates were identified with the TR463/Y121F/M172I/T289A/G448S mutation: one cultured in Leiden in 2013, one in Groningen in 2014 and one in Amsterdam in 2015. Although the number of isolates with this mutation is low, it was recovered from patients in geographically distinct hospitals. The phenotype of TR463/Y121F/M172I/T289A/G448S appears to be similar to that of the TR46/Y121F/T289A mutation.
In addition, another new mutation was found in a single clinical isolate, harboring four 46 bp repeats: TR464/Y121F/M172I/T289A/G448S. Although this mutation has not yet been recovered from the environment, these observations indicate that new azole resistance mutations continue to emerge in the environment.

Resistance frequency in specific populations

The resistance frequency observed in ICU and hematology patients in Erasmus Medical Center was very high (31.8%, Table 4.5.7.1). In seven patients direct azole resistance PCR was positive resulting in 10 azole-resistant cases among 29 patients (34.5%). This observation confirms a previous observation in the ICU in Leiden, which showed that among 38 patients with *A. fumigatus* culture-positive IA, 10 patients were infected with an azole-resistant isolates (26% of culture positive cases).[6] These resistance rates are higher than found in the unselected surveillance and several ICUs are considering moving away from azole monotherapy as first-line therapy or have done so.

Azole-susceptible and azole-resistant co-infections

In the Radboudumc three patients were identified with azole-susceptible and azole-resistant *A. fumigatus* co-infections.[7] Cultures from these patients were found to harbor both azole-susceptible and azole-resistant colonies. The presence of azole-resistance was initially not detected, but during voriconazole therapy azole-resistant colonies emerged. Despite increasing resistance in cultures, the patients continued to improve both clinically and radiologically. However, in one patient the infection unexpectedly disseminated and the distant fungal lesion was caused by the azole-resistant strain.[7]
We believe that individual pulmonary lesions may evolve from genetically different *A. fumigatus* spores. Lesions caused by azole-susceptible spores will improve during azole therapy, but those caused by azole-resistant spores may progress. This observation complicates not only patient management, but also surveillance studies, as it will be difficult to rule out resistance. In 2015 azole-susceptible and azole-resistant colonies in culture were found in 13 patients of 50 patients with azole-resistant cultures from three centers, which corresponds with a rate of 26%.

Conclusion

The problems due to azole resistance have increased: in several hospitals the resistance frequency was higher than in 2014, and evidence was found for the emergence of new resistance mutations. In addition, the number of resistant cases in specific high-risk groups may be high requiring alternative empiric treatment strategies. Patient management is also complicated by co-infections caused by to azole-susceptible and azole-resistant *A. fumigatus*.

Conclusions

1. The overall azole resistance frequency in 2015 was 10.7%, which is higher than observed in the two previous years
2. Evidence was found for new resistance mutations
3. In 13 patients of 50 (26%) patients with azole-resistant cultures from three centers mixed cultures of susceptible and resistant isolates were found, complicating testing and screening for resistance.

Table 4.5.7.1 Overview of number of *A. fumigatus* culture-positive patients and frequency of azole resistance in 5 UMCs in 2013 to 2015.

	2013		2014		2015	
	#patients screened	#patients with confirmed azole resistant isolates (%)	#patients screened	#patients with confirmed azole resistant isolates (%)	#patients screened	#patients with confirmed azole resistant isolates (%)
ErasmusMC	231	10 (4.3)	265	10 (3.8)	22	7 (31.8)*
LUMC	99	19 (19.2)	113	15 (13.3)	141	23 (16.3)
Radboudumc	123	6 (4.9)	143	7 (4.9)	145	12 (8.3)
UMCG	194	16 (8.2)	191	18 (9.4)	225	15 (6.7)
VuMC	113	8 (7.1)	104	9 (8.7)	89	14 (15.7)
Total	**760**	**58 (7.6)**	**814**	**59 (7.2)**	**600**	**64 (10.7)****

* *A. fumigatus isolates from 22 ICU and hematology patients were screened for azole resistance.*

** *Based on four centers where screening of unselected isolates took place.*

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5 Antimicrobial stewardship Monitor

Introduction

Antimicrobial stewardship is the persistent effort by a health care institution to optimize antimicrobial use among patients in order to optimize patient outcomes, contain healthcare costs and minimize unintended consequences of antimicrobial use, including toxicity and the emergence of resistance. In their 2012 vision document, drafted at the request of Dutch Health Care Inspectorate (IGZ), the SWAB has stressed the need to establish antimicrobial stewardship teams (A-teams) in every Dutch hospital responsible for the implementation of an antimicrobial stewardship program. Together with infection prevention and control, antimicrobial stewardship programs are essential to curb antimicrobial resistance and ensure the treatment of infections in the future. In response to the recommendation by SWAB, IGZ and the Minister of Health, A-teams have been established in the majority of hospitals in the Netherlands. Practical support is provided by the "Antimicrobial Stewardship Practice Guide for the Netherlands", available at www.ateams.nl. As of 2015, the SWAB has started to develop the Antimicrobial Stewardship Monitor program to measure the progress and impact of the national implementation of antimicrobial stewardship. This Antimicrobial Stewardship monitor will report yearly on:

1) The quality of antibiotic use in hospitals in the Netherlands
2) The stewardship activities employed by A-teams aimed at measuring and improving the quality of antimicrobial use

These data, combined with antibiotic consumption and resistance data, will provide insight into the process of implementation of the antimicrobial stewardship program in the Netherlands, and its impact.

The Antimicrobial Stewardship Monitor, developed by SWAB, will be published yearly in NethMap as from this year. Since the formation of antimicrobial stewardship program in hospitals is not yet complete, we here present data obtained in a pilot study conducted in 5 hospitals.

Methods

Twelve quality indicators (QI) were selected to monitor the appropriateness of hospital antibiotic use. Eleven were selected by a RAND-modified Delphi procedure among international experts1. These were complemented by a twelfth QI: "Perform a bedside consultation in case of *Staphylococcus aureus* bacteremia", since this is a well-documented intervention to reduce mortality among those patients (Table 5.1). The QI "Prescribe empirical antibiotic therapy according to local guideline" was only assessed for antibiotics on a list of "restricted" and "limited prescription" antibiotics. In the Antimicrobial Stewardship Practice Guide for the Netherlands, *Restricted Antimicrobial drugs* have been defined as drugs that only should be prescribed for microorganisms that are resistant to the usual drugs. *Limited Prescription Antimicrobial drugs* have been defined as drugs that are indicated for some indications but should not be used in other situations.

The possibilities of reporting these 12 QIs without performing extra effort was tested in a pilot study among five A-teams from two university hospitals, two teaching hospitals and one non-teaching hospital. Performance scores were calculated for the QIs that were documented for the period of January 2015 until December 2015.

Results

In this pilot study, activities were limited to five different QIs (Table 5.2), and two of these were performed by all A-teams. These two QIs were (1) the assessment of the appropriateness of prescription of Restricted Antibiotics and Limited Prescription Antibiotics and (2) bedside consultation for *S. aureus* bacteremia. Four of five A-teams could report data about the appropriateness of Restricted Antibiotics and Limited Prescription Antibiotics. For the other QIs, results could be reported for 2 out of 5 hospitals at maximum (Table 5.2).

Carbapenem prescriptions followed the local guideline or an expert's advice in 90% (range: 84-97%) of the cases (Figure 5.1). The appropriateness of glycopeptides prescription was generally high: 97% (range: 83-100%). However, the numbers of prescriptions in some of the hospitals were very low. Pre-authorisation for glycopeptides prescription was mandatory in one hospital, which resulted in 100% appropriateness (Figure 5.2). Fluoroquinolone prescription was appropriate in 79% (range: 68% to 100%) of the cases (Figure 5.3). Hospital D only monitored the use of levofloxacin.

Discussion and future directions

The primary goal of this pilot study for the national SWAB *Antimicrobial Stewardship Monitor* program was to assess the feasibility of registration and uniform reporting of antimicrobial stewardship activities and outcomes, in order to report the impact of the national antimicrobial stewardship program in NethMap on a yearly basis.

Most of the A-teams included in the pilot study could provide data about whether or not the use of Restricted Antibiotics and Limited Prescription Antibiotics was justified. In general, the indication for the use of glycopeptides was correct, whereas the prescriptions of carbapenems and particularly fluoroquinolones followed the local guidelines or an expert's advice less frequently. Notably, the number of A-teams included in this pilot study is small, and there is significant variation between hospitals. The latter could reflect differences in (the success of) antimicrobial stewardship programs, hospital type, complexity of the patients admitted and differences in (education of) hospital staff.

In addition, the completeness of the local guidelines and the way the review of antibiotic use was performed in each hospital may have influenced the performance scores. For example, antibiotics may

correctly follow ward-specific guidelines that are not (yet) incorporated into the local hospital guideline. However, if the local guideline is used as reference, these prescriptions will be scored as inappropriate. This underlines the necessity of uniform definitions.
A-teams that currently have successfully implemented an antimicrobial stewardship program often lack a systematic registration system incorporated in the daily work flow. As a result, they are not able to report outcomes for most QIs. Not only does this hamper the development of a national *Antimicrobial Stewardship Monitor*, more importantly, this implies that A-teams themselves have insufficient data to analyze where and how to intervene. Therefore, in the national SWAB *Antimicrobial Stewardship Monitor* program, SWAB will actively support the A-teams in collecting and reporting their activities in a structured and automated way. In order to simultaneously perform and document activities and to be able to extract data automatically, A-teams should establish a close collaboration with their local ICT experts.
As supported by the data from the pilot study presented, the national *Antimicrobial Stewardship Monitor* will initially focus on data that can be acquired relatively easily in the majority of hospitals. To assess what parameters to start with, input of the A-teams is of the utmost importance. In close collaboration with the A-teams, SWAB will establish a data set for the Antimicrobial Stewardship Monitor 2017, in which data for all Dutch hospitals will be reported in NethMap 2017.

Figure 5.1 Appropriateness of carbapenem prescriptions in 2015 in 4 hospitals. The number of prescriptions in the bar represent the number of prescriptions reviewed. One hospital (A) monitored during a four-month period, the others performed continuous monitoring and feedback.

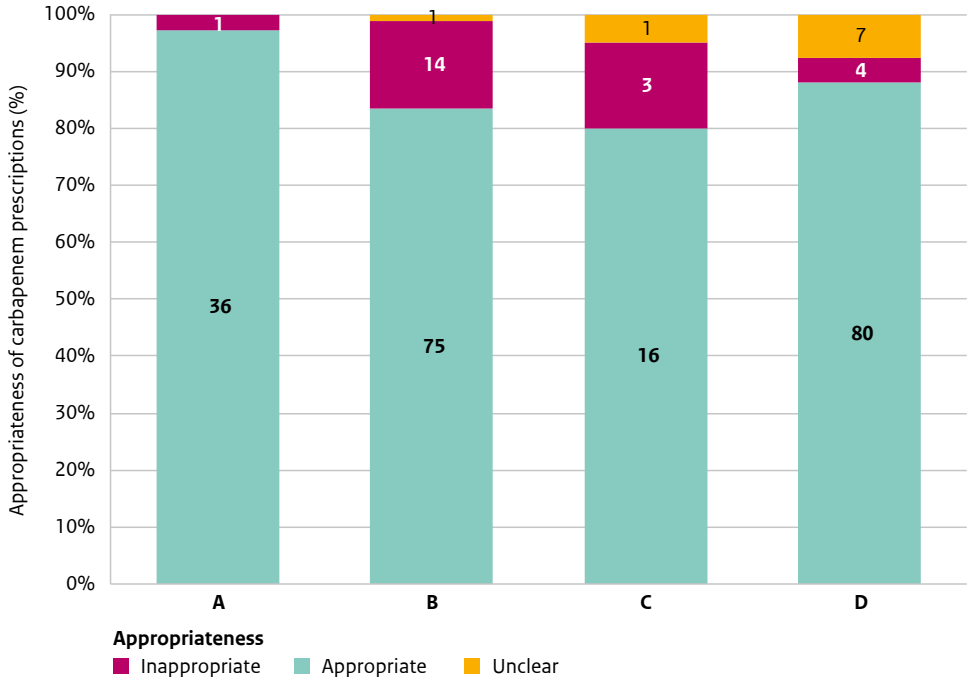

Figure 5.2 Appropriateness of glycopeptides prescriptions in 2015 in 4 hospitals. The number of prescriptions in the bar represent the number of prescriptions reviewed. One hospital (A) monitored during a four-month period, the others performed continuous monitoring and feedback.

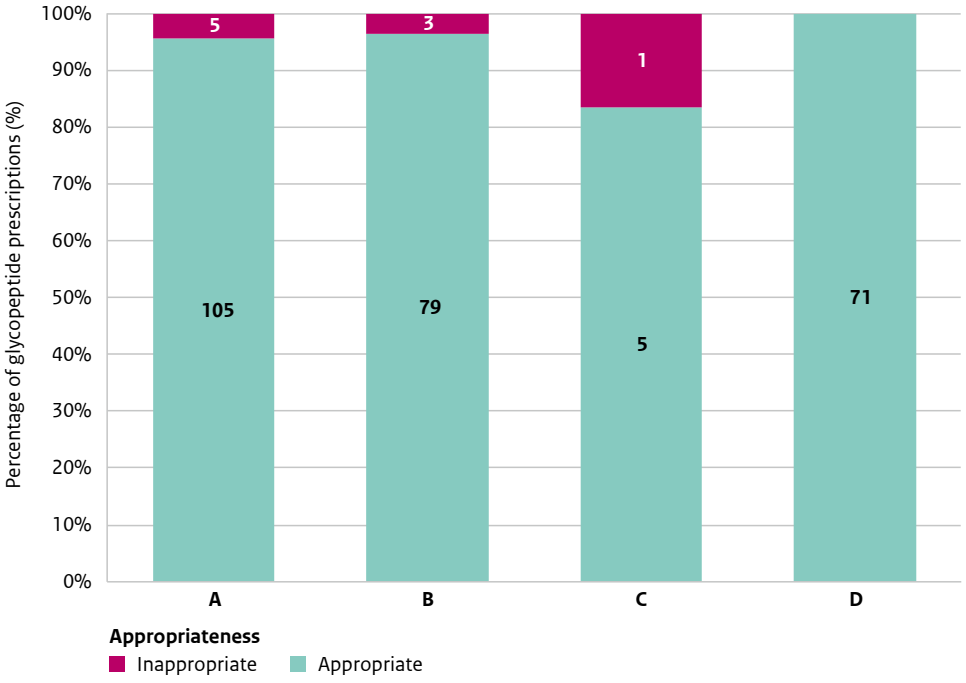

Figure 5.3 Appropriateness of fluoroquinolone prescriptions in 2015 in 4 hospitals. The number of prescriptions in the bar represent the number of prescriptions reviewed. One hospital (A) monitored during a four-month period, the others performed continuous monitoring and feedback.

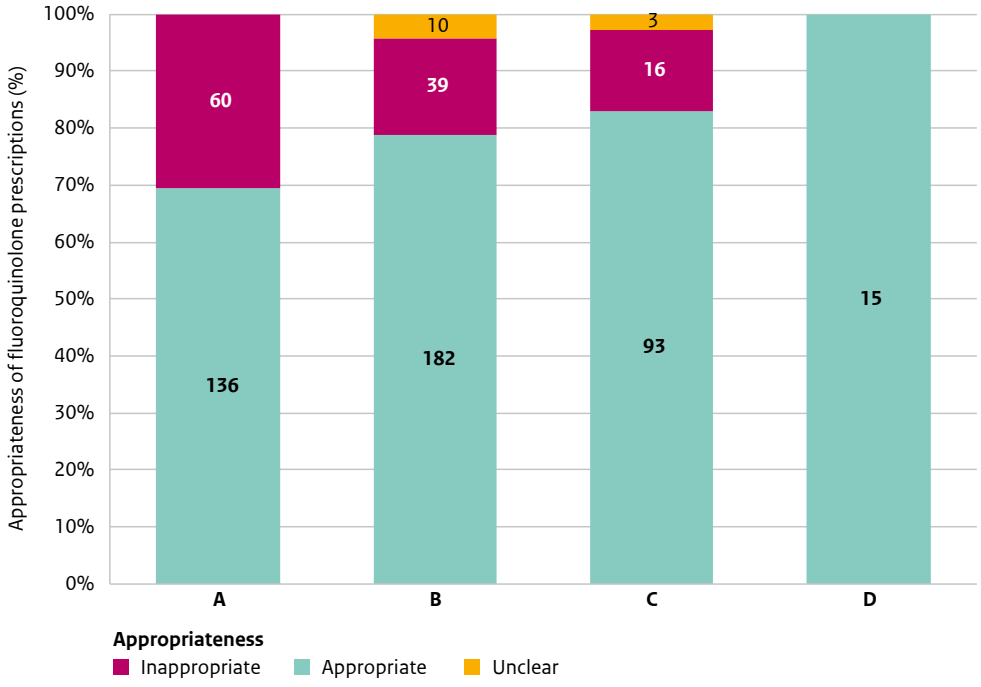

Table 5.1 List of generic quality indicators to monitor antibiotic use in hospitalized adult patients on non-ICU departments and their performance score.

Number	Quality indicator	Performance score
1	Take at least two sets blood cultures before starting systemic antibiotic therapy	Percentage of patients from whom blood cultures were taken before the first administration of antibiotics in the hospital
2	Take cultures from suspected sites of infection, preferably before antibiotics are started	Percentage of patients from whom cultures of suspected sites were taken
3	Prescribe empirical antibiotic therapy according to the local guideline*	Percentage of patients whose antibiotic prescription* was according to the local guideline or followed an expert's advice (microbiologist or infectious disease specialist)
4	Document antibiotic plan	Percentage of patients with a documented antibiotic plan
5	Switch from intravenous to oral therapy on the basis of the clinical condition and when oral treatment is adequate	Percentage of patients whose intravenous administration of antibiotics was changed after 48-72h to oral therapy based on clinical conditions
6	Change empirical to pathogen-directed therapy	Percentage of patients with positive cultures whose empirical therapy was changed correctly to pathogen-directed therapy
7	Adapt antibiotic dosage to renal function	Percentage of patients with a compromised renal function whose dosing regimen was adjusted to renal function
8	Perform therapeutic drug monitoring when the therapy is >3 days for aminoglycosides and >5 days for vancomycin	Percentage of patients who received aminoglycosides or vancomycine for whom at least one serum drug level was measured after >3 or >5 days of therapy, respectively
9	Discontinue antibiotic therapy if infection is not confirmed	Percentage of patients without an infection whose empirical therapy was discontinued within 7 days after starting empirical therapy
10	A local antibiotic guideline should be present	
11	The local guidelines should correspond to the national antibiotic guidelines	
12	perform bedside consultation in case of *Staphylococcus aureus* bacteremia	Percentage of patients with *Staphylococcus* aureus bacteremia for whom a bedside consultation by an infectious disease specialist was performed

** was only assessed for antibiotics on a list of "restricted" and "limited prescription" antibiotics*

Table 5.2 Antimicrobial stewardship teams activities and registration.

QI number	QI	Hospital	A	B	C	D	E	Total
1	Blood cultures taken?	Activity	-	-	-	+	-	1/5 (20%)
		Registration	-	-	-	+	-	1/5 (20%)
3	Use of restrictive list	Activity	+	+	+	+	+	5/5 (100%)
		Registration	+	+	+	+	-	4/5 (80%)
5	Switch intravenous to oral therapy	Activity	-	+	+	-	+	3/5 (60%)
		Registration	-	+	+	-	-	2/5 (40%)
8	Therapeutic drug monitoring	Activity	-	+	+	+	+	4/5 (80%)
		Registration	-	+	-	-	-	1/5 (20%)
12	Bedside consultation for *S. aureus* bacteremia	Activity	+	+	+	+	+	5/5 (100%)
		Registration	-	+	-	-	-	1/5 (20%)

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MARAN 2016

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2015

June 2016

Colophon

This report is published under the acronym MARAN-2016 by the Central Veterinary Institute of Wageningen University and Research Centre 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-2016 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-2016 is published in a combined back-to-back report with NETHMAP-2016. The combined report is available on the website of CVI-Lelystad at www.cvi.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-2016 can be ordered from the secretariat of CVI-Lelystad, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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Contents

1 Summary

Antibiotic Usage

Sales of antimicrobial veterinary medicinal products (206 tonnes) decreased in 2015 by 0.65%, compared to 2014 (207 tonnes). In relation to 2009, the index year used by the Ministry of Economic Affairs, in 2015 total sales decreased by 58.4%. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 64%. Sales and use of antimicrobial drugs of critical importance for human healthcare (fluoroquinolones and cephalosporins of 3rd and 4th generation) were further reduced in 2015 in the monitored animal sectors. In three sectors (pigs, cattle and broilers) an overall reduction in use of antimicrobials was realized. In veal calves and turkeys increased use was noted. The fraction of unmonitored use data increased. Therefore, surveys in some unmonitored sectors were initiated in 2015 and will be followed by others in 2016.

Antimicrobial resistance

In 2015 *S.* Typhimurium (N = 233) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 176), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (N=284) in second place. In pigs, *S.* Typhimurium and its monophasic variant dominated. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. In 2015, the number of *S.* Paratyphi B var. Java was substantially reduced and no longer predominant in poultry. Also *S.* Heidelberg, still predominant in 2014, was less frequently isolated in 2015. The prevalence of *S.* Enteritidis remained comparable to the prevalence in 2014 and was the most predominant serovar in poultry in 2015. Highest resistance levels were observed in the monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Heidelberg, *S.* Paratyphi B var. Java and other *S.* Typhimurium and to a lesser extent in *S.* Infantis, *S.* Brandenburg and *S.* Stanley. The dominant serovars of ciprofloxacin resistant isolates were *S.* Enteritidis (20%), *S.* Infantis (12%), *S.* Typhimurium (8%), *S.* Heidelberg (8%) and *S.* Paratyphi var. Java (7%), mainly from poultry and human sources. In 2015, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 36/1761 (2.0%), among eleven different serovars, predominantly isolated from poultry sources. In 2015 no carbapenemase producing *Salmonella* were found.

As a result of prioritization and changes in legislation, since 2014 the focus of the surveillance of antimicrobial resistance in *Campylobacter* is focused at poultry and poultry meat samples. Resistance rates in *C. jejuni* from broilers and poultry meat did not substantially change in 2015 as compared to 2014. Resistance rates for quinolones and tetracycline in *C. coli* from broilers considerably increased in 2015 as compared to 2014. Hence resistance rates became comparably high in broilers and poultry meat. In laying hens, resistance levels of *C. jejuni* for the quinolones and tetracycline were substantially lower compared to broilers. However, these differences were not observed with *C. coli*. Ciprofloxacin resistance in *Campylobacter* isolates is high and still rising in human patients which is a concern for public health. However, resistance to erythromycin, representing the first choice macrolide clarithromycin for treating campylobacteriosis, remained low. For *C. jejuni* from human patients, resistance levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

Over the last decade, STEC O157 isolates from humans show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim, resulting in approximately 15% resistance for all four antibiotics in 2015. Resistance profiles of STEC non-O157 isolates from raw beef were comparable to those of human isolates, except for the quinolones. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was 2.2% in meat isolates, but not detected in human STEC O157 isolates.

In 2015, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and veal calves and stabilized in pigs. In isolates from broiler meat, turkey meat, beef and pork, resistance stabilized. Resistance to third-generation cephalosporins was low (< 1%) in most animal species. In broiler isolates the resistance level stabilised at 2.5%. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from poultry sources and to a lesser extent from white veal calves. Among indicator *E. coli* from animals and meat, resistance to ampicillin, tetracycline, sulphonamides and trimethoprim was still commonly detected 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. Monitoring of herbs, included in the monitoring programme of 2015, revealed the occurrence of *E. coli* frequently resistant to ampicillin, tetracycline, sulphonamides, trimethoprim, chloramphenicol and ciprofloxacin.

In 2015, only enterococci isolates from veal calves were included. Susceptibility testing of enterococci is considered of lesser priority than *E. coli*, also in the new legislation. Therefore, from 2013 onwards poultry, pigs and cattle are sampled once every three years instead of annually. In veal calves, highest resistance levels were observed for tetracycline (52.9% in *E. faecalis* and 41.3% in *E. faecium*), erythromycin (41.2% in *E. faecalis* and 30.4% in *E. faecium*). In addition, high levels of resistance for chloramphenicol were observed in *E. faecalis* (29.4%) and for quinu/dalfopristin in *E. faecium* (72.8%). For two new antibiotics in the panel (daptomycin and tigecyclin) no resistance was observed in enterococci derived from faeces, but in meat resistance for tigecycline was incidentally observed in *E. faecalis* (0.7%) and for daptomycin in *E. faecium* (6.5%).

ESBL-producing *E. coli* represented 0.9% of randomly isolated *E. coli* , the lowest proportion observed since 2007. Selective isolation from livestock faeces indicated ESBL/AmpC producing *E. coli* prevalence of 56.5% in broilers, 12.3% in slaughter pigs, 17.3% in white veal calves, 10% in rosé veal calves and 9.3% in dairy cows. Classical human associated ESBL-types $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$ were found in *E. coli* isolates from broiler faeces, together with $bla_{CTX\text{-}M\text{-}55}$ not described before in Dutch broilers. ESBL/AmpC prevalence in *E. coli* isolates from prepared meat tended to be higher compared to raw meat, possibly due to cross-contamination during processing. ESBL/AmpC-prevalence in poultry meat decreased substantially compared to 2014. This decrease is most likely associated with the major reduction in antibiotic use in broilers since 2011 and the total ban on the use of ceftiofur at hatcheries in 2010.

In 2015 the prevalence of ESBL-producing *Salmonella* was 1.8%, confirming the decreasing trend observed in 2014 (2.1%) and 2013 (4%). Most frequently found ESBL-genes were $bla_{CMY\text{-}2}$, generally associated with *S.* Heidelberg, and $bla_{CTX\text{-}M\text{-}1}$ in *S.* Heidelberg and Enteritidis. In *Salmonella* isolates from human sources a variety of ESBL-genes were found: $bla_{CMY\text{-}2}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}65}$.
The majority of ESBL-*Salmonella* isolates were highly multidrug resistant, with an increased pattern of resistance to 5- 8 different antibiotics compared to 2014. No resistance to carbapenems was detected in *Salmonella*.

No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance using selective methodologies. Only 3 isolates of *Shewanella* spp holding chromosomal $bla_{OXA\text{-}48b}$ were detected in broilers and a veal calf.

The colistin resistance gene *mcr-1* was present at low level in *E. coli* from livestock (≤ 1%) and meat (2%) and in *Salmonella* from poultry meat (1%) in the period 2010-2015. In 2015, *mcr-1* was identified in sixteen *E. coli*, one *S.* Paratyphi B variant Java isolated and one *S.* Schwarzengrund, all isolated from poultry sources (chicken and turkey meat).

It can be concluded that the reduction in antibiotic sales for animals has almost stabilized in 2015. The reduction in use levelled off in most animal species except for veal calves and turkeys, species that showed an increase. In poultry the use decreased after the increase in 2014. This usage pattern was reflected in the resistance data of 2015 where resistance levels mostly stabilized in bacterial organisms sampled from all animal species. However the occurrence of ESBL/AmpC-producing *E. coli* in poultry products 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 colistin resistance gene *mcr-1*, were not detected or found at low levels, respectively. The current stabilization of antibiotic use and of resistance levels may warrant a re-evaluation of the current targets for antibiotic use in relation to targets for antimicrobial resistance in animals and food thereof.

2 Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2015

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data of all antimicrobial veterinary medicinal products on package level sold in the Netherlands in 2015, 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 88.7% of the sales in 2015.
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, which was launched by EMA in 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 Dutch livestock farming sector (pigs, poultry, veal calves, other cattle and sheep) shows annual variations (Table ABuse01). Overall, the total live weight of livestock produced in The Netherlands has remained stable, 2.5-2.6 million tons, although over the last four years a gradual increase of 6.5% was observable. All in all this indicates that the reported reduction over the years in sales of antimicrobials 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	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Piglets (less than 20 kg)	4,300	4,170	4,470	4,680	4,555	4,809	4,649	4,797	4,993	4,920	5,115	5,408
Sows	1,125	1,100	1,050	1,060	1,025	1,100	1,098	1,106	1,081	1,095	1,106	1,053
Fattening pigs	5,715	5,730	5,700	5,970	6,155	6,199	6,459	6,200	4,189	4,209	4,087	4,223
Other pigs	1,865	1,900	1,660	1,960	2,050	2,100	2,040	2,021	1,841	1,789	1,765	1,769
Turkeys	1,238	1,245	1,140	1,232	1,044	1,060	1,036	990	827	841	794	863
Broilers	43,854	45,525	42,529	44,487	50,270	52,323	54,367	57,811	43,912	44,242	47,020	49,107
Other poultry	42,922	48,695	50,666	49,992	47,914	46,383	48,218	40,442	52,356	54,345	56,924	58,636
Veal calves	775	813	824	860	913	886	921	906	908	925	921	909
Cattle	2,984	2,933	2,849	2,960	3,083	3,112	3,039	2,993	3,045	3,064	3,230	3,360
Sheep	1,700	1,725	1,755	1,715	1,545	1,091	1,211	1,113	1,093	1,074	1,070	1,032

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 2015 (206 tonnes) were slightly reduced (0.65%) , compared to 2014 (207 tonnes) . Total sales decreased by 58.4% over the years 2009-2015.
Some classes of antibiotics showed a decrease in 2015, but others increased (Figure ABuse02). Increased sales were noted for aminoglycosides (+44%), tetracyclins (+18%), quinolones (+13%), polymyxins (+10%), amphenicols (+4.8%) and combinations (+0.8%). Reductions in sales were realized for all cephalosporin, 1st and 2nd generation -6.7%, and 3rd and 4th generation -20%, for fluoroquinolones (-6.5%), macrolides (-17%), trimethoprim/sulfonamides (-14%), penicillins (-7.4%).

Tetracyclines
The total mass of tetracyclines sold increased, the fraction of doxycycline was stable with 42% of the total sales of tetracyclines (41% in 2014, 31% in 2013, 41% in 2012 and 34% in 2011).

Penicillins
Second place in mass, penicillin sales decreased to the level of 2013. 70% of the mass in this group consists of broad spectrum penicillins.

Trimethoprim/sulfonamides
The use of trimethoprim/sulfonamides decreased further in 2015, now being third in mass sold.

Figure ABuse01 Antimicrobial veterinary medicinal product sales from 1999-2015 in kg (thousands).

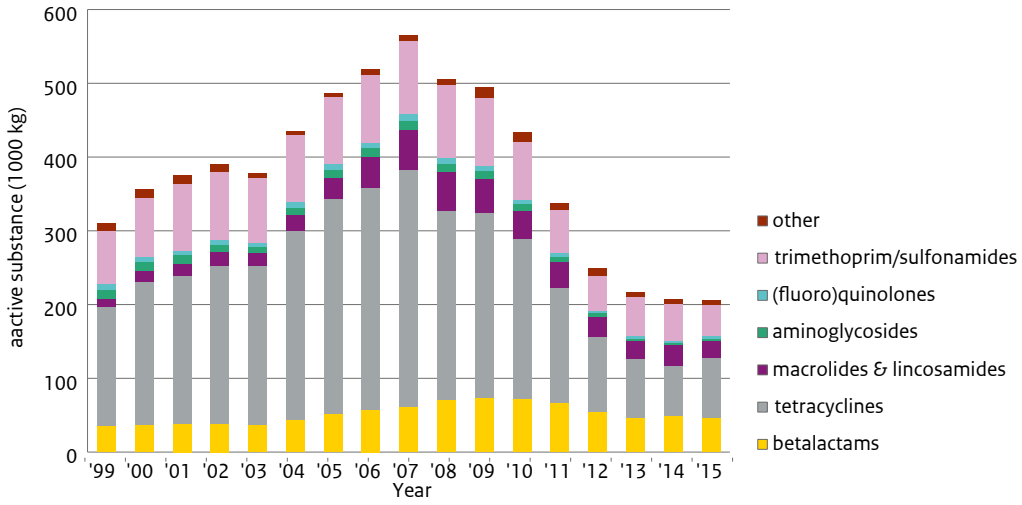
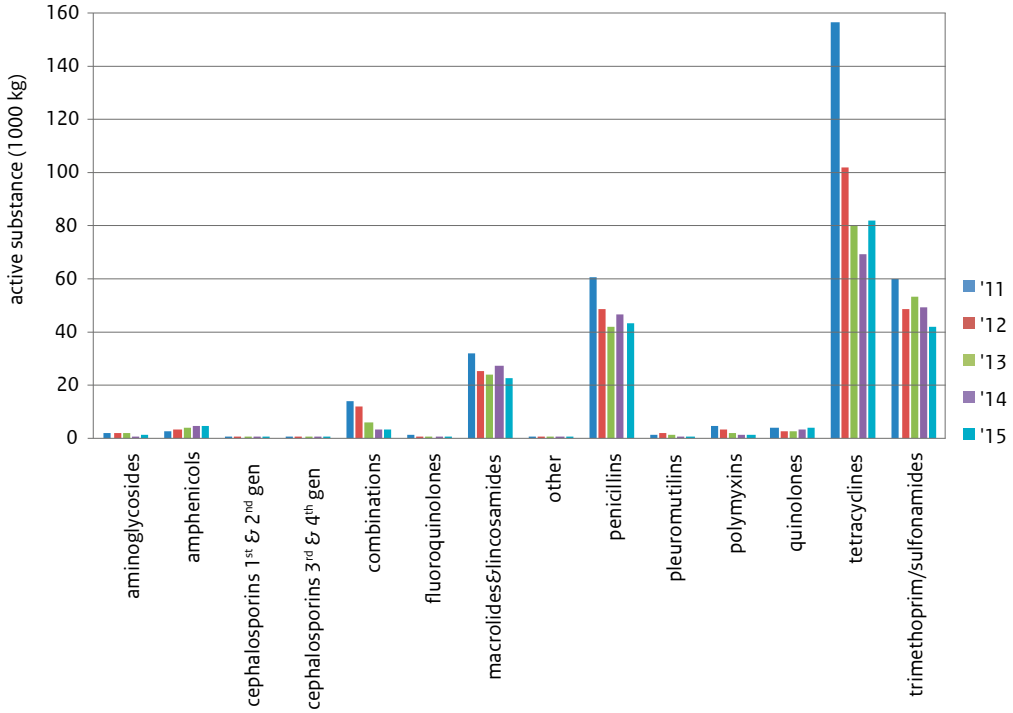


Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2015 in kg (thousands) (FIDIN, 2015).

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

Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmacotherapeutic class from 2011-2015 in kg (thousands).



(Fluoro)quinolones

The sales of fluoroquinolones decreased with 27 kg in 2015. An overall reduction of 74% was realized in comparison with 2011. The sales of quinolones increased again. When compared with 2011 an increase of 4.2% occurred. 33.2% of the sales are applied in the monitored sectors.

Cephalosporins

The sales of 1st and 2nd generation cephalosporins increased in 2014 due to underreporting in previous years; two presentations of veterinary medicinal product for companion animals were reported for the first time. The sales of these VMP's was stable with a slight decrease in 2015. The sales of 3rd and 4th generation cephalosporins decreased in 2015 with 3 kg, a reduction of 98.8% was achieved since 2011. Only 5.2% of the sold mass was used in the monitored sectors, 83.5% of these sales are applied outside the food producing animal sectors and companion animals.

Polymyxins

Colistin use increased in some sectors, but compared to 2011 a reduction of 68% was accomplished.

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

Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands 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. While the calculation method for treated body mass (numerator) is the same, totalized for all farms per sector, the denominator is represented by 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 2012-2015 (pigs, veal calves, cattle, broilers and turkeys). Table ABuse04 depicts the animal bodyweights applied in the calculation of the denominator. In Table ABuse05 the resulting $DDDA_{NAT}$ are shown. In three sectors (pigs, cattle and broilers) a reduction in consumption was realized. In veal calves and turkeys increased consumption is noted.
The trends in the number of defined daily dosages animal for the veal farming, sows/piglets farming, fattening pigs farming and broiler farming sectors as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2015 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 kg's 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 2015, CBS (Centraal Bureau voor de Statistiek, National Institute of Statistics) data for number of animals are used in the calculations for broilers and veal calves, 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.

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. From these detailed prescription data the mass of sold cephalosporins 3rd and 4th generation in the monitored

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

Sector	2012	2013	2014	2015
pigs	710,688	710,802	704,937	706,025
sow/piglets	328,408	332,661	368,935	358,841
fatttening pigs	382,280	378,141	336,003	347,184
veal calves	156,602	159,547	158,828	156,751
cattle	1,522,500	1,532,000	1,615,000	1,680,000
diary cows	924,600	958,200	966,000	1,030,200
other cattle	597,900	573,800	649,000	649,800
broilers	43,846	44,242	47,020	49,107
turkeys	4,961	5,046	4,763	5,178

Figure ABuse03 Animal-defined daily dosages for veal calves (blue), broiler (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 2011-2015 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).

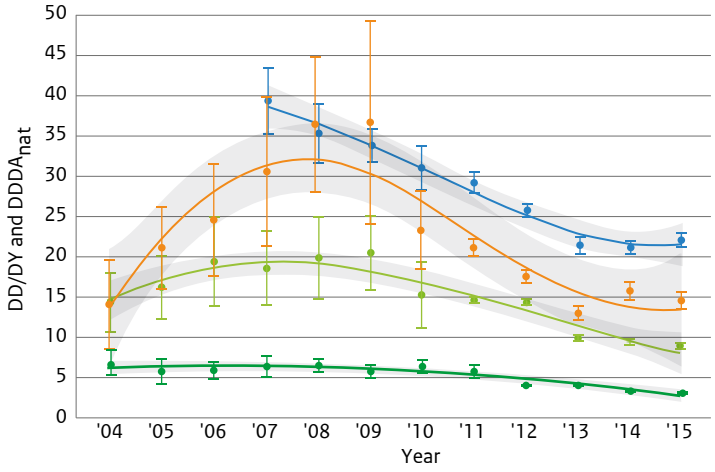


Table ABuse04 Trends in $DDDA_{nat}$ in the Netherlands in livestock.

	Animalsector																		
	Pigs				Veal calfs*				Cattle				Broilers				Turkeys		
Year	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2013	2014	2015
Number of farms with prescriptions	6425	6588	6072	5824	2175	2125	2061	1978	32254	31650	31223	30708	732	770	797	816	48	41	40
Pharmacotherapeutic group																			
Aminoglycosides	-	-	0.01	0.01	0.81	0.53	0.34	0.19	0.01	0.01	0.01	0.01	0.58	0.03	0.03	0.02	1.24	0.40	0.71
Amphenicols	0.06	0.09	0.17	0.18	1.23	1.23	1.52	1.63	0.05	0.07	0.08	0.08	-	-	-	-	0.02	-	-
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	-	-	0.02	0.02	0.01	0.01	-	-	-	-	-	-	-
Cefalosporins 3rd & 4th generation	-	-	-	-	0.00	0.00	0.00	-	0.03	0.00	0.00		-	-	-	-	-	-	-
Combinations	0.27	0.10	0.05	0.04	0.42	0.09	0.01	-	0.85	0.66	0.30	0.28	0.52	0.37	0.08	0.11	-	-	-
Fluoroquinolones	0.00	0.00	0.00	-	0.31	0.03	0.02	0.02	0.01	0.00	0.00		0.80	0.24	0.18	0.07	1.76	1.29	1.20
Macrolides/lincosamides	1.39	1.02	1.09	1.04	3.91	3.84	3.72	3.88	0.09	0.12	0.14	0.13	1.06	0.31	0.35	0.48	3.55	2.12	1.98
Penicillins	2.91	2.17	2.05	1.93	2.80	2.11	2.15	2.33	1.22	1.45	1.27	1.26	7.46	6.34	9.96	8.44	9.34	14.89	16.61
Pleuromutilins	0.35	0.12	0.09	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12
Polymyxins	0.58	0.44	0.34	0.38	0.73	0.36	0.15	0.19	0.05	0.02	0.01	0.01	0.84	0.08	0.05	0.06	0.18	0.08	0.63
Quinolones	0.03	0.03	0.05	0.03	0.27	0.30	0.49	0.58	0.00	0.00	0.01	0.01	1.97	1.65	2.22	2.86	0.23	0.02	0.10
Tetracyclines	6.79	4.58	4.34	4.15	12.61	10.87	10.66	11.01	0.48	0.48	0.42	0.41	2.40	2.52	1.77	1.49	11.19	9.58	12.57
Trimethoprim/sulfonamides	1.92	1.40	1.33	1.20	2.76	2.14	2.08	2.22	0.18	0.20	0.19	0.20	1.97	1.46	1.45	1.07	1.80	2.37	2.01
Total	14.32	9.97	9.52	9.05	25.85	21.50	21.15	22.05	3.00	3.04	2.44	2.38	17.61	13.01	15.76	14.59	29.31	30.74	35.94

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

animal sectors could be attributed to the treatment of 686 animals in the cattle sector (almost exclusively to dairy cows), 94 courses of injections and 592 intra-mammary treatment courses. For more details, annual reports of the SDa can be consulted (http://autoriteitdiergeneesmiddelen.nl/en/publications).

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The decrease in sales of antibiotics licenced for therapy in the Netherlands has levelled off further, as already was noticed starting from 2013. The calculation of the consumption is based on national conversion factors (DDDA's) of authorized drugs. A comparison with the internationally established ESVAC DDDvet will be produced later in 2016 and included in SDa reports.
The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) in the monitored sectors is limited to indications without alternative treatments. Consumption of antibiotics in unmonitored sectors is under investigation. Consumption monitoring is initiated for rabbits in 2016. In other sectors surveys are held (companion animals and horses) or are being proposed or in preparation.

Table ABuse05 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	

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		10.5
		female		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

3 Resistance data

Susceptibility test results as determined in 2015 for the food-borne pathogens *Salmonella enterica*, *Campylobacter* spp. and Shiga-toxin producing *E. coli* (STEC), and the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis* are presented in this chapter. 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 values are in most cases lower than clinical breakpoints, and therefore, depending on the antibiotic, non-wild type susceptible isolates 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*

Resistance percentages of *Salmonella* are presented in this chapter. The tested *Salmonella* isolates were sampled from humans suffering from clinical 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 2015 *S.* Typhimurium (N = 233) together with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 176), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (N=284) in second place.
2. In pigs, *S.* Typhimurium and its monophasic variant dominated. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated.
3. In 2015, the number of *S.* Paratyphi B var. Java was substantially reduced and no longer predominant in poultry. Also *S.* Heidelberg, still predominant in 2014, was less frequently isolated in 2015. The prevalence of *S.* Enteritidis remained comparable to the prevalence in 2014 and was the most predominant serovar in poultry in 2015.
4. Highest resistance levels were observed in the monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Heidelberg, *S.* Paratyphi B var. Java and other *S.* Typhimurium and to a lesser extent in *S.* Infantis, *S.* Brandenburg and *S.* Stanley.
5. The dominant serovars of ciprofloxacin resistant isolates were *S.* Enteritidis (20%), *S.* Infantis (12%), *S.* Typhimurium (8%), *S.* Heidelberg (8%) and *S.* Paratyphi var. Java (7%), mainly from poultry and human sources.
6. In 2015, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 36/1761 (2.0%), among eleven different serovars, predominantly isolated from poultry sources.
7. In 2015 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 (swine, cattle and poultry) is presented in Table S01.

Human isolates (N = 1141 in 2015) were a selection of all isolates sent to the RIVM by regional public health and other clinical laboratories. All strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N = 51) and cattle (N = 54) 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 chickens (broilers, including poultry products, N = 60; layers, reproduction animals and eggs, N = 37) 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 = 354 from animal feed and food products; other animals from animal husbandry (e.g. horses, sheep, goats, ducks) and pets, samples from the environment etc. Traditionally, *S.* Enteritidis or *S.* Typhimurium were most frequently isolated from human clinical infections. In 2015, *S.* Typhimurium (19%) 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, with *S.* Enteritidis (24%) in second place.

The relative contribution of different animal species to infections in humans varied by serovar. *S.* Typhimurium and its monophasic variant were predominantly associated with pigs and to a lesser extent cattle, but was also found in poultry. *S.* Enteritidis was mainly present in poultry and more specifically in layers and contaminated eggs (Table S01).
In pigs, *S.* Typhimurium and its monophasic variant remained most predominant, whereas presence of *S.* Derby was substantially decreased as compared to 2014. In cattle was, besides the *S.* Typhimurium variants, *S.* Dublin most commonly isolated. In poultry the presence of *S.* Paratyphi B var. Java (*S.* Java) was substantially reduced and it was no longer the most predominant serovar in poultry comparable to *S.* Infantis. Also *S.* Heidelberg, still predominant in 2014, was less frequently isolated in 2015. In 2015, the prevalence of *S.* Enteritidis remained comparable to the prevalence in 2014, hence *S.* Enteritidis became the most predominant serovar in poultry.

Depending on the serotype, reported travel contributed up to 39% of the cases of human salmonellosis over the years 2012-2015. Relative high contributions (≥25%) were noted for the serovars Paratyphi B var Java, Mbandaka, Typhi, Livingstone, Kentucky, Virchov, Corvallis, Bredeney, Poona and Haifa.
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 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*. 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 2014 and 2015 from humans, pigs, poulty, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2012-2015	2014	2015	2014	2015	2014	2015
N Total			1182	1203	83	54	47	54
N tested	Tested		1091	1141	74	51	45	54
Enteritidis	623	16%	265	284	1			
Typhimurium	611	6%	191	233	28	28	25	30
SI 1,4,5,12:i:-	507	3%	234	176	31	22	8	7
Infantis	172	6%	30	52				
Paratyphi B. var. Java	118	25%	10	19	2			
Dublin	94	2%	29	21			12	15
Derby	73	5%	15	18	9	2		
Senftenberg	70	20%	4	3	1			
Heidelberg	69	4%	46	4				
Agona	66	21%	9	12				
Brandenburg	52	2%	21	8	4		1	1
Typhi	46	39%	25	20				
Mbandaka	38	32%	10	2				
Livingstone	36	30%	4	4	1	1		
Saintpaul	33	20%	18	13				
Stanley	33	18%	10	21				
Napoli	32	6%	13	16				
Chester	31	8%	17	14				
Newport	31	14%	14	14			1	
Kentucky	29	28%	9	11				
Thompson	24	2%	10	8				
Oranienburg	23	18%	6	18				
Tennessee	22	14%	3					
Hadar	21	13%	5	15				
Braenderup	19	21%	4	9				
Montevideo	19	16%	3	5				
Goldcoast	19	3%	2	11	3	1		1
Panama	19	19%	6	7				
Virchow	18	33%	9	6				
Corvallis	17	30%	10	7				
Schwarzengrund	17	22%	1	5				
London	16	3%	9	3	1			
Rissen	15	11%	1	10				
Anatum	14	13%	1	4				
Bovismorbificans	14	21%	8	5				
Indiana	13	9%	1	4				
Muenchen	13	13%	2	8				
Putten	13	n.a.						
Bredeney	11	36%	1	5				
Javiana	10	8%	6	6				
Manhattan	10	6%	4	3	1			
Mikawasima	10	0%	2	7				
Ohio	10	19%	4					

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2014 and 2015 from humans, pigs, poulty, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2014	2015	2014	2015	2014	2015	2014	2015
N Total	315	209	135	81	52	39	948	933
N tested	222	160	91	60	50	37	244	354
Enteritidis	45	41	10	8	25	20	27	212
Typhimurium	19	9	8	2	4	5	82	39
SI 1,4,5,12:i:-	10	10	7	9	2		34	2
Infantis	48	32	28	20	3	3	49	58
Paratyphi B. var. Java	81	27	26	14	2		19	38
Dublin	1	1		1	1		11	5
Derby	3	9	2	1		1	10	2
Senftenberg	4	1	1	1	2		89	69
Heidelberg	40	18	24	8			5	14
Agona	7	5	5	2	1	2	27	25
Brandenburg	1	2		2			7	26
Typhi								
Mbandaka	3	2	1		2	2	25	36
Livingstone	2	2	2	1			135	44
Saintpaul	1	1	1				2	3
Stanley							2	3
Napoli		1						2
Chester		1		1				1
Newport		1					1	1
Kentucky	1	1	1				3	4
Thompson	1	3	1			2	4	1
Oranienburg							4	8
Tennessee	2		1		1		20	34
Hadar	5		2				4	4
Braenderup	2	1			2	1	3	1
Montevideo	3	1	2				9	1
Goldcoast	2		2				1	2
Panama							16	5
Virchow		2					4	7
Corvallis	2	1		1				1
Schwarzengrund	1	2					1	5
London	1	1		1				12
Rissen	1				1		4	3
Anatum	2	1		1	2		162	16
Bovismorbificans		1		1			4	1
Indiana	8		4		1		3	1
Muenchen		1						2
Putten	3	5	2	1			5	2
Bredeney	6						2	3
Javiana								1
Manhattan							2	1
Mikawasima							2	1
Ohio		6		1			4	4

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2014 and 2015 from humans, pigs, poulty, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2012-2015	2014	2015	2014	2015	2014	2015
N Total			1182	1203	83	54	47	54
N tested	Tested		1091	1141	74	51	45	54
Bareilly	9	18%	2	5				
Blockley	9	8%		10				
Give	9	13%	1	4				
Poona	9	29%	5	2				
Goettingen	8	0%	1	5				
Kottbus	8	20%	4	2				
Jerusalem	5	n.a.						
Haifa	4	33%	5	1				
Gallinarum	3	n.a.						
Other serovars	242	15%	92	83	1			

MIC-distributions and resistance percentages of 1761 *Salmonella's* from different sources tested for susceptibility in 2015 are presented in Table S02. Highest levels of resistance were observed for sulfamethoxazole, tetracycline, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid and trimethoprim. The levels of resistance to ciprofloxacin and cefotaxime/ceftazidime have slightly decreased compared to 2014, but are still higher than in 2012. None of the isolates were resistant to the carbapenem antibiotic meropenem, indicating that carbapenemase producers were not present in the tested isolates (see also appendix 1 screening for carbapenemases). A few isolates (1.6%) were found resistant to tigecycline. Using the tentatively set epidemiological cut off value of 16 mg/L for azithromycin, 0.2% of the isolates (all human origin) were found resistant.

Resistance profiles varied considerably among serovars as shown in Table S03. Resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2015 are presented in this table. High resistance levels (66.1-86.9%) were observed in the monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Heidelberg and *S.* Paratyphi B var. Java and to a lesser extent (30.4-42.6%) in *S.* Typhimurium, *S.* Infantis, *S.* Brandenburg and *S.* Stanley.
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.* Heidelberg, *S.* Infantis, *S.* Stanley, *S.* Paratyphi B var. Java, *S.* Agona and to a lesser extent *S.* Enteritidis and *S.* Brandenburg. Except for *S.* Stanley, resistance to the fluoroquinolones was most prominent in isolates from poultry, hence reflecting the usage of quinolones in poultry production. As in 2013 and 2014, isolates suspected to be ESBL producing (cefotaxime resistant) dominated in *S.* Heidelberg from imported poultry products from Brazil.

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2014 and 2015 from humans, pigs, poulty, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broiler		Layer		Other	
	2014	2015	2014	2015	2014	2015	2014	2015
N Total	315	209	135	81	52	39	948	933
N tested	222	160	91	60	50	37	244	354
Bareilly								3
Blockley								
Give							2	5
Poona							2	4
Goettingen		2				1		
Kottbus	1	1	1	1				2
Jerusalem	2	2	1		1	1		3
Haifa								
Gallinarum	2				2		1	
Other serovars	5	15	3	4		1	161	162

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* spp. with low-level ciprofloxacin resistance (MIC >0/06 mg/L) (www.eucast.org). Using the EUCAST recommended epidemiological, cut off value of 0.06 mg/L as breakpoint, 13.9% of *Salmonella* isolates (N =245/1761), demonstrated a resistant phenotype for ciprofloxacin (Table S02). The dominant serovars of ciprofloxacin resistant isolates were S. Enteritidis (20%), S. Infantis (12%), S. Typhimurium (8%), S. Heidelberg (8%) and S. Java (7%), mainly from poultry and human sources.

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 of the possibilities for effective treatment of human infections (WHO, factsheet 139, 2005). In 2015, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 36/1761 (2.0%), among eleven different serovars. Fourteen isolates were derived from humans (eight S. Typhimurium, three monophasic S. Typhimurium, one S. Corvallis, one S. Enteritidis and one S. Oranienburg), almost all other isolates (n=22) were derived from poultry sources (fourteen S. Heidelberg, two S. Paratyphi B var. Java, two S. Infantis, one S. Dublin, one S. Molade, one S. Schwarzengrund and one monophasic S. *enterica subspecies enterica* 1,4,[5],12:i:-). Again, like in 2013 and 2014, S. Heidelberg derived from poultry products imported from Brazil were most predominant. Cefotaxime resistant S. Heidelberg comprised 58% of total S. Heidelberg isolated and cefotaxime resistant S. Paratyphi B var. Java comprised 4% of total S. Paratyphi B var. Java isolated.

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* (N=1761) tested for antibiotic susceptibility during 2015

Salmonella	MIC (%) distribution mg/L																		R%	95% CI
N = 1688	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							38.0	35.0	2.6	0.3				24.1					24.1	22.1-26.1
Cefotaxime					95.9	2.0	0.2		0.1	1.8									2.0	1.4-2.7
Ceftazidime						95.8	2.4	0.3	0.3	0.2	1.0								1.5	1-2
Gentamicin						72.3	23.9	1.6	0.3	0.1	0.6	0.3	0.7						2.1	1.5-2.8
Tetracycline								67.9	4.8	0.6		0.6	2.1	24.1					26.8	24.8-28.9
Sulfamethoxazole										40.1	23.5	7.6	1.1	0.2	0.1			27.5	27.5	25.4-29.6
Trimethoprim					68.5	20.0	0.7	0.1					10.6						10.6	9.2-12.1
Ciprofloxacin	35.2	49.4	1.5	1.3	5.7	4.7	1.0	0.4		0.5	0.3								13.9	12.3-15.6
Nalidixic acid									78.4	7.6	2.2	1.2	0.2	0.7	9.7				11.8	10.3-13.3
Chloramphenicol										88.1	6.8	0.5	0.3	0.6	3.6				5.1	4-6.1
Azitromycin*								0.6	65.4	31.2	2.6	0.2		0.1					0.2	0.1-0.5
Colistin**							42.7	42.4	11.3	3.5	0.2								-	-
Meropenem		89.8	10.1	0.1															0.0	0-0
Tigecyclin					47.8	44.2	6.4	1.6	0.1										1.6	1.1-2.3

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, 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 2015 (N tested).

	Enteritidis (322)	Typhimurium (319)	1,4,[5],12:i:- (229)	Infantis (93)	Paratyphi B var Java (56)	Derby (44)	Dublin (40)	Senftenberg (37)	Agona (33)	Heidelberg (24)	Brandenburg (23)	Stanley (22)
Ampicillin	6.2	42.6	80.3	5.4	16.1	9.1	2.5	2.7	15.2	62.5	17.4	22.7
Cefotaxime	0.3	2.5	1.7	2.2	3.6	0.0	2.5	0.0	0.0	58.3	0.0	0.0
Ceftazidime	0.3	0.0	1.7	2.2	3.6	0.0	0.0	0.0	0.0	58.3	0.0	0.0
Gentamicin	0.3	2.5	2.2	2.2	3.6	0.0	0.0	0.0	3.0	8.3	13.0	0.0
Tetracycline	2.2	38.6	86.9	37.6	3.6	18.2	2.5	0.0	15.2	75.0	8.7	18.2
Sulfamethoxazole	2.2	39.5	82.1	40.9	41.1	15.9	7.5	0.0	15.2	75.0	21.7	9.1
Trimethoprim	0.6	16.3	8.7	21.5	66.1	11.4	2.5	0.0	12.1	0.0	4.3	9.1
Ciprofloxacin	15.0	6.0	5.2	32.3	28.6	4.5	2.5	0.0	27.3	79.2	8.7	31.8
Nalidixic acid	15.0	4.4	4.8	32.3	28.6	0.0	0.0	0.0	15.2	79.2	8.7	27.3
Chloramphenicol	0.3	13.5	7.4	3.2	1.8	6.8	10.0	0.0	3.0	0.0	0.0	4.5
Azithromycin	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.3	1.6	1.3	12.9	0.0	2.3	0.0	0.0	0.0	20.8	0.0	0.0

S. Typhimurium

As shown in Table S01, *S.* Typhimurium represents 19.4% (233/1203) of all human *Salmonella* isolates as characterized by the RIVM in 2015. This is slightly more than in 2014 (16.2% (191/1182)). In animals *S.* Typhimurium is a common serotype. If the monophasic *S*I 1,4,[5],12:i:- 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 sulfonamides (Table S04). Resistance to chloramphenicol and trimethoprim remained common. About 12% of the *S.* Typhimurium isolates exhibited the resistance profile Ampicillin-Chloramphenicol-Sulfamethoxazole-Tetracycline (ACST). Although, streptomycin is not tested anymore these figures indicate that the proportion of the penta-resistant phenotype (ACSuST) is relatively low compared to previous years. This is in line with the internationally reported decrease in occurrence of *S.* Typhimurium DT104, which has this penta-resistance phenotype. Resistance to the clinical important drug cefotaxime was only seen in isolates from humans at a low level (3.4%). Resistance to fluoroquinolones was at a low level in isolates from humans (7.7%) and pigs (3.8%) and absent (0.0%) in isolates from cattle. Low-level resistance to tigecycline was found in *S.* Typhimurium derived from humans (1.7%) and pigs (3.8%).

Regarding the trends, resistance levels in *S.* Typhimurium isolates from human samples have increased over the years until 2010 after which resistance showed a tendency to decrease until 2013. In 2014 resistance levels for almost all antimicrobials tested were comparable to 2013 or tended to increase again. However, in 2015 all resistance levels, except for a slight increase to cefotaxime, showed again a decrease (Figure S01). Resistance levels in *S.* Typhimurium isolates from animal samples have varied considerably over the years due to the relatively small number of animal isolates per year. As from 2013-2014, levels show a tendency to decrease or at least to stabilize. However, given the relatively small number of the isolates per year it warrants caution with regard to the interpretation of these data.

S. Enteritidis

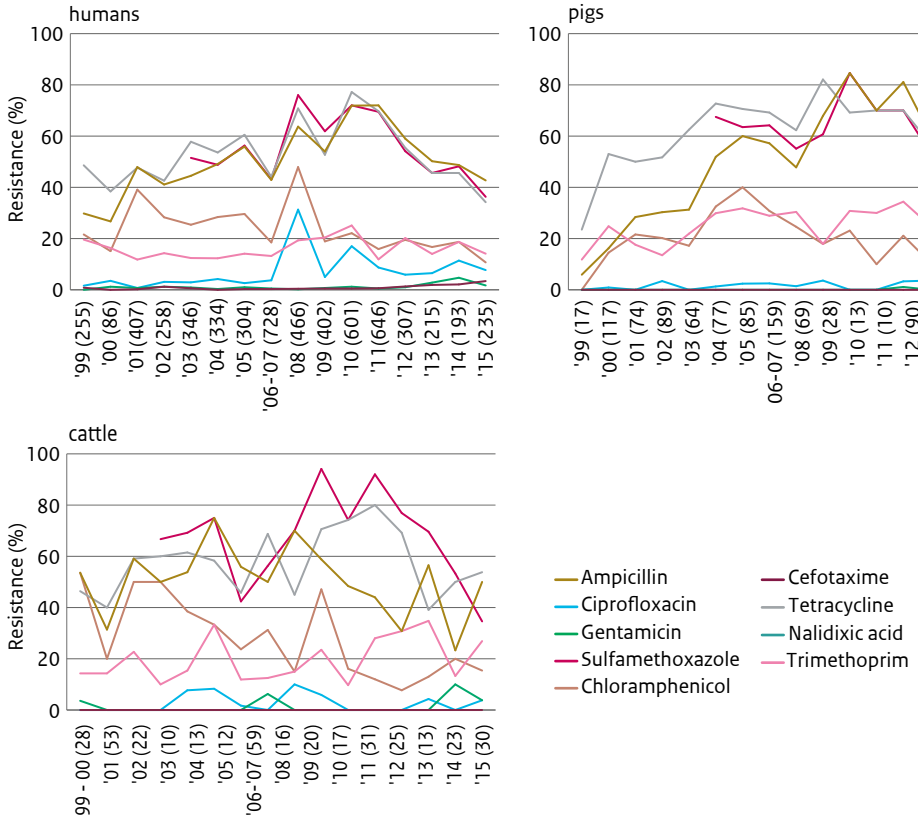
In the Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs and to a lesser extent poultry meat products. Phage typing, that was used to differentiate between types isolated from Dutch broilers and humans has been replaced by MLVA-typing. 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 (mainly laying hens) and were similar to the most predominant MLVA types in 2013 and 2014. Interesting is the moderate resistance of strains from human infections compared to the lack of resistance in Dutch layers, indicating other sources of infection. Other sources are considered to be consumption of contaminated imported eggs and poultry food products and travel abroad (Table S01). Although *S.* Enteritidis prevalence varies over the years, it is traditionally much higher in layers than in broilers.
Compared to other *Salmonella* serovars, resistance in *S.* Enteritidis is very low. As shown in Table S05, resistance to the quinolones was absent in isolates from laying hens and moderately high (15.9%) in isolates from humans.

Table S04 Resistance percentages of *S.* Typhimurium (N tested) isolated from different sources in 2015.

	S. Typhimurium (319)			
	Humans (234)	Cattle (30)	Pigs (26)	Other sources* (29)
Ampicillin	42.7	23.3	50.0	55.2
Cefotaxime	3.4	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0
Gentamicin	1.7	10.0	3.8	0.0
Tetracycline	34.2	50.0	53.8	48.3
Sulfamethoxazole	36.3	53.3	34.6	55.2
Trimethoprim	14.1	13.3	26.9	27.6
Ciprofloxacin	7.7	0.0	3.8	0.0
Nalidixic acid	5.6	0.0	3.8	0.0
Chloramphenicol	10.7	20.0	15.4	27.6
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.7	0.0	3.8	0.0

** Other sources includes laying hens, poultry products and other food products.*

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999-2015.



The trends in resistance of *S.* Enteritidis over the years are summarized in Figure S02. Apart from this, similar to the situation for *S.* Typhimurium, resistance levels vary considerably over the years due to the relatively small number of animal isolates per year. Hence, interpretation should be done with great caution. In humans, the onset of a slight decrease in resistance to quinolones in 2014 continued in 2015.

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

Prevalence of *S.* Java, the most predominant serovar isolated in broiler production until 2014, was substantially decreased in 2015 (Table S01). From poultry, 56 *S.* Java strains were included for susceptibility testing (Figure S03). In 2015, resistance levels of *S.* Java isolated from poultry sources demonstrated a remarkable decrease, irrespective of antimicrobial (Figure S03). Resistance levels in 2014 reduced in 2015 from 100% to 66% for trimethoprim, from 83% to 41% for sulfamethoxazole, from 50% to 16% for ampicillin, from 11.9% to 3.6% for cefotaxime/ceftazidime (ESBL-producers), 9.5% to 3.6% for gentamicin. Resistance levels were, after an unexplained increase in 2014, substantially lower in 2015 than in 2013. Hence, the data suggest that a trend to decrease was set after 2013 and the 2014

Table S05 Resistance percentages of *S.* Enteritidis (N tested) isolated from different sources in 2015.

	S. Enteritidis (321)		
	Humans (251)	Laying hens (19)	Other sources* (51)
Ampicillin	5.2	5.3	11.8
Cefotaxime	0.4	0.0	0.0
Ceftazidime	0.4	0.0	0.0
Gentamicin	0.4	0.0	0.0
Tetracycline	2.8	0.0	0.0
Sulfamethoxazole	2.8	0.0	0.0
Trimethoprim	0.8	0.0	0.0
Ciprofloxacin	15.9	0.0	15.7
Nalidixic acid	15.9	0.0	15.7
Chloramphenicol	0.4	0.0	0.0
Azithromycin	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	0.4	0.0	0.0

** other sources includes broilers, poultry meat and other food products*

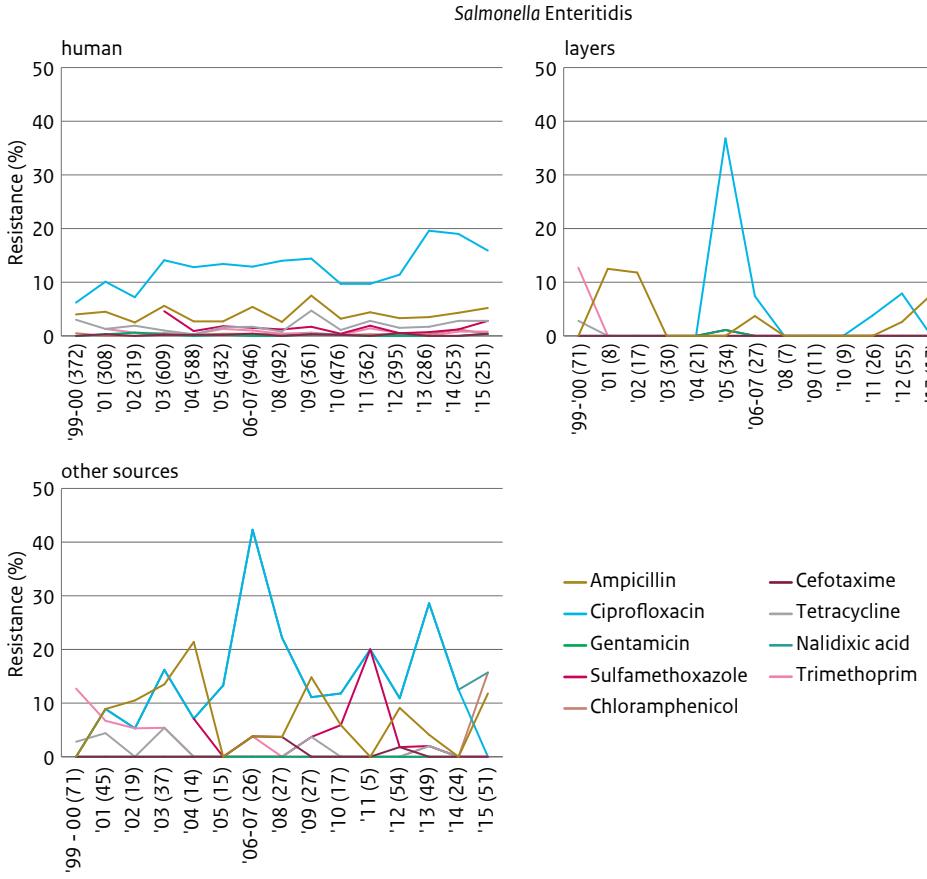
results may partially be the result of a sampling bias. Resistance levels in 2015 further reduced from 43% to 29% for both quinolones ciprofloxacin and nalidixic acid, from 4.8% to 3.6% for tetracycline and from 2.4% to 1.8% for chloramphenicol.

A number of *S.* Java strains were isolated from human infections in 2015 (n=18). All strains tested were trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related.

Salmonella in raw meats (poultry, pork, other sources), vegetables and spices

Resistance data in raw meat products are presented (Table S06, Figure S03). In 2015 *S.* Infantis (64%) was the dominant serovar found in raw meat products, followed by *S.* Java (23%), mainly isolated from poultry sources.
In general, resistance levels in pork meat are lower than in meat from poultry and other raw meat sources. Noteworthy are the high level of resistance to the quinolones (ciprofloxacin and nalidixic acid) and the relatively high level of resistance to tigecycline and cephalosporins (cefotaxime and ceftazidime) in poultry and other raw meat products, but absent in pork meat.
Moderately high resistance levels to the quinolones (ciprofloxacin and nalidixic acid) and tigecycline and low resistance to cephalosporins (cefotaxime and ceftazidime) were also shown in isolates from herbs and vegetables. Seventeen different *Salmonella* serotypes were found among 19 samples from herbs and vegetables. Among those were four of the twelve most prevalent serotypes described earlier in Table S03: *S.* Infantis (n=1), *S.* Paratyphi B var. Java (n=1), S.Dublin (n=1), *S.* Senftenberg (n=1).

Figure S02 Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other sources from 1999-2015.



The overall resistance levels of *Salmonella* from poultry products over the years are shown in Figure S04. After substantial reductions observed in 2013, the level tend to increase again for sulfamethoxazole, ciprofloxacin, tetracycline, ampicillin and cefotaxime. This increase could reflect the relative high proportion of strains from imported poultry products (52%) included in the survey of 2015.

Figure S03 Trends in resistance (%) of S. Paratyphi B var. Java isolated from poultry sources from 1999-2015 and humans (Separate data on the rigt indicate all human S. java isolates from 1999-2015).

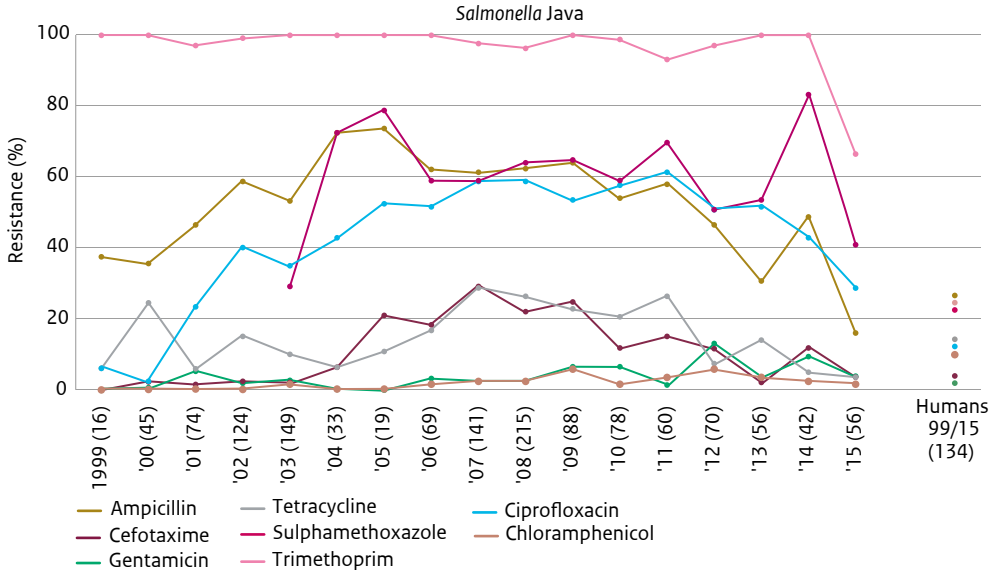
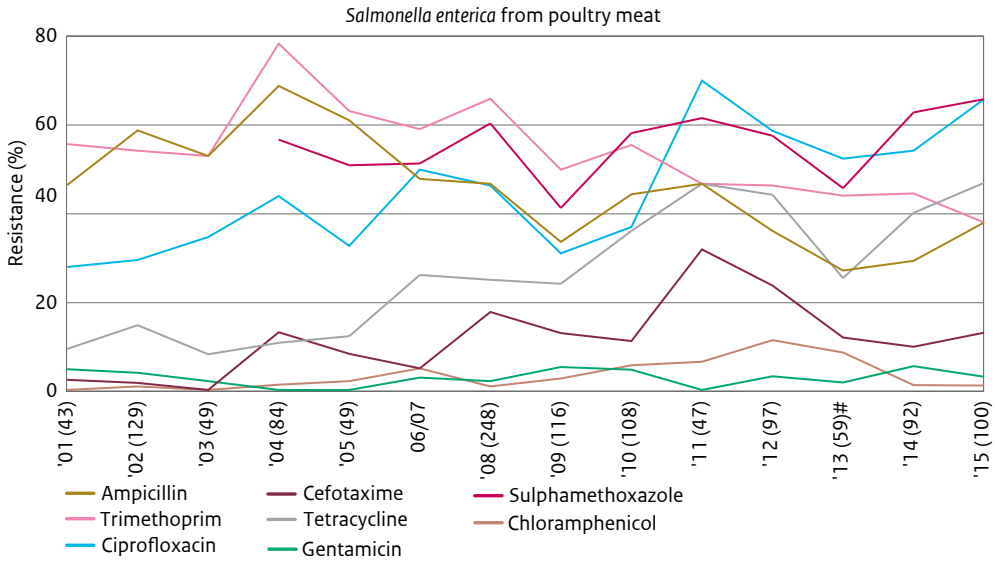


Table S06 Resistance (%) of *Salmonella* enterica isolated from raw meat from poultry, pork and other meat sources, herbs and spices and animal feed in the Netherlands in 2015.

	poultry meat all serovars N = 100	pork meat all serovars N = 53	other raw meat all serovars N = 26	herbs and vegetables all serovars N = 19
Ampicillin	38.0	45.3	42.3	15.8
Cefotaxime	13.0	0.0	19.2	5.3
Ceftazidime	13.0	0.0	19.2	5.3
Gentamicin	3.0	0.0	26.9	10.5
Tetracycline	47.0	41.5	34.6	21.1
Sulfamethoxazole	66.0	43.4	53.8	26.3
Trimethoprim	38.0	18.9	11.5	21.1
Ciprofloxacin	64.2	0.0	57.7	15.8
Nalidixic acid	63.0	0.0	50.0	10.5
Chloramphenicol	1.0	13.2	7.7	15.8
Azithromycin	1.0	0.0	0.0	10.5
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	16.0	0.0	15.4	10.5

Figure S04 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meat in the Netherlands from 2001-2015.



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

3.1.2 *Campylobacter*

Antimicrobial resistance in *Campylobacter jejuni* and *C. coli* are described in this chapter. *C. 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 broiler chickens, laying hens were included in the surveillance.

The MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated at CVI from caecal samples of broilers in 2015 are summarized in Table C01. More detailed resistance profiles of *C. jejuni* and *C. coli* from different sources (caecal samples from broilers and layer hens and poultry meat) are shown in Table C02. Trends over the last decade in resistance of *C. jejuni* and *C. coli* from broilers and broiler meat products are depicted in Figures C01 and C02.
National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and from 2005 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 at poultry and poultry meat.
2. Resistance rates in *C. jejuni* from broilers and poultry meat did not substantially change in 2015 as compared to 2014.
3. Resistance rates for quinolones and tetracycline in *C. coli* from broilers considerably increased in 2015 as compared to 2014. Hence resistance rates became comparably high in broilers and poultry meat.
4. In laying hens, resistance levels of *C. jejuni* for the quinolones and tetracycline were substantially lower compared to broilers. However, these differences were not observed with *C. coli*.
5. Ciprofloxacin resistance in *Campylobacter* isolates is high and still rising in human patients which is a concern for public health. However, resistance to erythromycin, representing the first choice macrolide clarithromycin for treating campylobacteriosis, remained low.
6. For *C. jejuni* from human patients, resistance levels were higher for all three antimicrobials tested in travel related infections compared to domestically acquired campylobacteriosis.

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. Six out of twelve antimicrobials (ampicillin, chloramphenicol, clarithromycin, tulathromycin, sulfamethoxazole and neomycin) have no longer been included in the monitoring programme of *Campylobacter* spp, since the implementation. 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. Resistance in

Table C01 MIC distribution (in %) for *Campylobacter jejuni* (N = 135) and *C. coli* (N = 21) isolated from caecal samples of broilers in 2015.

C. jejuni	MIC (%) distribution mg/L												R%	95% CI
(N = 135)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	27.4	1.5	1.5			0.7	31.1	15.6	22.2				69.6	61.7 - 77.5
Nalidixic acid					11.1	20.0	1.5	0.7			66.7		66.7	58.5 - 74.7
Erythromycin				86.7	13.3								0.0	0 - 2.7
Gentamicin	65.2	34.1	0.7										0.0	0 - 2.7
Streptomycin		5.2	78.5	16.3									0.0	0 - 2.7
Tetracycline			40.0	11.9	0.7	0.7			3.7	4.4	38.5		48.1	39.5 - 56.7

C. coli	MIC (%) distribution mg/L												R%	95% CI
(N = 21)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	28.6					19.0	38.1	9.5	4.8				71.4	51.7 - 91.1
Nalidixic acid						19.0	9.5				71.4		71.4	51.7 - 91.1
Erythromycin				71.4	19.0	4.8			4.8				4.8	0 - 14
Gentamicin	4.8	90.5	4.8										0.0	0 - 16.1
Streptomycin			4.8	90.5					4.8				4.8	0 - 14
Tetracycline			23.8								76.2		76.2	57.6 - 94.7

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 *Campylobacter jejuni* and *C. coli* isolated from poultry meat and caecal samples from broilers and layers in 2015

	C. jenuni			*C. coli*		
	Broilers	Layers	Poultry meat	Broilers	Layers	Poultry meat
N	135	121	188	21	75	50
Ciprofloxacin	69.6	36.4	66.0	71.4	69.3	78.0
Nalidixic acid	66.7	37.2	67.6	71.4	69.3	84.0
Erythromycin	0.0	0.8	4.3	4.8	6.7	20.0
Gentamicin	0.0	0.8	2.1	0.0	1.3	4.0
Streptomycin	0.0	0.8	1.6	4.8	1.3	18.0
Tetracycline	48.1	18.2	38.8	76.2	58.7	76.0

C. jejuni from broilers and poultry meat seems to have stabilized for tetracycline, erythromycin, streptomycin and gentamicin. Resistance to ciprofloxacin showed more fluctuation over the years and an increase since 2014. Over the years more fluctuation was observed in *C. coli* than in C. *jejuni*, especially in isolates from broilers, probably due to the relative low number of isolates in the survey. However, resistance in *C. coli* from broilers seemed to stabilize for erythromycin, streptomycin and gentamicin, but since 2014 showed again a substantial increase for ciprofloxacin and tetracycline. In 2015 the highest resistance levels of *C. jejuni* in poultry were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table CO1). Resistance of *C. jejuni* for erythromycin, streptomycin and gentamicin was low in poultry meat and was not detected in the caecal samples from broilers. In laying hens, resistance levels of *C. jejuni* for the quinolones and tetracycline were substantially lower compared to broilers (Table Co2). Resistance of *C. jejuni* for erythromycin, streptomycin and gentamicin, was not detected in broilers and in only one isolate in laying hens. The highest resistance levels of *C. coli* in poultry were, as for C. *jejuni*, detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table CO1). High resistance levels were shown in broilers as well as in layer hens. Overall, resistance levels were higher in *C. coli* than in *C. jejuni* isolates. Higher resistance levels for erythromycin were commonly observed in *C. coli*, particularly in isolates from poultry meat. Streptomycin resistance in *C. coli* from poultry meat showed a substantial increase from 4.8% to 18.0% in 2015. Like in C. *jejuni*, no resistance was detected for gentamicin in *C. coli* from broilers and the resistance in *C. coli* from layers and poultry meat slightly increased, but remained low.

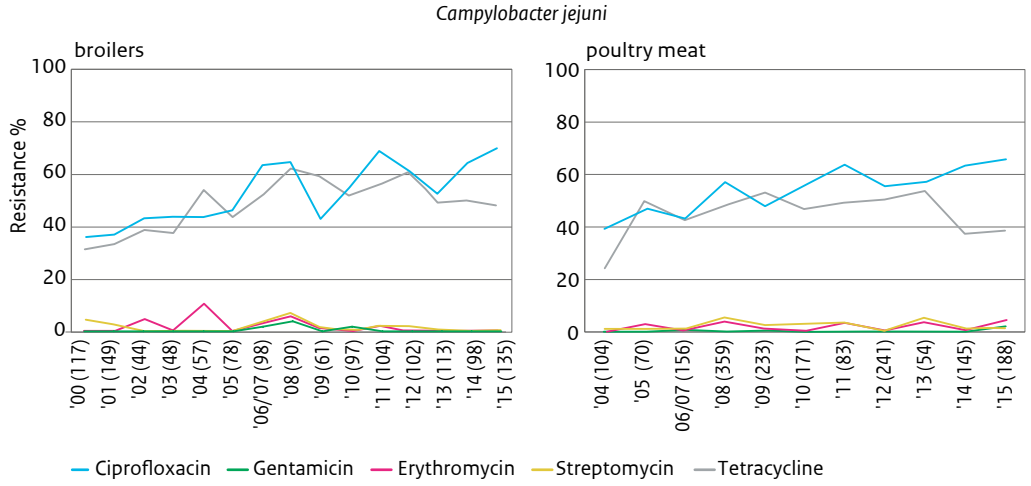
Quinolones

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. The resistance level of *C. jejuni* from poultry meat is comparably high and also showed an increase (63.4% in 2014 and 66.0% in 2015). Ciprofloxacin resistance rates in *C. jejuni* isolates from laying hens were relatively high and increased from 34.4% in 2014 to 36.4% in 2015. Increasing high levels of quinolone resistance were also observed in *C. coli* isolates from broilers (51.3% in 2014 and 71.4% in 2015), poultry meat (76.2% in 2014 and 78.0% in 2015) and laying hens (53.3% in 2014 and 69.3% in 2015). The resistance levels for fluoroquinolone were also high in human isolates of *Campylobacter* spp and slightly increased from 60.7% in 2014 to 61.4% in 2015. These figures indicate that ciprofloxacin resistance in *Campylobacter*s is still rising, both in poultry (products) and human patients.

Macrolides

Clarithromycin (a macrolide), is 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 1.6% of strains from animal origin in 2015 and 2.4% of human isolates from 2011-2015 (n=13113) were classified resistant. It should be noted that for human isolates more sensitive breakpoints for resistance have 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*).

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



As in former years, erythromycin resistance is low in *C. jejuni* with no resistance in broilers, 0.8% in layers and 4.3% in poultry meat (Table Co2). In contrast, erythromycin resistance is more frequently present in *C. coli* from broilers (4.8%), layers (6.7%) and poultry meat (20.0%). All *C. coli* isolates from poultry meat were obtained from fresh retail meat produced in EU countries (majority from NL). So, the difference in macrolide resistance of *C. coli* from animals and meat products remains unexplained.

Broiler chickens, laying hens and poultry meat

In *Campylobacter* spp from poultry, resistance profiles were determined for isolates recovered from animals as well as from meat samples. In 2015, *Campylobacter* spp. isolated from faecal samples of broilers and laying hens were included. 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. However, resistance rates of *C. coli* isolates from broilers and laying hens were more comparable. Resistance rates for tetracycline and the quinolones in *C. jejuni* and *C. coli* isolates from broilers and poultry meat were rather similar. Differences in resistance rates between meat and broilers were shown for erythromycin, gentamicin and streptomycin. In particular, resistance in *C. coli* for erythromycin and streptomycin was clearly higher in poultry meat than in broilers. In general, higher resistance rates were observed for most antimicrobials in *C. coli* from poultry meat compared to *C. jejuni* from the same sources. 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.

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

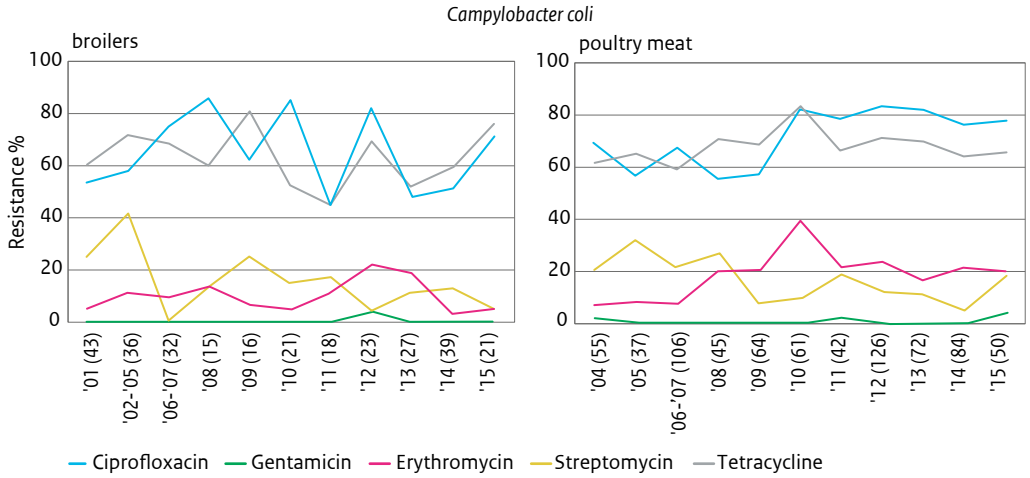


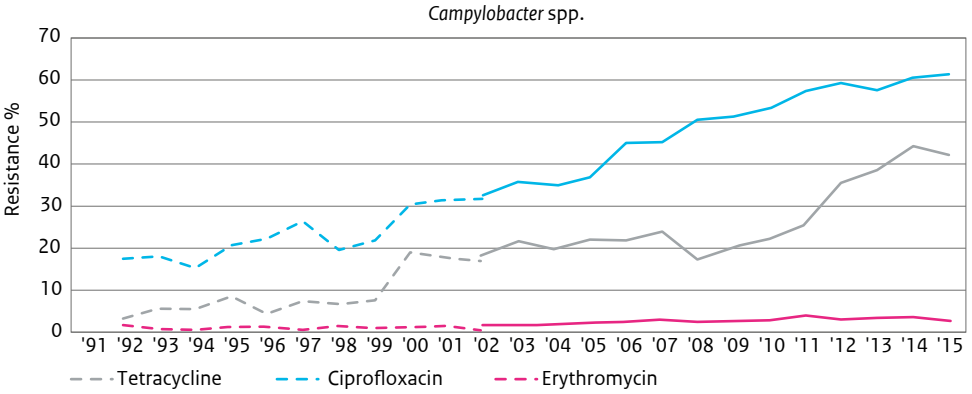
Table C03 Domestically acquired and travel related resistance in C. *jejuni* and *C. coli* isolated from humans from 2005-2015 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2005-2010							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	14701	46.2	1135	46.8	785	60.9	83	57.8
Tetracycline	9600	19.5	876	28.4	324	30.2	57	19.3
Erythromycin	12131	1.9	324	5.5	617	3.7	70	7.1

	2011-2015							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	14562	58.4	958	61.2	733	72.3	102	65.7
Tetracycline	8249	36	521	53.2	223	52.9	38	65.8
Erythromycin	12518	2.3	756	14.8	595	3.9	86	25.6

	Campylobacter spp. (R%)					
	2015	2014	2013	2012	2011	2005/10
Fluoroquinolone	61.4	60.7	57.6	59.4	57	47.2
Tetracycline	42.2	44.3	38.5	35.4	25.5	21.4
Erythromycin	2.9	3.4	3.2	3	3.7	2.5

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



Campylobacter in humans

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 continuous increasing trend of ciprofloxacin resistance in *Campylobacter* spp. isolated from human patients. Resistance to tetracycline slightly decreased in 2015. Resistance to erythromycin seemed to have stabilized at a low level.

In Table C03 resistance levels are specified according to the most probable infection route, i.e. whether the infection was acquired domestically or abroad. For C. *jejuni*, resistance levels were higher for all three antimicrobials in travel related infections compared to those domestically acquired. For *C. coli*, based on a relatively low number of isolates, this difference seemed less straightforward.

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

Highlights

1. Over the last decade, STEC O157 isolates from humans show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim, resulting in approximately 15% resistance for all four antibiotics in 2015.
2. Resistance profiles of STEC non-O157 isolates from raw beef were comparable to those of human isolates, except for the quinolones.
3. Resistance for the quinolones (ciprofloxacin and nalidixic acid) was 2.2% in meat isolates, but 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 2015, *E. coli* non-O157 isolates were also obtained from raw beef (including calf meat) and tested for susceptibility. Resistance percentages of human and meat isolates are presented in Table STEC02.

Human STEC O157 isolates

Traditionally, resistance levels in human STEC O157 have been very low. However, since last year resistance rates of human isolates showed a tendency to increase for ampicillin, tetracycline, sulfamethoxazole and trimethoprim (Figure STEC01). After finding low resistance levels for quinolones in two subsequent years (4.2% in 2013 and 2.4% in 2014), resistance for ciprofloxacin and nalidixic acid was not detected in 2015. As in former five 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 1999-2015.

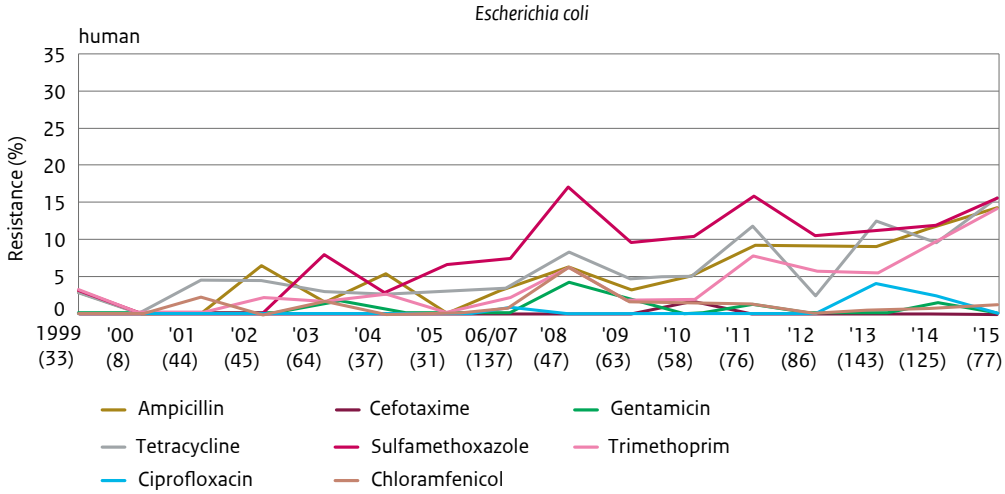


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=77) isolated from humans the Netherlands in 2015.

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								1.3	84.4					14.3					14.3	6.3 - 22.2
Cefotaxime					100														0.0	0 - 0
Ceftazidime						100													0.0	0 - 0
Gentamicin						83.1	16.9												0.0	0 - 0
Tetracycline								33.8	49.4	1.3				15.6					15.6	7.3 - 23.8
Sulfamethoxazole										84.4								15.6	15.6	7.3 - 23.8
Trimethoprim					85.7								14.3						14.3	6.3 - 22.2
Ciprofloxacin	87.0	13.0																	0.0	0 - 0
Nalidixic acid									97.4	2.6									0.0	0 - 0
Chloramphenicol										77.9	20.8				1.3				1.3	0 - 3.8
Azithromycin*								44.2	51.9	3.9									0.0	0 - 0
Colistin							98.7	1.3											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.

Table STEC02 Resistance (%) of *E. coli* STEC O157 isolated from humans and *E. coli* STEC non-O157 from raw beef and calf meat in the Netherlands in 2015.

Faecal samples	Human	Raw beef
	N = 77	N = 91
Ampicillin	14,3	7,7
Cefotaxime	0,0	0,0
Ceftazidime	0,0	0,0
Gentamicin	0,0	0,0
Tetracycline	15,6	14,3
Sulfamethoxazole	15,6	14,3
Trimethoprim	14,3	5,5
Ciprofloxacin	0,0	2,2
Nalidixic acid	0,0	2,2
Chloramphenicol	1,3	1,1
Azithromycin	0,0	0,0
Colistin	0,0	0,0
Meropenem	0,0	0,0
Tigecycline	0,0	0,0

STEC non-O157 isolates from raw beef

Resistance profiles of STEC non-O157 isolates from raw beef were comparable to those of human isolates; The highest resistance was shown for tetracycline and sulfamethoxazole followed by ampicillin and trimethoprim. In contrast to human STEC O157 isolates, beef STEC isolates showed low resistance (2.2%) for the quinolones ciprofloxacin and nalidixic acid.

3.2 Commensal indicator organisms

The susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals are described in this chapter. 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.

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].
This monitoring is conducted since 1998 in slaughter pigs and broilers. From 2005 onwards, resistance in isolates from both dairy cattle and 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 was stopped in 2012. From that date samples of veal calves were taken at slaughterhouses and resistance levels were reported separately for white veal calves and rosé veal calves. In addition to food animals, herbs were included in the surveillance programme of 2015. Laying hens, included in the surveillance programme of 2014, were not monitored in 2015.

It should be noted, that the sampling strategies used are inherently insensitive to detect resistance 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 set of selected isolates is intended to represent the *E. coli*, or *Enterococcus* species 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*

Highlights

1. In 2015, resistance levels of indicator *E. coli* from faecal samples showed a tendency to decrease in broilers and veal calves and stabilized in pigs.
2. In isolates from broiler meat, turkey meat, beef and pork, resistance stabilized.
3. Resistance to third-generation cephalosporins was low (< 1%) in most animal species. In broiler isolates the resistance level stabilised at 2.5%.
4. Although resistance to fluoroquinolones is decreasing, it was still commonly present in indicator *E. coli* from poultry sources and to a lesser extent from white veal calves.
5. Among indicator *E. coli* from animals and meat, resistance to ampicillin, tetracycline, sulphonamides and trimethoprim was still commonly detected in broilers, turkey, pigs and veal calves.
6. Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.
7. Monitoring of herbs, included in the monitoring programme of 2015, revealed the occurrence of *E. coli* frequently resistant to ampicillin, tetracycline, sulphonamides, trimethoprim, chloramphenicol and ciprofloxacin.

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, is described in this chapter.
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 with mandatory panels of antimicrobials. As a result for *E. coli*, in 2014 three antibiotics (streptomycin, kanamycin and florfenicol) were excluded from the national monitoring and three new antibiotics (meropenem, azithromycin and tigecycline) were included. Carbapenems (including meropenem), azithromycin and tigecycline are important in human medicine and used for treatment of infections with highly resistant Gram-negative bacteria.

Resistance levels

Resistance levels of a total of 1283 *E. coli* isolates obtained from broilers, pigs, dairy cattle, and veal calves 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 2015 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.
Resistance levels of 956 *E. coli* isolates collected from raw meat products and an additional 39 *E. coli* isolates collected from herbs are, as resistance percentages per product, presented in Table Eco03. Trends in the Netherlands from 2002 to 2015 in resistance of *E. coli* isolated from beef, pork and raw meat products of poultry and turkey are presented in Figure Eco03.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1283) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2015.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1283	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							1.1	21.5	45.5	4.0	0.1		0.1	27.7					27.9	25.3 - 30.4
Cefotaxime					99.1		0.1	0.1	0.2	0.6									0.9	0.3 - 1.4
Ceftazidime						99.1	0.2	0.2		0.5	0.1								0.9	0.3 - 1.4
Gentamicin						25.7	62.5	10.2	0.2	0.1	0.5	0.5	0.2						1.6	0.8 - 2.2
Tetracycline								42.4	25.3	0.5	0.5	0.2	4.9	26.2					31.8	29.2 - 34.4
Sulfamethoxazole										69.8	0.1		0.2		0.1			29.9	29.9	27.3 - 32.4
Trimethoprim					44.2	30.0	1.2	0.1					24.6						24.6	22.1 - 26.9
Ciprofloxacin	73.9	11.1	0.4	1.4	6.9	4.2	0.8	0.2		0.9	0.4								14.7	12.6 - 16.6
Nalidixic acid									83.5	2.0	0.5	0.2	1.6	4.7	7.5				14.0	12 - 15.8
Chloramphenicol										79.6	11.8	1.6	1.7	1.1	4.2				8.6	7 - 10.1
Azithromycin*								3.6	57.8	35.0	2.7	0.4	0.4	0.2					1.0	0.4 - 1.5
Colistin							89.3	10.7											0.0	0 - 0
Meropenem		99.7	0.3																0.0	0 - 0
Tigecycline					84.6	15.0	0.4												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.

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

Table Eco02 Resistance (in %) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2015.

Faecal samples	Broilers	Pigs	Dairy cows	Veal calves	
	N = 400	N = 298	N = 292	White, N = 150	Rosé, N = 143
Ampicillin	53.3	28.9	1.4	26.7	10.5
Cefotaxime	2.5	0.3	0.3	0.0	0.0
Ceftazidime	2.5	0.3	0.3	0.0	0.0
Gentamicin	4.0	0.7	0.0	1.3	0.0
Tetracycline	35.8	45.3	3.8	64.0	16.1
Sulfamethoxazole	47.0	40.3	2.4	33.3	13.3
Trimethoprim	41.5	35.9	1.4	24.7	0.7
Ciprofloxacin	44.0	0.7	0.0	6.7	0.0
Nalidixic acid	42.0	0.7	0.0	6.0	0.0
Chloramphenicol	11.0	9.4	1.4	15.3	7.7
Azithromycin	2.5	1.0	0.0	0.0	0.0
Colistin	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0

For most drugs or drug classes there are notable variations in resistance levels between the different animal species (Table Eco02). Highest levels are recorded for broilers, slaughter pigs and white veal calves, lower levels for rosé veal calves and traditionally lowest levels are observed for dairy cattle. In general, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These include the drug classes that are most frequently used in veterinary medicine.

Quinolones

Resistance to quinolones was most pronounced in *E. coli* from broiler chickens; 44% resistance to ciprofloxacin and 42% resistance to nalidixic acid. Although resistance rates to quinolones were still high, the recent reduction in usage of quinolones in broiler chickens may have contributed to a further decrease of resistance compared to 2013 and 2014 (54% and 46%, respectively). In 2015, high level resistance (MIC >1 mg/L) to ciprofloxacin in broiler chickens was detected in 6.5% (26/400) of the isolates, which is similar to former years. In 2015, resistance to ciprofloxacin showed a further decrease in *E. coli* isolates from white veal calves, became undetectable in isolates from rosé veal calves and remained low in isolates obtained from pigs.

Resistance to quinolones in *E. coli* from meat samples showed minor differences in 2015, as compared to 2014. Resistance levels remained high in poultry and turkey meat products. Compared to the previous year, resistance slightly increased in meat products of poultry and turkey and in beef samples, slightly decreased in pork and became undetectable in meat samples of veal. The percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 32.3% and 28.6%, respectively in poultry and 40.0%

and 30.0%, respectively in turkey. Resistance to quinolones was substantial in herbs. The percentage resistance of *E. coli* in herbs was, as in poultry and turkey meat products, higher ciprofloxacin than for nalidixic acid; 20.5% and 7.7%, respectively. This is probably due to the increase of PMQR genes exhibiting resistance to ciprofloxacin, but no to nalidixic acid.

Cefotaxime resistance

Resistance to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL producing *E. coli*, remained at a low level in isolates from broiler chickens, pigs and dairy cows. Cefotaxime resistance was not detected in veal calves, indicating a reduction in white veal calves as compared to 2014. Resistance levels in *E. coli* were 2.5% in broilers, 0.3% in pigs and 0.3% in dairy cows for both, cefotaxime and ceftazidime. The 2.5% cefotaxime resistance in broiler chickens demonstrates a stabilization of cefotaxime resistance since 2013 and 2014 (2.7% and 2.9%, respectively) (Figure Ec001).

Resistance to third generation cephalosporins in meat samples remained low and was detected in pork, beef, poultry and turkey meat samples. Resistance to cefotaxime in commensal *E. coli* randomly obtained from poultry meat showed a substantial decrease from 22.5% to 1.9% between 2011 and 2014, but a tendency to increase to 4,3% in 2015 (Figure Ec003).
The reduction in cefotaxime resistance, determined in randomly selected *E. coli* isolates cultured on non-selective media, strongly 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 poultry meat samples using selective media decreased from 67% in 2014 to 39% in 2015 (see appendix 1). 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.

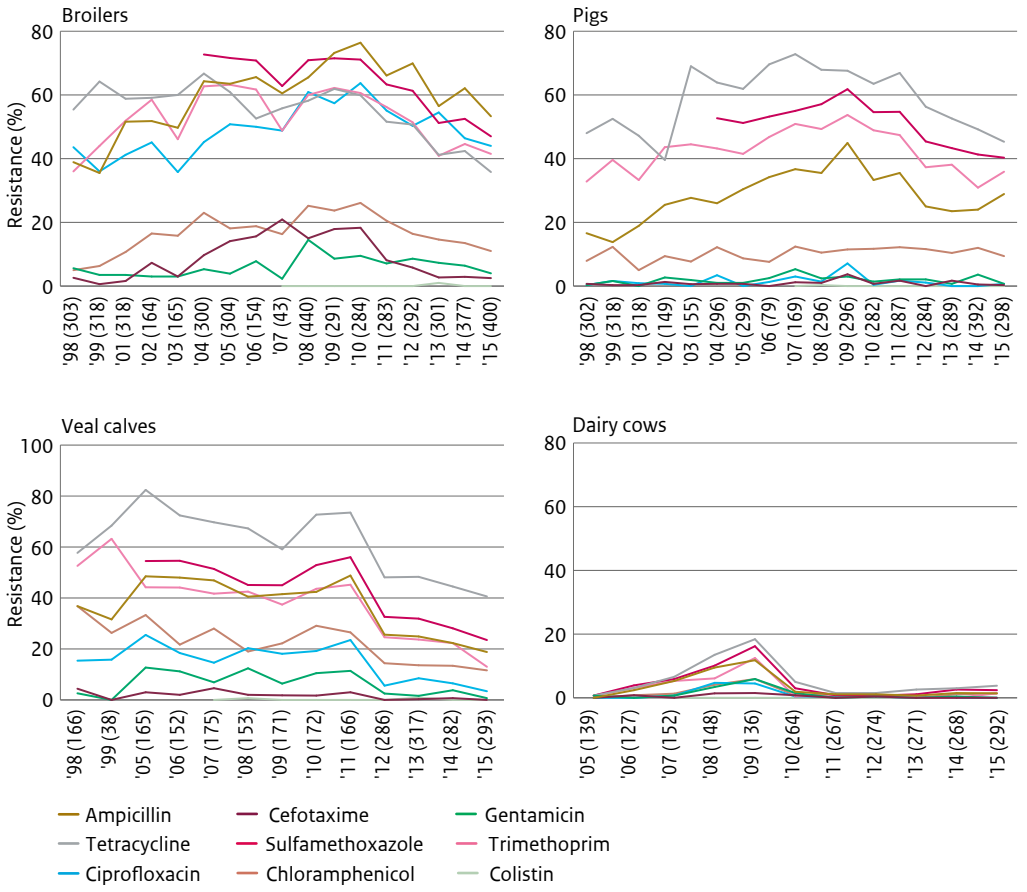
Broiler chicken

Commensal *E. coli* isolated from caecal samples from broiler chickens showed resistance to all commonly tested antimicrobials (Table Ec002). Overall resistance tended to decrease, but level of resistance to ampicillin (53.3%), tetracycline (35.8%), sulfamethoxazole (47.0%), trimethoprim (41.5%) and the quinolones ciprofloxacin (44.0%) and nalidixic acid (42.0%) remained quite high. Cefotaxime resistance remained stable at a low level (2.5%).

Slaughter pigs

Resistance against tetracycline, sulfamethoxazole, trimethoprim and ampicillin remained high in 2015 in *E. coli* isolates from swine and was 45.3%, 40.3%, 35.9% and 28.9%, respectively. All four antibiotics showed an ongoing tendency to decrease since 2011. However, in 2015 a further decrease was only shown for tetracycline and sulfamethoxazole, whereas a slight increase was shown for ampicillin and trimethoprim (Figure Ec001).
Resistance to the 3rd generation cephalosporins was low and showed a further decrease to 0.3% in 2015, still indicating that ESBLs are present, but in lower concentrations.

Figure Eco01 Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2015.



Veal calves

Since 2012, resistance data on the two veal calf husbandry types 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 calf categories most antibiotics are administered during the starting period. Rosé calves are slaughtered at an older age, which has as a consequence that on average in white veal calves more antibiotics are used. This results in two distinct data sets revealing a clear difference in resistance levels between the two husbandry types. For most antibiotics included, a much higher resistance level was recorded for white than for rosé veal calves (Table Eco02).

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 1998-2015.

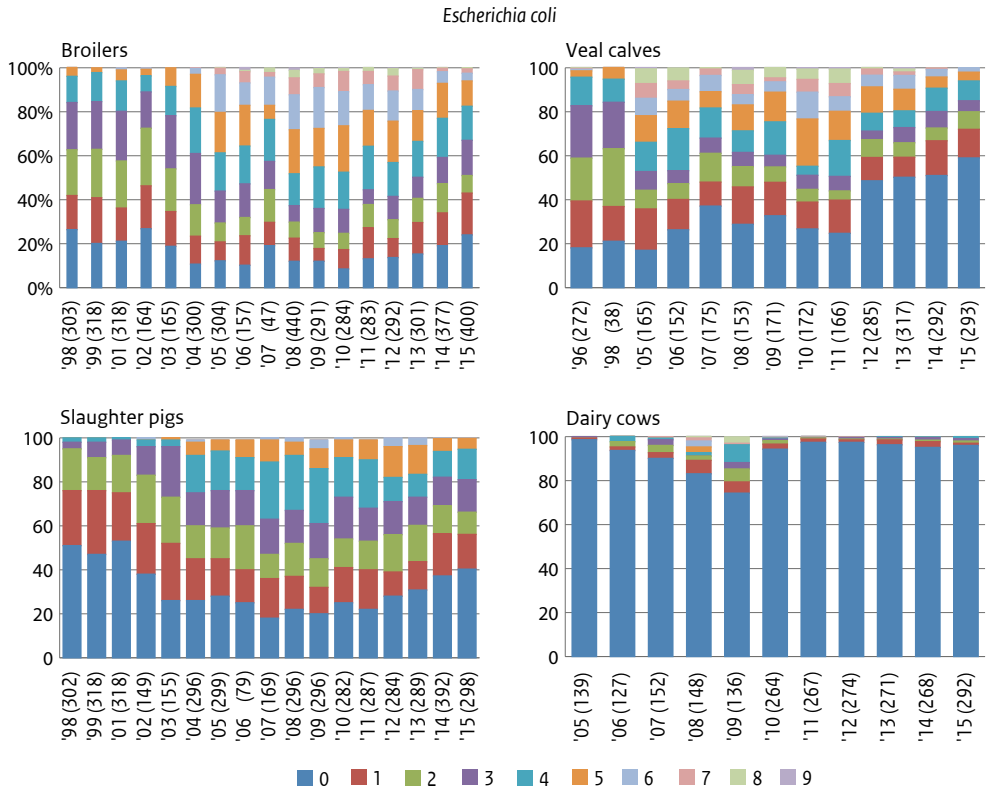


Figure Eco01 illustrates the trends in resistance in *E. coli* isolated from both types of veal calves combined. Resistance levels have been relatively stable over time, with a clear decrease in 2012, which was also the year in which the sampling strategy changed (see the description at the beginning of chapter 3.2). The changed strategy from sampling at farm to sampling at slaughterhouse might have influenced the results from 2012 and onwards. After 2012, resistance levels stabilised again. Slight decreases are shown for most tested antimicrobials. In 2015, resistance against the 3rd generation cephalosporins further decreased in *E. coli* isolates from white veal calves and became, as in rosé veal calves, under the detection level. Overall resistance levels decreased in *E. coli* isolated from veal calves, but due to higher resistance levels, the decrease was more prominent observed in isolates from white than from rosé veal calves.

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 relative low use of antibiotics in this husbandry system. Resistance rates showed slight in- or decreases compared to 2014, but overall rates remained below 4%. Furthermore, one isolate (0.7%) exhibited resistance to cefotaxime, and no resistance to ciprofloxacin was detected.

Multidrug resistance

Due to the implementation of new antimicrobial susceptibility testing panels for *E. coli*, the data to determine multidrug resistance have been adjusted backwards starting from 2014. For this reason, trends in multidrug resistance should be interpreted with care. The data with the determined level of multidrug resistance over the years are shown in Figure Eco02.

The data in 2015 indicate a still decreasing trend in the level of multidrug resistance in broilers, pigs and veal. However, levels of multidrug resistance (resistant to three or more classes of antibiotics) remained still high among *E. coli* originating from broilers (49.0%), pigs (33.9%) and veal calves (20.0%). In dairy cattle multidrug resistance in *E. coli* remained rare with 2.0% of the isolates showing resistance to three or four classes of antimicrobials.

Moreover, the overall increasing tendency of the number of completely susceptible *E. coli* isolates in all animal species (Figure Eco02) included in the survey (especially in broilers an pigs) 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.

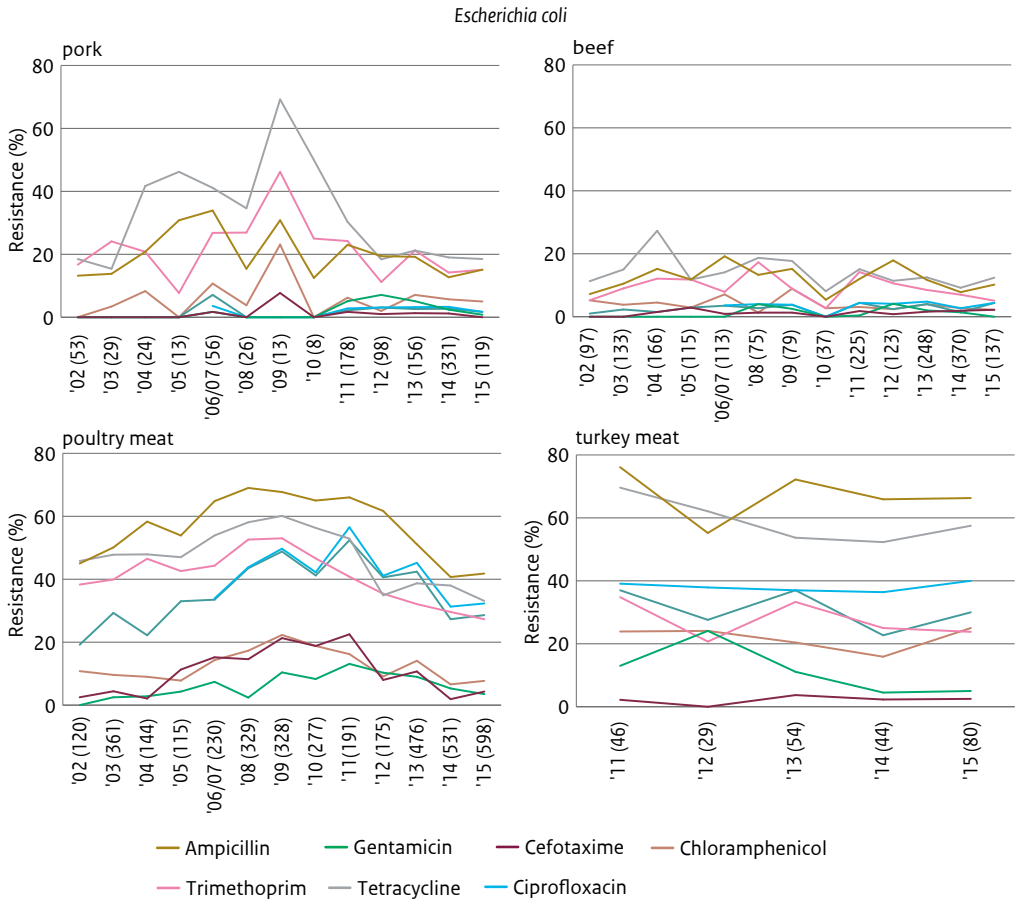
3.2.2 *E. coli* in raw meat products of food-animals

Resistance percentages of *E. coli* isolated from raw meat products (including poultry, pork, veal, beef, lamb and turkey) sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA) are shown in Table Eco03 The trends in resistance are presented in Fig Eco03. In 2015, trends for veal and lambs are no longer included in Figure Eco03, because of the high uncertainty in the interpretation due to continuous low number of isolates over the years. Instead, the resistance rates in trends in resistance of isolates from turkey meat are depicted for the first time. After a tendency to decrease over the last 4-5 years, resistance rates in poultry, pork and beef seem to have stabilized in 2015. In turkey meat, resistance rates have been at a constant high level in the past five years. As a result the number of multidrug resistant *E. coli* isolates is among the highest of all animal species included (data not shown). Cefotaxime resistance in *E. coli* isolates from poultry products showed after a rapid decrease from 10.7% in 2013 to 1.9% in 2014, a slight increase to 4.3% in 2015. Fluctuations in the resistance rates might be caused by year-to-year differences in the proportion of foreign poultry and turkey products included in the survey. Nevertheless, the prevalence of ESC-resistant *E. coli* on meat decreased substantially compared to 2014 from 67% in 2014 to 39% in 2015 (see appendix I, table ESBL04) suggesting that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is reduced. Isolates from pork and beef remained incidentally resistant to 3rd generation cephalosporins. Compared to the other types of meat, resistance rates of *E. coli* from beef are traditionally among the lowest and remained at a constant low level over the years. In pork, resistance for most antibiotics noticeably decreased from 2009 to 2012 and has stabilized at a lower level in the last two years. Interpretation of data from veal and lamb remains complicated because of the low yearly numbers of isolates obtained and tested.

Table Eco03 Resistance (in %) of *E. coli* isolated from raw meat products and herbs at retail in the Netherlands in 2015.

Meat products	Chicken	Pork	Veal	Beef	Lamb	Turkey	Herbs
	N = 598	N = 119	N = 6	N = 137	N = 16	N = 80	N = 39
Ampicillin	41.8	15.1	33.3	10.2	0.0	66.3	35,9
Cefotaxime	4.3	0.0	0.0	2.2	0.0	2.5	0,0
Ceftazidime	5.0	1.7	0.0	2.9	0.0	2.5	0,0
Gentamicin	3.5	0.8	0.0	0.0	0.0	5.0	5,1
Tetracycline	33.1	18.5	33.3	12.4	0.0	57.5	35,9
Sulfamethoxazole	36.3	19.3	50.0	11.7	0.0	45.0	33,3
Trimethoprim	27.3	15.1	50.0	5.1	0.0	23.8	33,3
Ciprofloxacin	32.3	1.7	0.0	4.4	0.0	40.0	20,5
Nalidixic acid	28.6	1.7	0.0	4.4	0.0	30.0	7,7
Chloramphenicol	7.7	5.0	16.7	2.2	0.0	25.0	23,1
Azithromycin	1.2	0.8	0.0	0.0	0.0	3.8	2,6
Colistin	1.7	0.8	0.0	0.7	0.0	7.5	2,6
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0,0
Tigecycline	3.3	2.5	0.0	2.9	12.5	2.5	5,1

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from pork, beef and raw meat products of poultry and turkey in the Netherlands from 2002-2015.



3.2.3 *Enterococcus faecalis* and *E. faecium*

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 in this chapter. From 2013 onwards, as a result of less priority for including enterococci in the surveillance, poultry, pigs and cattle and meat thereof are sampled once every three years. In 2015 *Enterococcus faecalis* and *E. faecium* were isolated from faecal samples of veal calves only. Supplementary to isolates from live animals, susceptibility profiles of *E. faecalis* and *E. faecium* isolated from raw beef products are presented as well.

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. As a result for the monitoring of enterococci, three antimicrobials were excluded (florfenicol, salinomycin and streptomycin) and three new antimicrobials (teicoplanin, daptomycin and tigecycline) were included. All three antimicrobials are used for treatment of human infections with resistant enterococci. Implementation of these new antimicrobials into the monitoring programme is important for early detection of possible spread of resistance for these new agents in bacteria from food-producing animals.

Highlights

1. In 2015, only enterococci isolates from veal calves were included. Susceptibility testing of enterococci is considered of lesser priority than *E. coli*, also in the new legislation. Therefore, from 2013 onwards poultry, pigs and cattle are sampled once every three years instead of annually.
2. In veal calves, highest resistance levels were observed for tetracycline (52.9% in *E. faecalis* and 41.3% in *E. faecium*), erythromycin (41.2% in *E. faecalis* and 30.4% in *E. faecium*). In addition, high levels of resistance for chloramphenicol were observed in *E. faecalis* (29.4%) and for quinu/dalfopristin in *E. faecium* (72.8%).
3. For two new antibiotics in the panel (daptomycin and tigecyclin) no resistance was observed in enterococci derived from faeces, but in meat resistance for tigecycline was observed in *E. faecalis* (0.7%) and for daptomycin in *E. faecium* (6.5%).

Resistance levels

In 2015 MIC values have been determined for 17 *E. faecalis* and 92 *E. faecium* strains isolated from caecal samples of veal calves as well as for 137 *E. faecalis* and 46 *E. faecium* isolates from beef samples. MIC-distributions for *E. faecalis* and *E. faecium* isolated from veal calves are presented in Table Ento1 and the resistance percentages specified for the isolates from slaughter veal calves are presented in Table Ento2. Trends over the years are depicted in Figure Ento1.
Data for 2015 on *E. faecalis* and *E. faecium* from beef products are presented in Table Ento3. Trends over the years for enterococci from beef sources are presented in Figure Ento2.

Veal calves

High resistance levels in *E. faecalis* as well as in *E. faecium* were observed for tetracycline (52.9% and 41.3%) and erythromycin (41.2% and 30.4%) (Table Ento2). In addition, high levels of resistance for chloramphenicol were observed in *E. faecalis* (29.4%) and for quinu/dalfopristin in *E. faecium* (72.8%). Over the last years, resistance in *E. faecalis* to most tested antimicrobials remained relatively stable but, showed a tendency to decrease. However, because of the low number of isolates included (n = 17) the resistance rates should be interpreted with care. Also in *E. faecium* were the resistance levels to most tested antimicrobials relatively stable. Resistance for tetracycline and linezolid showed a minor increase in *E. faecium*, as compared to 2012. Ampicillin resistance in *E. faecium*, decreasing since 2009, became undetectable in 2015.Vancomycin resistance in *E. faecium* has not been detected since 2009 (Figure Ento1).

Raw bovine meat (beef) products

Resistance percentages of *E. faecalis* and *E. faecium* strains isolated from raw beef products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (NVWA) are shown in Table Ento3.

For most antimicrobials, differences were observed in resistance level between enterococci obtained from faecal samples and meat samples. Overall, the resistance rates of enterococci were lower in meat than in faeces. Resistance rates of *E. faecalis* in beef samples were substantial lower for chloramphenicol, erythromycin, gentamicin and tetracycline compared to isolates from faeces. Comparing resistance rates of *E. faecalis* obtained from faeces and meat should be done with great care, because of the low number of fecal isolates included. Resistance rates of *E. faecium* in beef samples were substantial lower for erythromycin, quinu/dalfopristin and tetracycline compared to faeces. In addition, low resistance to chloramphenicol, gentamicin and linozelid, as shown in isolates from faeces, was not detected in *E. faecium* isolated from beef samples.

Furthermore, in meat samples *E. faecalis* is more frequently isolated than in faeces. This suggests that *E. faecalis* may be more adapted to circumstances during meat processing and has more chances to survive. The result is that the MIC-data from meat samples cannot be directly compared to data from faeces and that data from faeces cannot be one-to-one translated to data from meat and should only be compared on bacterial species level. For two new antibiotics in the panel (daptomycin and tigecyclin) no resistance was observed in enterococci derived from faeces, but in meat resistance for tigecycline was observed in *E. faecalis* (0.7%) and for daptomycin in *E. faecium* (6.5%).

The overall differences between resistance levels in faecal samples and meat remain noteworthy and might suggest that certain selection pressures could favor the selection of certain biotypes in meat. Also meat from foreign origin may have biased the results.

In *E. faecalis* resistance rates seem to have stabilized at a relatively low level. In *E. faecium* resistance rates decreased or tend to stabilize at a relative low level (Figure Ento2).

Table Ent01 MIC distributions (in %) for *E. faecalis* (N = 17) and *E. faecium* (N = 92) isolated from veal calves in the Netherlands in 2015.

E. faecalis	MIC (%) distribution mg/L																		R%	95% CI
N = 17	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						17.6	64.7	17.6											0.0	
Chloramphenicol									11.8	58.8			23.5	5.9					29.4	
Ciprofloxacin						29.4	64.7												0.0	
Daptomycin					5.9	5.9	58.8	29.4											0.0	
Erythromycin							23.5	23.5	11.8		5.9				35.3				41.2	
Gentamicin										70.6	23.5							5.9	5.9	
Linozelid						5.9	17.6	76.5											0.0	
Quino/dalfopristin*								5.9	11.8	41.2	41.2								-	
Teicoplanin						100													0.0	
Tetracycline							47.1				5.9	5.9		41.2					52.9	
Tigecycline		5.9	82.4	11.8															0.0	
Vancomycin							23.5	76.5											0.0	

E. faecium	MIC (%) distribution mg/L																		R%	95% CI
N = 92	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						14.1	31.5	43.5	10.9	0.0									0.0	
Chloramphenicol									28.3	57.6	3.3	9.8	1.1						1.1	
Ciprofloxacin					3.3	5.4	41.3	18.5	29.3	2.2									2.2	
Daptomycin							12.0	23.9	64.1										0.0	
Erythromycin							27.2	20.7	21.7	5.4			1.1		23.9				30.4	
Gentamicin										87.0	9.8	1.1			2.2				2.2	
Linozelid							1.1	90.2	2.2	5.4									5.4	
Quino/dalfopristin						10.9	16.3	12.0	55.4	4.3	1.1								72.8	
Teicoplanin						98.9	1.1												0.0	
Tetracycline							58.7						2.2	30.4	8.7				41.3	
Tigecycline		17.4	46.7	33.7	2.2														0.0	
Vancomycin							89.1	10.9											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.

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

Table Ent02 Resistance percentages (%) of *Enterococcus faecalis* and *E. faecium* isolated from veal calves in the Netherlands in 2015.

	Veal calves	
	E. faecalis (N = 17)	*E. faecium* (N = 92)
Ampicillin	0.0	0.0
Chloramphenicol	29.4	1.1
Ciprofloxacin	0.0	2.2
Daptomycin	0.0	0.0
Erythromycin	41.2	30.4
Gentamicin	5.9	2.2
Linozelid	0.0	5.4
Quinu/dalfopristin*	-	72.8
Teicoplanin	0.0	0.0
Tetracycline	52.9	41.3
Tigecycline	0.0	0.0
Vancomycin	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 1998-2015.

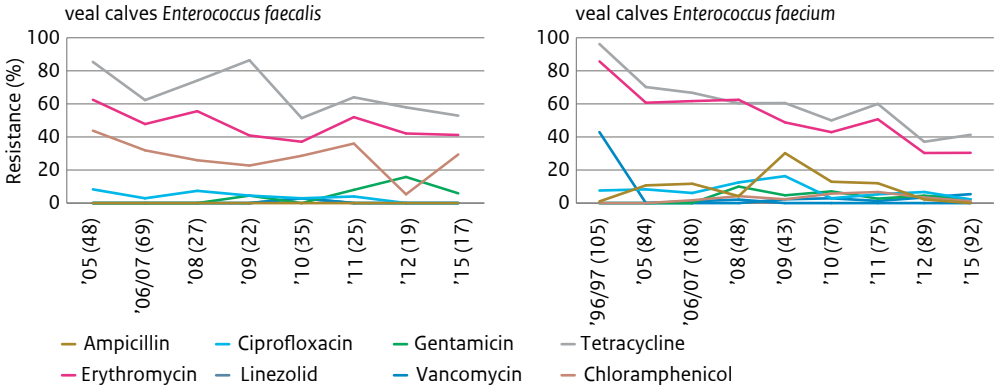
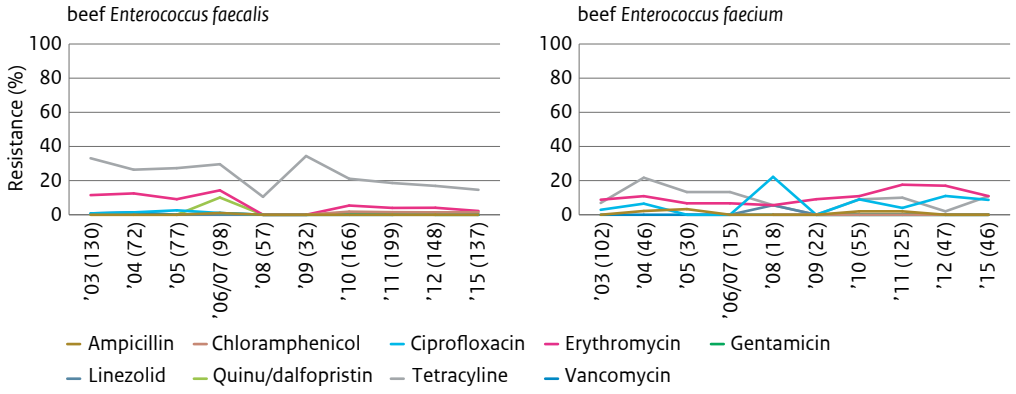


Table Ent03 Resistance % of *Enterococcus faecalis* and *E. faecium* strains isolated from raw beef in the Netherlands in 2015.

	Bovine meat (beef)	
	E. faecalis (N = 137)	*E. faecium* (N = 46)
Ampicillin	0.0	0.0
Chloramphenicol	1.5	0.0
Ciprofloxacin	0.0	8.7
Daptomycin	0.0	6.5
Erythromycin	2.2	10.9
Gentamicin	0.0	0.0
Linozelid	0.0	0.0
Quinu/dalfopristin*	-	54.3
Teicoplanin	0.0	0.0
Tetracycline	14.6	10.9
Tigecycline	0.7	0.0
Vancomycin	0.0	0.0

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

Figure Ent02 Trends in resistance percentages in *E. faecalis* and *E. faecium* isolated from raw meat products from beef in the Netherlands from 2003-2015.



4 Appendix I

Results of the screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant *Enterobacteriaceae* in food and food producing animals in the Netherlands in 2015

Highlights

1. ESBL-producing *E. coli* represented 0.9% of randomly isolated *E. coli*, the lowest proportion observed since 2007.
2. Selective isolation from livestock faeces indicated ESBL/AmpC producing *E. coli* prevalence of 56.5% in broilers, 12.3% in slaughter pigs, 17.3% in white veal calves, 10% in rosé veal calves and 9.3% in dairy cows.
3. Classical human associated ESBL-types $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$ were found in *E. coli* from broiler faeces, together with $bla_{CTX\text{-}M\text{-}55}$ not described before in Dutch broilers.
4. ESBL/AmpC prevalence in *E. coli* from prepared meat tended to be higher compared to raw meat, possibly due to cross-contamination during processing. ESBL/AmpC-prevalence in poultry meat decreased substantially compared to 2014. This decrease is most likely associated with the major reduction in antibiotic use in broilers since 2011 and the total ban on the use of ceftiofur at hatcheries in 2010.
5. The prevalence of ESBL-producing *Salmonella* in 2015 was 1.8%, confirming the decreasing trend observed in 2014 (2.1%) and 2013 (4%). Most represented ESBL-genes were $bla_{CMY\text{-}2}$, generally associated with S. Heidelberg, and $bla_{CTX\text{-}M\text{-}1}$ in S. Heidelberg and Enteritidis.
6. In *Salmonella* isolates from human sources a variety of ESBL-genes were found: $bla_{CMY\text{-}2}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}65}$.

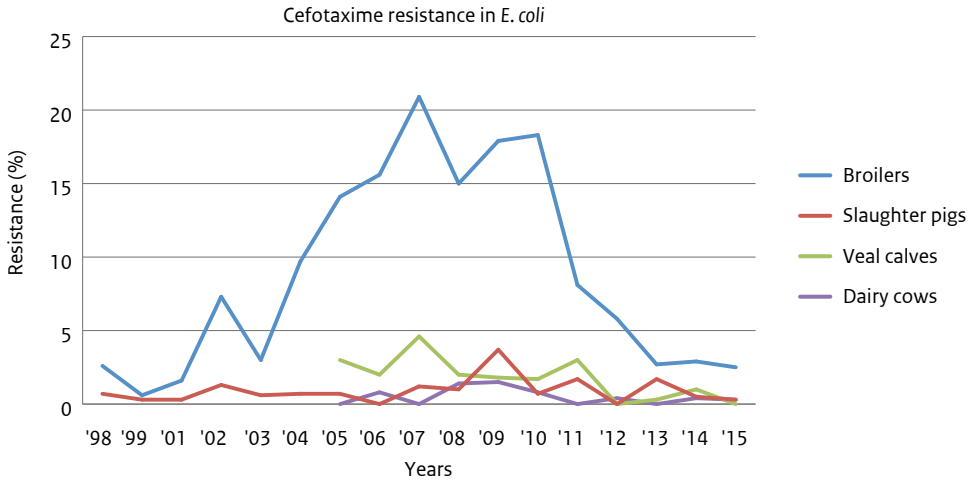
7. The majority of ESBL-*Salmonella* isolates were highly multidrug resistant, with an increased pattern of resistance to 5- 8 different antibiotics compared to 2014. No resistance to carbapenems was detected in *Salmonella*.
8. No carbapenemase-producing *Enterobacteriaceae* were detected in active surveillance. Only 3 isolates of *Shewanella* spp holding chromosomal $bla_{OXA-48b}$ were detected in broilers and veal calf.
9. The colistin resistance gene *mcr-1* was present at low level in *E. coli* from livestock (≤ 1%) and
10. In 2015, *mcr-1* was identified in sixteen *E. coli*, one *S.* Paratyphi B variant Java isolated and one S. Schwarzengrund, all isolated from poultry sources (chicken and turkey meat).

4.1 ESBL-producing bacteria

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 EFSA guidelines . These isolates are tested for susceptibility to cefotaxime and ceftazidime. Proportions of resistant isolates are determined based on EUCAST 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 with a resistance phenotype in randomly picked *E. coli* isolated from non-selective media derived from broilers, slaughter pigs (1998-2015), veal calves and dairy cows (2005-2015). In broilers after 2003 an apparent increase in cefotaxime resistance was observed up to levels that varied between 15-20%, with the highest peak observed in 2007. The prevalence in broilers declined to 2.7% in 2013, to steadily level off to 2.9-2.5% in 2014 and 2015, respectively. 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 ceftiofur (off label) use was stopped at Dutch hatcheries. In 2014, the decrease in usage stopped in broilers, which may have resulted in the levelling off observed in the past two years.
From a total of 1283 randomly selected *E. coli* isolates that were tested in 2015, twelve displayed non-wildtype susceptibility to cefotaxime (see also 3.2.1). Ten were isolated from broilers, one from a slaughter pig and one from a dairy cow (Table ESBL01). In veal calves no ESBL-suspected *E. coli* isolates were found in 2015. All non-wildtype susceptible isolates were screened for beta-lactamase gene families using PCR or microarray (Check-Points CT103). Subsequently the genes were identified by dedicated PCR and sequence analysis. All isolates with a negative 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 poultry isolates five plasmid mediated ESBL genes were present: $bla_{CTX-M-1}$ (n=2), $bla_{CTX-M-9}$ (n=2), $bla_{CTX-M-15}$ (n=1), bla_{SHV-12} (n=1), and bla_{CMY-2} (n=1). Mutation in the chromosomal *ampC* gene was detected in three of the broiler isolates. 2015 is the first year in which $bla_{TEM-52c}$ was not found in cefotaxime resistant isolates from broilers derived from the monitoring program. An increase in ESBL gene variability was

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

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



registered compared to 2014 when only two types of plasmid mediated ESBL genes were detected. $bla_{TEM-52c}$ was detected in both milk cow and pig isolates, together with bla_{CMY-2} in the latter. Conversely from previous years, when $bla_{CTX-M-1}$ was the most detected gene in isolates from food-producing animals, two isolates harbouring $bla_{CTX-M-14}$ (CTX-M-9 group) were found in broiler isolates, together with the reappearance of bla_{CMY-2} in both broilers and slaughter pigs, undetected in 2014 (results not shown).

It can be concluded that by random isolation, only nine plasmid mediated ESBLs were found in 1283 isolates in 2015 (0.9%), the lowest observed since 2007. This confirms the already promising results of 2014, when 1.1% ESBL/AmpC-producing isolates were detected, a major improvement compared to 2008 when ESBL/AmpC-producing isolates added up to 7.6%, before antibiotic usage reduction started in Dutch livestock.

Active surveillance by selective isolation of ESBL/Ampc producing *E. coli* in 2015

As of 2014 an active surveillance for ESBL/AmpC producers in broilers, pigs, veal calves and dairy cows was implemented in the monitoring program as a mandatory part of the surveillance. Faecal samples taken for monitoring at slaughterhouse (slaughter pigs, white and rosé veal calves, and broilers) and at farm (dairy cows) were also used for ESBL/AmpC-producing *E. coli* detection by selective methods. Screening of faecal samples was done by overnight non-selective enrichment in Buffered Pepton Water (BPW) followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime. This resulted in the screening of 1300 faecal samples (Table ESBL02).

In 2015, also 3909 meat samples (Table ESBL04) were analysed for ESBL/AmpC-producing *E. coli*. Meat samples were pre-enriched in 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.

Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkeyLegend. Turkeys only in 2011, not tested in 2012 during 2007-2015.

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
Total	263	26	30	8	6	333	131	19	3	37	2	42	9	43	19	28		

 # All were $bla_{CTX\text{-}M\text{-}1}$, only in 2011 one $bla_{CTX\text{-}M\text{-}3}$ gene was found in an isolate from veal calves.
Three combinations (all in broiler isolates) were found: in 2008: $bla_{CTX\text{-}M\text{-}1}$ with $bla_{CTX\text{-}M\text{-}2}$; in 2009: $bla_{CTX\text{-}M\text{-}1}$ with $bla_{SHV\text{-}12}$ and $bla_{CTX\text{-}M\text{-}1}$ with $bla_{SHV\text{-}12}$ and $bla_{CMY\text{-}2}$.

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

n.t. : not tested

Table ESBL02 Prevalence of *E. coli* isolates showing reduced susceptibilty to cefotaxime derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves and dairy cows taken at slaughter in 2015.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	400	235	226	56.5
Pigs	300	56	37	12.3
Veal calves				
white	150	28	26	17.3
rosé	150	15	15	10.0
Dairy cows	300	33	28	9.3
Total	1300	367	332	25.5

Results of active surveillance 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 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 1300 samples analysed for ESBL-producing *E. coli*, 25.5% were positive, mainly due to the high prevalence in broilers (56.5%). As already observed in the past, ESBL-producing *E. coli* levels in white veal calves were higher than in rosé veal calves (respectively 17.3% and 10%). A stable reduction in ESBL-producing *E. coli* in broilers was observed in 2015, with a 10% reduction compared to 2014 (66% in 1601 samples) and an overall 30% reduction from 2009 (Dierikx *et al.*, 2013). Prevalence in pigs remained the same between 2014 and 2015 (12.3%), whereas prevalence in dairy cows slowly decreased from 14% in 2011 to 6% in 2014, to unexpectedly increase to 9.3% in 2015.

ESBL/AmpC genes detected in animal faeces are reported in Table ESBL03. The increase in ESBL types variation observed in 2014 compared to former years (MARAN 2011 and 2013) continued in 2015, likely dependent on 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, $bla_{\text{CTX-M-1}}$ was the dominant ESBL-variant in all animal species examined, followed by $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{TEM-52c}}$. The high variation in ESBL-types observed in broilers during passive surveillance (see Table ESBL01) mirrored the results of the active surveillance with 12 ESBL-types detected. The more classical human associated ESBL-types $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{CTX-M-15}}$ were described in isolates from broiler faeces, as in 2014, together with $bla_{\text{CTX-M-55}}$ not previously described in Dutch broilers. Interestingly, the increased prevalence in isolates from dairy cows matched an increase in ESBL-types with $bla_{\text{CTX-M-1}}$ being the predominant beta-lactamase gene, and $bla_{\text{SHV-12}}$ detected for the first time since 2011 (MARAN 2010 and 2011). Pig and veal calf isolates didn't show significant differences compared to previous years, whereas chromosomal *ampC* types seem to play a larger role in conferring cefotaxime resistance compared to 2014, with relatively high numbers in pig and cow isolates (34% and 15%, respectively). Combination of ESBL-types was rare, with only two broiler isolates exhibiting $bla_{\text{TEM-52c}}$ together with $bla_{\text{SHV-12}}$.

Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, slaughter pigs, veal calves and dairy cows in 2015. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Slaughter pigs	Veal calves White	Veal calves Rose	Dairy cows	Total
CTX-M-1 group							
	CTX-M-1	110	21	10	6	11	158
	CTX-M-3	1					1
	CTX-M-15	6	1	5	1	3	16
	CTX-M-32	1		1		1	3
	CTX-M-55			1	1		2
CTX-M-9group							
	CTX-M-9	1	1			1	3
	CTX-M-14	2		3	3	1	9
	CTX-M-27		1			1	2
	CTX-M-65		1	1	2	1	5
TEM							
	TEM-52	3					3
	TEM-52c	21	7			3	31
	TEM-52cVar	4	1	1			6
SHV							
	SHV-2A	2					2
	SHV-12	23	1	3		2	29
CMY							
	CMY-2	50	3	1	2	4	60
Combinations							
	TEM-52c&SHV	2					2
Chromosomal ampC							
	ampC-type-3	7	19	1		4	31
	ampC-type-3-like					1	
	ampC-type-18	2		1			3
Total		235	56	28	15	33	367

Table ESBL04 ESBL-suspected and confirmed *E. coli* isolates from raw meat products in the Netherlands in 2015.

Animal source		N screened	N suspected ESBL	% suspected ESBL
Cattle				
	fresh meat	467	8	1.7
	preparation	585	28	4.8
Calf				
	fresh meat	21	0	0.0
	preparation	26	1	3.8
Pig				
	fresh meat	779	6	0.8
	preparation	559	8	1.4
Lamb				
	fresh meat	47	1	2.1
	preparation	26	0	0.0
Chicken				
	fresh meat	587	231	39.4
	preparation	674	290	43.0
	import	43	26	60.5
Turkey				
	fresh meat	80	18	22.5
	preparation	12	2	16.7
	import	3	2	66.7
Total		3909	621	15.9

Results of active surveillance of ESBL/AmpC-producing *E. coli* in raw meat and meat products

All *E. coli* selectively isolated on MacConkey with 1 mg/L cefotaxime demonstrating resistance to either cefotaxime or ceftazidime were considered ESBL-suspected *E. coli*. Prevalence of ESBL-suspected isolates in fresh and frozen (preparation) raw meat are shown in Table ESBL04. Out of 3909 meat samples (consisting of fresh meat, meat preparation and imported frozen meat), 621 were positive for ESBL suspected *E. coli*. Although the highest prevalence was observed in imported poultry meat (60.5%), the decreasing trend of ESBL-suspected isolates in fresh broiler meat started in 2012 (83%) and continued in the past two years (73% and 67% in 2013 and 2014, respectively). Turkey meat showed a high variability in suspected ESBL-producing *E. coli* prevalence depending on the source with 22.5% prevalence in fresh meat versus 66.7% in frozen meat. Meat preparations of chicken and turkey (depicted as meat products in 2014) showed a high prevalence of 43-17%, respectively. While in cattle, pig and lamb meat ESBL-suspected prevalence was comparable to 2014 (1.7-4.8%, 0.8-1.4, and 2.1%, respectively), incidence in processed calf meat was significantly lower than 2014 (from 21% to 3.8%). In general, prevalence in processed meat tended to be higher compared to raw meat, likely due to cross-contamination during processing.

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2015.

ESBL gene		Chicken	Beef	Pork	Lamb	Turkey	Total
CTX-M-1 group							
	CTX-M-1	35	14	5		9	63
	CTX-M-15		7			2	9
	CTX-M-32		2				2
	CTX-M-55	2					2
CTX-M-2 group							
	CTX-M-2	2	1				3
CTX-M-8/25 group							
	CTX-M-8	1	1				2
CTX-M-9 group							
	CTX-M-14		2				2
	CTX-M-27					1	1
TEM							
	TEM-52c	5	1			1	7
	TEM-52cVar	6					6
SHV							
	SHV-12	11	1	1	1	4	18
CMY							
	CMY-2	37	1	1		1	40
Chromosomal ampC							
	ampC-type-3		2	2		1	5
Total		99	32	9	1	19	160

Given the high number of suspected ESBL-producing *E. coli*, 160 isolates were selected for molecular typing and confirmed by MALDI-TOF as *E. coli*. Table ESBL05 shows the different ESBL/AmpC types detected in meat. Most of ESBL-types found in beef were also found in faecal samples of veal calves ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{SHV\text{-}12}$, and $bla_{CMY\text{-}2}$) strongly suggesting faecal contamination during slaughter and/or meat processing. Chicken and pork meat displayed less ESBL-types than broiler or slaughter pig faecal samples, and chromosomal *ampC* types were detected in 6 isolates. Conversely from 2014, the dominant human $bla_{CTX\text{-}M\text{-}15}$ was not detected in chicken meat but only in broiler faecal samples (see Table ESBL03).

Other frequent ESBL-types were $bla_{CMY\text{-}2}$ and $bla_{SHV\text{-}12}$ typically associated with food-producing animals the meat originated from, that were reported in higher percentages compared to 2014. In contrast, $bla_{CTX\text{-}M\text{-}2}$ was found in only 3 meat samples (1.8%), with a significantly lower prevalence than in 2014 (17.5%).

Table ESBL06 Beta-lactamases in *Salmonella* isolated in 2015.

					CTX-M-1 group				CTX-M-9 group			TEM	CMY	Combinations		
Serovar	**Humans**	**Poultry**	**Turkey**	**Unspecified**	**CTX-M-1**	**CTX-M-15**	**CTX-M-55**	**CTX-M2**	**CTX-M-9**	**CTX-M-14**	**CTX-M-65**	**TEM-52Var**	**CMY-2**	**TEM-52Var & CMY-2**	**CTX-M-1 & CMY-2**	**Total**
1,4,5,12:i:-	1	1			1					1						2
4,12:i:-	2						1				1					2
Corvallis	1					1										1
Dublin				1			1									1
Enteritidis	1												1			1
Heidelberg		10	2	2	2	2		1		1	1	1	6			14
Infantis		1		1	1								1			2
Molade				1											1	1
Oranienburg	1													1		1
Paratyphi B variatie Java		2									1		1			2
Schwarzengrund			1										1			1
Typhimurium	6				2	1		1	1				1			6
Total	**12**	**14**	**3**	**5**	**6**	**4**	**2**	**2**	**1**	**2**	**3**	**1**	**11**	**1**	**1**	**34**

Table ESBL07 Resistance and multidrug resistance percentages of ESBL-producing *Salmonella* in the Netherlands in 2015.

Antimicrobials	R%
Ampicillin*	97.1
Cefotaxime	100.0
Ceftazidime	76.5
Gentamicin	20.6
Tetracycline	73.5
Sulfamethoxazole	79.4
Trimethoprim	38.2
Ciprofloxacin	73.5
Nalididixic acid	58.8
Chloramphenicol	20.6
Azithromycin	0.0
Colistin	8.8
Meropenem	0.0
Tigecycline	14.7

Multi drug resistance	N = 36
0	0%
1	0%
2	12%
3	6%
4	9%
5	29%
6	26%
7	0%
8	15%
9	3%
10	0%

** One CTX-M-55 harbouring S. Dublin was susceptible for ampicillin (MIC: 8 mg/L)*

ESBL/AmpC-producing *Salmonella*

Surveillance of resistance to extended spectrum cephalosporins is also done in *Salmonella enterica* isolated in the Netherlands. In 2015 a selection of 1761 *Salmonella* isolates sent to RIVM for sero- or MLVA-typing were tested for susceptibility to cefotaxime and ceftazidime. Cefotaxime resistant *Salmonella* were isolated only in 34 samples mainly from human (n= 12), poultry (n=14) and turkey (n=3) sources (Table ESBL06). The prevalence of ESBL-producing *Salmonella* was 1.8%, confirming the decreasing trend observed in 2014 (2.1%), almost half the amount of 2013 (4%). Next to *S.* Heidelberg (n=14), a wide variation of eleven other serovars was identified to carry ESBLs genes, identified as *E. coli* as described above.

The most represented ESBL-types were: i) bla_{CMY-2}, generally associated with *S.* Heidelberg but also present in other 5 serovars; ii) and $bla_{CTX-M-1}$ in *S.* Heidelberg and Enteritidis. Compared to 2014, prevalence of bla_{CMY-2} dropped from 58% to 35% with the appearance of previously undetected ESBL-types such as $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$. On the other hand, bla_{CMY-2} was also detected in combination with $bla_{CTX-M-1}$ or $bla_{TEM-52Var}$. In isolates from human origin a variety of ESBL-genes were found: bla_{CMY-2}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, $bla_{CTX-M-15}$, $bla_{CTX-M-55}$ and $bla_{CTX-M-65}$ (Table ESBL06).

All cefotaxime resistant *Salmonella* isolates were highly multidrug resistant, as shown in Table ESBL07. Compared to 2014, when most of the isolates were resistant to 3 to 5 antibiotics (83%), the majority of 2015 isolates showed resistance to 5, 6 or 8 different antibiotics, accounting for 70% of the total. Three isolates were resistant to 9 out of 10 antibiotics, but no resistance was detected against meropenem in any of the isolates.

Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime during 2007-2015.

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	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
Total	93	45	8	20	45	4	10	113	3	341		

\# *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*

ESBL-types found in *Salmonella* since 2007 are summarized in Table ESBL08. Every year genes belonging to bla_{CMY-2}, bla_{TEM-52}, the $bla_{CTX-M-1}$-group and the $bla_{CTX-M-9}$-group were found in *Salmonella* isolates derived from different sources. After no detection in 2014, $bla_{CTX-M-2}$ was again detected this year in one human Typhimurium isolate and one Heidelberg obtained from turkey meat. The relatively high prevalence of bla_{CMY-2} positive isolates observed in 2014 and attributed to the (compulsory) extra sampling of imported meat from South America, was not reported in 2015.
In conclusion, ESBL/AmpC-producing *E. coli* and *Salmonella* are widespread in Dutch food-producing animals and in raw meat products mainly of poultry origin. ESBL-prevalence was 0.9% of total *E. coli* in passive surveillance using random isolation, the lowest observed since 2007. Also active surveillance in faecal samples of food-producing animals using selective culturing showed an apparent decline for broilers with a 10% prevalence decrease compared to 2014.
The dominant ESBL-types were confirmed to be $bla_{CTX-M-1}$ and bla_{CMY-2}, in all animal species independently on the source of isolation, whereas an increased detection of $bla_{CTX-M-14}$ was registered in both *E. coli* and *Salmonella*. The dominant human ESBL-gene $bla_{CTX-M-15}$ was incidentally found in broiler faecal samples but not in chicken products, conversely from previous years.

4.2 Carbapenemases

Carbapenemases are extended spectrum beta-lactamases that can also hydrolyse carbapenems. This class of antibiotic is a last-resort in human therapy, therefore their use is restricted to human medicine only. Nevertheless, carbapenemase-producing microorganisms in food-producing animals and in the environment are increasingly reported (Woodford *et al.*, 2014), fuelling a debate on the actual risks for public health (Poirel *et al.*, 2014). Carbapenemase producing *E. coli* and *Salmonella* were found in samples derived from pigs, broilers and dogs in Germany (Fischer *et al.*, 2012, 2013, Stolle *et al.*, 2013). Since The Netherlands has intensive contact with Germany in terms of trade of live animals, and a risk of introduction cannot be ruled, from 2012 onwards extra screening was conducted with the aim to detect carbapenemase-producing *Enterobacteriaceae* in food-producing animals.
In 2015, 1300 faecal samples were screened for carbapenemase producing bacteria using RT PCR, a quite sensitive method in an environment with very low anticipated prevalence of carbapenem resistance. Samples were grown overnight in Buffered Pepton 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 performed according to manufacturer's instructions on isolated DNA. If RT-PCR gave suspicious or positive results, a three step analysis was performed to confirm the results:

1. A DNA-lysate was used with CT102 micro array (Check-Points), to confirm bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48} presence.
2. If micro array was positive, results were further confirmed by dedicated PCR and 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).

The 2015 carbapenemase screening resulted in three bla_{OXA-48}-positive samples (two broilers and one veal calf faecal samples). Bacterial isolates were cultured from positive samples and identified as

Shewanella xiamenensis (n=2, broilers) and *Shewanella oneidensis* (n=1, veal calf) with chromosomally located $bla_{OXA-48b}$ genes. This gene is closely related to bla_{OXA-48} (> 99% nucleotide homology) and has also been found in faecal samples in 2013 (MARAN 2013). Plasmid transformation and conjugation were not successful in transferring the bla_{OXA48b} genes to an *E. coli* K12 recipient for all *Shewanella* isolates, and chromosomal isolation was demonstrated. Considering that $bla_{OXA-48b}$ genes were located on the chromosome of *Shewanella* spp., the progenitor of this carbapenemase family (Zong, 2012), these genes were considered of environmental origin and not a public health risk. Importantly, no carbapenemase producing *Enterobacteriaceae* were detected in the faecal samples from livestock in 2015.
Screening for carbapenemase producing isolates in faecal samples of food-producing animals and in food products will continue in 2016, to monitor potential carbapenemase gene spread among environmental and clinically relevant bacteria.

4.3 Colistin resistance

Colistin has been extensively used in veterinary medicine for treatment of diarrhoeal diseases in livestock. In human medicine, colistin is nowadays often 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 transferable resistance gene has generated renewed attention to this "old'' compound. Quickly after the finding of a plasmid mediated colistin resistance gene (*mcr-1*) in *Enterobacteriaceae* in livestock and humans in China (Liu *et al.*, 2015) *mcr-1* was also reported from several European countries (Skov *et al.*, 2016).
In response we started a retrospective study to screen for *mcr-1* in all colistin resistant *E. coli* and *Salmonella* isolates in our strain collection from 2010-2015. This study revealed the presence of *mcr-1* in *E. coli* isolates obtained from livestock and meat, as well as in *Salmonella* isolates obtained from poultry and turkey meat at a low prevalence. However, the colistin resistance gene was not detected in human *Salmonella* isolates. The results of the retrospective study are shown in Table Col01.
In 2015, *mcr-1* was identified in sixteen *E. coli*, one *S.* Paratyphi B variant Java and one *S.* Schwarzengrund, all isolated from poultry sources (chicken and turkey meat), but *mcr-1* was not identified in randomly isolated *E. coli* from 1300 faecal samples of livestock (specific data not shown) .
Additional molecular characterization revealed that *mcr-1* was present on different types of conjugative plasmids (IncX4, IncHI2 and IncI2) in both *E. coli* and *Salmonella*, often flanked by insertion sequence IS*Apl1*. These results demonstrate that *mcr-1* circulates on different conjugative plasmids in *Enterobacteriaceae* in the digestive tract of livestock (Veldman *et al.*, 2016).
In 2016, a prospective study has been started on the presence of *mcr-1* in faecal and meat samples as part of the national surveillance program on antibiotic resistance in animals to reveal the current spread of this gene in livestock and meat.

Table Col01 Results of retrospective screening of *mcr-1* in colistin resistant *E. coli* and *Salmonella* isolates obtained from various sources from 2010-2015

Bacteria	Period	Source	Total number	Colistin R (N)	*mcr-1* (N)	*mcr-1* (%)
E. coli	2010-2015	broilers	2226	16	10	0,4
E. coli	2011	turkeys	192	1	1	0,5
E. coli	2014	layers	190	0	0	0,0
E. coli	2010-2015	slaughter pigs	1832	3	0	0,0
E. coli	2010-2015	calves	1525	23	15	1,0
E. coli	2010-2015	cattle	1634	3	0	0,0
E. coli	2012-2015	chicken meat	1860	52	40	2,2
E. coli	2012-2015	turkey meat	201	23	20	10,0
E. coli	2012-2015	pork	726	4	1	0,1
E. coli	2012-2015	beef	862	5	1	0,1
E. coli	2012-2015	veal, retail	60	1	1	1,7
E. coli	2012-2015	lamb	72	0	0	0,0
S. enterica	2010-2015	human	7719	136	0	0,0
S. enterica	2010-2015	chicken	1227	53	11	0,9
S. enterica	2010-2015	turkey	32	2	2	6,3
S. enterica	2010-2015	cattle	326	19	0	0,0
S. enterica	2010-2015	pigs	422	13	0	0,0
S. enterica	2010-2015	other animals	26	4	0	0,0
S. enterica	2010-2015	food	821	31	0	0,0
S. enterica	2010-2015	feed	840	6	0	0,0
S. enterica	2010-2015	other materials	35	1	0	0,0
S. enterica	2010-2015	unknown source	798	34	0	0,0

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Erratum in report 2016-0060: NethMap 2016: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands / MARAN 2016: Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2015

In NethMap 2016, page 27, table 3.3, the names, codes and numbers in the lower 6 rows were displayed incorrectly.
The correct version of the table is shown below.

Table 3.3 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1000 inhabitant-days), 2005-2014 (Source: SWAB).

ATC Group	Therapeutic group	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	0.027	0.027	0.025	0.023	0.025	0.027	0.026	0.024	0.022	0.023
J01CA	Penicillins with extended spectrum	0.106	0.113	0.110	0.101	0.111	0.110	0.103	0.100	0.099	0.101
J01CE	Beta-lactamase sensitive penicillins	0.021	0.022	0.020	0.019	0.023	0.023	0.020	0.023	0.023	0.028
J01CF	Beta-lactamase resistant penicillins	0.089	0.091	0.087	0.086	0.093	0.097	0.089	0.093	0.100	0.105
J01CR	Penicillins + beta-lactamase-inhibitors	0.231	0.239	0.233	0.229	0.241	0.256	0.223	0.211	0.199	0.187
J01DB-DE	Cephalosporins	0.121	0.127	0.124	0.118	0.137	0.147	0.145	0.158	0.164	0.176
J01DF	Monobactams	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J01DH	Carbapenems	0.008	0.009	0.010	0.011	0.014	0.015	0.018	0.019	0.020	0.019
J01EA	Trimethoprim and derivatives	0.009	0.009	0.009	0.007	0.007	0.009	0.006	0.005	0.004	0.003
J01EC	Intermediate-acting sulphonamides	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.000
J01EE	Sulphonamides + trimethoprim	0.035	0.034	0.033	0.029	0.030	0.030	0.026	0.024	0.024	0.022
J01FA	Macrolides	0.042	0.040	0.040	0.037	0.039	0.041	0.037	0.038	0.034	0.034
J01FF	Lincosamides	0.030	0.031	0.031	0.029	0.033	0.035	0.032	0.031	0.032	0.028
J01GB	Aminoglycosides	0.038	0.039	0.041	0.048	0.055	0.058	0.054	0.044	0.045	0.044
J01MA	Fluoroquinolones	0.115	0.121	0.124	0.139	0.129	0.138	0.127	0.124	0.116	0.112
J01MB	Other quinolones	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000
J01XA	Glycopeptide antibacterials	0.010	0.011	0.011	0.012	0.015	0.016	0.017	0.017	0.018	0.018
J01XB	Polymyxins	0.005	0.005	0.006	0.008	0.009	0.006	0.003	0.002	0.003	0.002

ATC Group	**Therapeutic group**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
J01XD	Imidazole derivatives	0.024	0.027	0.027	0.025	0.026	0.030	0.027	0.029	0.030	0.030
J01XE	Nitrofuran derivatives	0.017	0.016	0.018	0.016	0.017	0.018	0.015	0.018	0.016	0.018
J01XX08	Linezolid	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	other antibiotics	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**0.931**	**0.965**	**0.952**	**0.941**	**1.008**	**1.061**	**0.971**	**0.963**	**0.951**	**0.954**