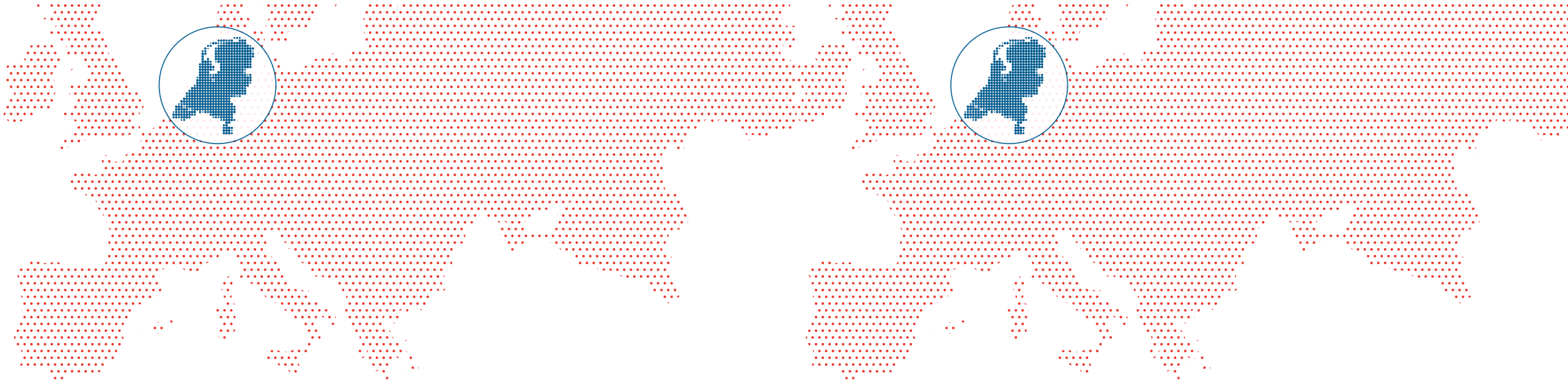
NethMap 2015

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

MARAN 2015

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2014



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NethMap 2015

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in The Netherlands
in 2014

June 2015

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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.
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1 Introduction

This is NethMap 2015, the SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in The Netherlands in 2014 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 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 and a nationwide surveillance system for antibiotic use.

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 2015 extends and updates the information of the annual reports since 2003. Since the introduction of a more concise format last year, 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 reader is encouraged to visit www.isis-web.nl for tailored overviews of resistance development.

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Lately, the appearance of highly resistant microorganisms (HRMO's) has received significant attention and has become a significant public health issue. The epidemiological background of these micro-organisms is increasingly complex, as are the challenges to antimicrobial treatment. We therefore provide in a separate chapter a comprehensive overview covering the major trends in antimicrobial resistance, consequences for therapeutic choices and these may serve as a basis for public health policies.
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

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2
Extensive summary

In the Netherlands, several surveillance programs have been developed 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, including conformation 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 2.1 an overview is provided of surveillance programs that are included in Nethmap 2015.

2.1 Most important trends in antimicrobial use

In GPs

- Antibiotic use declined for the third successive year from 11.37 DDD/1000 inhabitants per day in 2011 to 10.54 DDD/1000 inhabitants per day in 2014.
- The use of azithromycin increased whereas the use clarithromycin declined further.
- There was a 13% increase in use of ciprofloxacin which may be related to the decrease in the use of norfloxacin and levofloxacin; overall quinolone use increased 3%.

In nursing homes

- The mean use based on 34 nursing homes was 65 DDD/1000 residents/day but varied widely between 14 and 165 DDD/1000 residents/day.
- The most frequently used antibiotics are combinations of penicillins (mainly amoxicillin with clavulanic acid), with 18.9 DDD/1000 residents/day, nitrofurantoin derivates (13.7 DDD/1000 residents/day) and fluoroquinolones (7.9 DDD/1000 residents/day).

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Table 2.1 Overview of Surveillance programs in the Netherlands.

Surveillance program[1]	Origin of isolates	availability	Sources 2014	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in major pathogens					
SERIN	GP	1996-	42 GP practices from NIVEL	Central testing	Microdilution
ISIS-AR	GP, Hospital, Nursing homes	2008-	33 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-	89% (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-	18 hospitals	(de)central testing	E-test and ribotyping
Azole resistance in *Aspergillus fumigatus*	Hospital	2011-	8 University hospitals	Central testing	EUCAST methodology

[1] *SERIN = Surveillance of Extramural Resistance in The Netherlands; ISIS-AR = Infectious Disease Surveillance Information System on Antibiotic Reistance*

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

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In hospitals

- The in-patient use of antibiotics in 2013 increased from 71.3 DDD/100 patient days in 2012 to 74.7 DDD/100 patient days in 2013.
- Antibiotic use per 100 admissions was 307.8 DDD/100 admissions which is higher than in 2012 (295.7 DDD/100 admissions) but comparable to 2011 (306.4 DDD/100 admissions in 2011).
- After a peak in total use of 1.061 DDD/1000 inhabitants/day in 2010, this value decreased further in 2013 to 0.951 DDD/1000 inhabitants/day.
- Carbapenem use, especially meropenem, was again slightly increased, although, in a European context, it's use is still low. University hospitals account for most of the meropenem use.
- The point prevalence study in 51 hospitals (twice as many as 2013) by the PREZIES network showed that 32% of all admitted patients (N=12,329 patients) received antibiotics, the same figure as last year and the year before. Most often used antibiotics were amoxicillin with clavulanic acid (18%), ciprofloxacin (11%) and cefuroxim (8%).

2.2 Most important trends in antimicrobial resistance

In GPs

- For most antimicrobials, there are no significant shifts in resistance levels since 2010. The exceptions are nitrofurantoin, with slowly rising levels up to 3% in *E. coli* and trimethoprim and co-trimoxazol that show a decrease in resistance, although still between 20-30% for most species. There appears an increase in resistance to fosfomycin and amoxicillin with clavulanic acid 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 microorganisms (HRMO) and multi-drug resistance remained relatively low (< 4%) in all Enterobacteriaceae.
- The Gonococcal Resistance to Antimicrobials Surveillance (GRAS) reported no resistance to ceftriaxone and spectinomycin found.

In hospitals

- Compared to 2010, overall resistance rates for many antimicrobials were similar or slightly lower. The major exception was nitrofurantoin, which is, similar to GP, slightly increasing and 4% in outpatient departments for *E. coli*. A similar trend is observed for fosfomycin and amoxicillin with clavulanic acid in some species.
- The percentage of HRMO was highest among *K. pneumoniae* i.e. 8% (excl. ICU departments) and 11% (ICU) .
- Carbapenem resistance in *P. aeruginosa* increased from 2% to 4% (excl ICU).
- CRE were a rare occurrence in the Netherlands; 0.01% of *E. coli* and 0.15% of *K. pneumoniae* were non-susceptible to carbapenems. OXA-48 was the most prevalent carbapenemase detected.
- The prevalence of MRSA remains low.
- Resistance to vancomycin remained rare in enterococci (<0.5%).
- Resistance to penicillin (<0.5%) in pneumococci was still rare in the Netherlands.
- Resistance to penicillin in *N. meningitidis* was not found in 2014.
- For *C. difficile*, the prevalence of ribotype 027 was stable at 3% and no indications for clinical relevant resistance to metronidazole, vancomycin, and fidaxomicin.
- The overall frequency of azole resistance in *A. fumigatus* in 2014 was 7.2% compared to 7.8% in 2013.

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2.3 Antibiotic use and resistance in the veterinary sector

In 2014 the sales of antimicrobial veterinary medicinal products (207 tonnes) decreased by 4.4%, compared to 2013 (217 tonnes). The total sales decreased from 2009, the index year as defined by the Ministry of Economic Affairs, to 2014 by 58.1%. The policy objective for 2015, a 70% reduction compared to 2009, will therefore be a challenge. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 63%. In most livestock sectors reductions in antibiotic use levelled off in comparison with 2013, except for poultry and dairy cattle. In poultry antibiotic use increased again in 2014, probably as a result of changes in prescription patterns. In dairy cattle a substantial reduction in use was noted and a shift in antibiotic use from 3rd and 2nd choice to 1st choice antibiotics, particularly in dry cow treatment.

- Resistance levels in *S. Typhimurium* isolates from human samples have increased over the years until 2010 after which a constant tendency to decrease was observed until 2013. In 2014 resistance levels for almost all antimicrobials tested stabilized.
- In 2014 the resistance rates seem to have stabilized in *C. jejuni* from broilers and poultry meat. Ciprofloxacin resistance was at a high level and still rising in *Campylobacter* spp. causing infections in human patients (>60%). However, resistance to erythromycin, macrolides being the first choice antibiotic in human infections, was still low. For *Campylobacter* from human patients, resistance levels were higher for travel related infections compared to domestically acquired campylobacteriosis.
- Over the last decade, *Shigella* Toxin producing *E. coli* (STEC) isolates show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim. Resistance to the quinolones (ciprofloxacin and nalidixic acid) decreased in 2014. As in the former four years, no ESBL-producing isolates were detected
- In most animal species the resistance levels of indicator *E. coli* from fecal samples stabilized in 2014. This may reflect the use patterns of antibiotics in the different livestock species. In isolates from broiler meat, beef and pork, resistance showed a tendency to decrease.
- The decrease in cefotaxime resistant *E. coli* from 2008 – 2013, has levelled off in 2014. The prevalence of livestock being positive for ESBL/AmpC producing *E. coli* in the faeces was 67% in broilers, 34% in laying hens, 18% in slaughter pigs, 23% in white veal calves, 14% in rosé veal calves and 9% in dairy cows. Poultry meat was most frequently contaminated (67%), which was slightly lower than found in former years (83% in 2013 and 73% in 2012).
- The dominant human ESBL-gene ($bla_{CTX\text{-}M\text{-}15}$) was more frequently found in animals or their products. This is an unwanted development that warrants extra attention in the surveillance in food-animal sources. In 2014 in 1601 fecal samples from broilers, veal calves, slaughter pigs and dairy cows no carbapenemase-producing Enterobacteriaceae were detected.

These findings indicate that reductions in the total quantity of antibiotics used in the Netherlands and in 3rd and 4th generation cephalosporins are associated with a reduction of the general levels of antimicrobial resistance and the levels of ESBLs. These associations are indicative of a direct causal association between usage of antibiotics and antimicrobial resistance. This view is supported by the current levelling off in antibiotic use directly followed by a stabilization of resistance levels. This may warrant a re-evaluation of the current targets for antibiotic use in relation to targets for antimicrobial resistance in animals and food thereof.

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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 2014 did not increase further for most antibiotics. Yet, there is a continuing concern. For some microorganisms where resistance rates are apparently similar over the last years, a MIC creep is observed since last year below the clinical breakpoint, indicating that resistance may appear in the coming years. Although resistance has not increased further, empiric (mono) therapy for some of these agents is now unjustified in the severely ill patient for many of the antibiotics that were long considered as first line of treatment. Routine culturing with antibiograms remains mandatory to tailor therapy to the individual patient. If broad spectrum therapy is initially chosen, antibiograms should be used to narrow down antimicrobial therapy to prevent even further emergence of resistance and culture repeated if indicated. It should be realized that the resistance rates reported are for one isolate per patient, and only the first one, and that resistance in the individual patient, especially those that stay longer in the hospital, is significantly higher than reported here. 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 make these agents less suitable for empirical treatment in UTI both in children and adults. However, resistance to trimethoprim and co-trimoxazole has been decreasing for several years and is increasingly an option.
- The best suitable treatment options for uncomplicated UTI are still nitrofurantoin (3% resistance in *E. coli*, and slowly increasing) and fosfomycin (1% resistance in *E. coli*, but >30% in *K. pneumoniae*).
- Resistance to amoxicillin with clavulanic acid was ≥ 10% in *Enterobactereaceae* indicating that care should be taken with empirical treatment without further diagnostic work-up.
- The results indicate sampling for antimicrobial susceptibility testing becomes increasingly important in the treatment of UTI.

In hospitals

Outpatient departments

- Except for nitrofurantoin and fosfomycin, high levels of resistance preclude empirical treatment with oral agents for UTI; culture and tailored therapy are necessary.
- Resistance rates in the three major species are comparable to, or slightly higher than in GP patients, thus the treatment strategies will be largely similar for these species
- The species distribution in UTI in outpatient departments is significantly different from that of GP's reflecting more complicated patients. The resistance rates differ significantly by species and cultures are required for tailored therapy.

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Unselected hospital patient departments

- High levels of resistance to amoxicillin, amoxicillin with clavulanic acid, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections. The ciprofloxacin resistance rate of 17% in *E.coli* has further increased and is especially worrisome.
- Piperacillin/tazobatam, 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.

Intensive care patients

- There are no significantly important shifts in 2014. High levels of resistance to amoxicillin, amoxicillin with clavulanic acid, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections. The ciprofloxacin resistance rate of 13% in *E.coli* is similar to 2013.
- There are significant differences in resistance 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 between 1 and 5%. 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.

2.5 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. At the European level there has been a significant increasing trend in the percentages of *K. pneumoniae* resistant to fluoroquinolones, third-generation cephalosporins and aminoglycosides, as well as combined resistance to all three antibiotic groups over the last years. During the same period, resistance to third-generation cephalosporins increased significantly. The most worrying however, is the increase in the percentage of carbapenem resistance in *K. pneumoniae* which causes serious concern and a threat to patient safety in Europe.

In the Netherlands, with a few exceptions, no major shifts in resistance rates have occurred over the last five years. The resistance rates in 2014 did not increase further for most antibiotics. Yet, there is a continuing concern. For some microorganisms where resistance rates are apparently similar over the last years, an MIC creep is observed since last year below the clinical breakpoint, indicating that resistance may appear in the coming years.

The current measures to control the increase in antimicrobial resistance follow the perspective of human medicine: prudent antibiotic use, and screening and isolation of (hospitalised) patients at-risk.

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However, introductions of resistant bacteria from abroad, from livestock, from the environment and from the general population play a role in the spread of resistance. Consequently, a much wider variety of control measures to reduce the population at risk is needed.
To take adequate interventions to control this spread, harmonized and integrated surveillance 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. To achieve this, intensive collaboration between professionals in the private and public domain in both human and veterinary health care will be necessary.

Conclusions

The data presented in NethMap 2015 demonstrate that the continuing shifts in patterns of antibiotic use and resistance require a rethinking of antimicrobial use and policy, including restricted use of some classes of antibiotics, in particular those that are employed as a last line of defense. To control the increase and spread of antibiotic resistance, trends in resistance and antibiotic use should be carefully monitored to allow intervention if necessary.

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3
Use of Antimicrobials

Introduction

In this chapter the use of antimicrobials over the past decade is reported. First, extramural antibiotic use from 2005 until 2014 is presented, including total antibiotic use, as well as use of subgroups and individual antibiotics. Second, antibiotic use in hospital care from 2004 until 2013 is reported using several measures: DDD/100 patient days, DDD/100 admissions, as well as in DDD/1000 inhabitant days (DID). Third, antibiotic use data from the point prevalence study of the PREZIES network are reported. Finally, we report data of antibiotic use in nursing homes in the Netherlands. For the first time, we present also point prevalence data on antibiotic use in nursing homes, collected by PREZIES.

3.1 Primary care

Methods

Dutch data of 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 data from 90% of the Dutch community pharmacies (serving 91.5% of the Dutch population) and extrapolates the data to 100%. Data are presented as DDD per 1000 inhabitants per day (DID).

Results

Compared to 2013, total community antibiotic use in 2014 showed a small decrease of 0.27 DID to 10.54 DID. After years of increases in antibiotic use from 10.51 in 2005 to 11.37 DID in 2011, total use declined for the third successive year (Table 3.1).

Broken down by groups of antibiotics, decreases were seen for the use of amoxicillin with clavulanic acid (8% to 1.55 DID), for macrolides (4% to 1.22 DID), for amoxicillin and for tetracyclines (mainly doxycycline). Of the macrolides, use of azithromycin increased to 0.73 DID, whereas the use of

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Table 3.1 Ten years data on the use of antibiotics for systemic use (J01) in primary care (DDD/1000 inhabitant-days), 2005-2014 (Source: SFK).

ATC Group*	Therapeutic group	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	2.41	2.37	2.57	2.66	2.67	2.67	2.60	2.49	2.33	2.23
J01CA	Penicillins with extended spectrum	1.86	1.87	1.91	1.91	1.89	1.81	1.91	1.94	1.99	1.94
J01CE	Beta-lactamase sensitive penicillins	0.44	0.50	0.46	0.42	0.39	0.37	0.35	0.33	0.31	0.30
J01CF	Beta-lactamase resistant penicillins	0.29	0.31	0.32	0.36	0.38	0.38	0.39	0.41	0.41	0.44
J01CR	Penicillins + beta-lactamase-inhibitors	1.50	1.59	1.66	1.71	1.74	1.80	1.82	1.82	1.67	1.55
J01D	Cephalosporins	0.05	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04
J01EA	Trimethoprim and derivatives	0.25	0.23	0.22	0.21	0.21	0.20	0.20	0.19	0.17	0.16
J01EC	Intermediate-acting sulphonamides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01EE	Sulphonamides + trimethoprim	0.38	0.37	0.36	0.36	0.35	0.35	0.34	0.33	0.29	0.28
J01FA	Macrolides	1.42	1.39	1.39	1.36	1.33	1.31	1.34	1.34	1.22	1.18
J01FF	Lincosamides	0.08	0.09	0.10	0.11	0.12	0.14	0.15	0.16	0.17	0.18
J01GB	Aminoglycosides	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03
J01MA	Fluoroquinolones	0.84	0.87	0.91	0.89	0.86	0.85	0.82	0.80	0.76	0.79
J01MB	Other quinolones	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.00
J01XB	Polymyxins	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
J01XE	Nitrofuran derivatives	0.90	1.00	1.07	1.13	1.17	1.23	1.31	1.38	1.37	1.40
J01XX05	Methenamine	0.02	0.03	0.03	0.02	0.03	0.04	0.03	0.04	0.03	0.03
J01	**Antibiotics for systemic use (total)**	**10.51**	**10.73**	**11.10**	**11.24**	**11.21**	**11.23**	**11.37**	**11.34**	**10.81**	**10.54**

* *From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

clarithromycin further declined to 0.38 DID. Use of ciprofloxacin increased by 13% compared to 2013. Use of nitrofurantoin is still increasing in the Netherlands.

Discussion

The positive news is that after years of increase in antibiotic use until 2011, now for the third year a slight decrease was seen in overall use of antibiotics in the Dutch community. Nevertheless, it has to be mentioned that use of three specific antibiotics increased: azithromycin, ciprofloxacin and nitrofurantoin. For azithromycin, this is partly due to shifts in specific antibiotics used within the subgroup of macrolides, but the 13% increase in use of ciprofloxacin needs further assessment. Also of interest is the steadily increasing use of nitrofurantoin.

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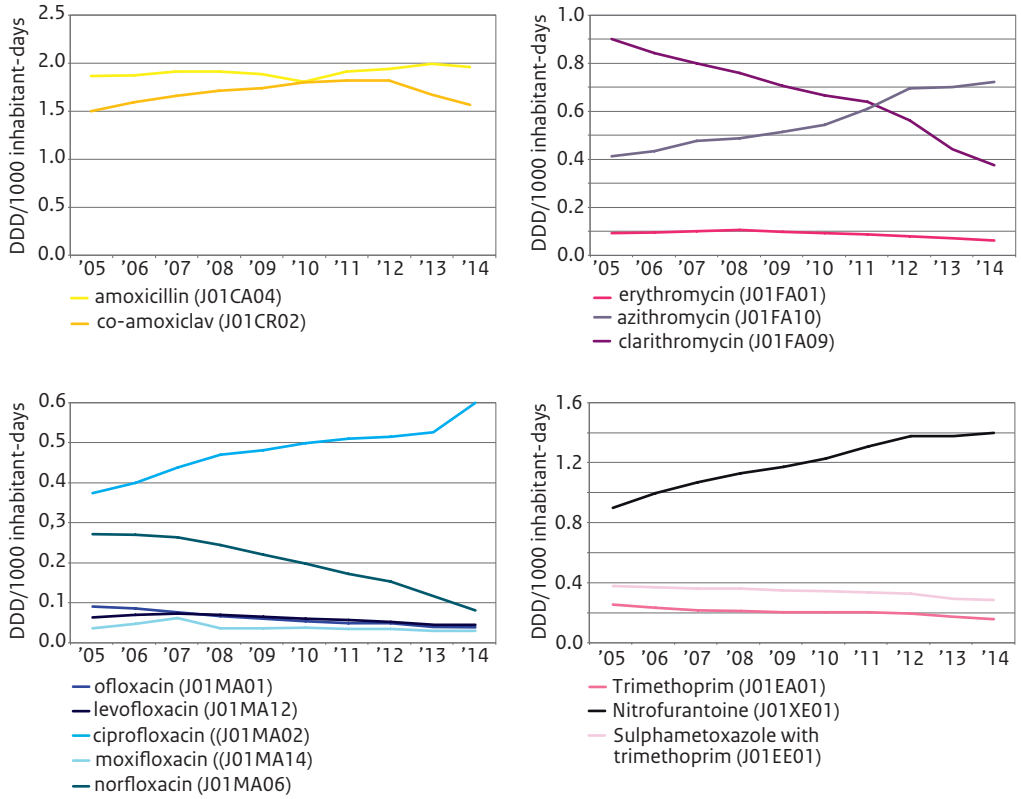
Figure 3.1 a-d Use of antibiotics for systemic use in primary health care, 2005-2014 (Source:SFK).



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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

Compared to 2012, the in-patient use of antibiotics in 2013 increased from 71.3 DDD/100 patient-days to 74.7 DDD/100 patient-days (Table 3.2). From 2004 to 2009, there was a steady increase in total use from 52 to about 71 DDD/100 patient-days. Between 2009 and 2012, use remained about stable around 71 DDD/100 patient-days. Antibiotic use per 100 admissions also increased to 307.8 DDD/100 admissions, after years of declines to a minimum of 295.7 DDD/100 admissions in 2012.

Broken down by hospital category, university hospitals used the least antibiotics (72.5 DDD/100 patient-days), whereas large teaching hospitals the most (76.0 DDD/100 patient-days). General hospitals used 74.8 DDD/100 patient-days on average. With respect to the ATC-4 level figure 3.2 shows the distribution of use per antibiotic subgroup for these different types of hospitals in 2013. Notable is the large difference in the relative use of combinations of penicillins (mainly amoxicillin with clavulanic acid) between university hospitals (15.2%), large teaching hospitals (17.9%) and general hospitals (24.0%). Most carbapenems and glycopeptides were used in university hospitals and relatively more nitrofuran derivates in general hospitals. Large teaching hospitals were the highest users of cephalosporins.

The increase in antibiotic use in 2013 is mainly due to a substantial increase in the use of cephalosporins (Fig. 3.3 and 3.4), with an increase, compared with 2012, of 2.0% for first-generation (cefalexin, cefalotin and cefazolin were used). A higher increase was seen on second- and third-generation cephalosporins

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Table 3.2 Ten years use of antibiotics for systemic use (J01) in hospitals, 2004-2013 (Source: SWAB).

ATC Group*	Therapeutic group	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	1.5	1.6	1.6	1.4	1.7	1.6	1.7	1.8	1.7	1.7
J01CA	Penicillins with extended spectrum	6.0	6.7	7.6	7.3	6.5	7.6	7.3	7.3	7.6	8.0
J01CE	Beta-lactamase sensitive penicillins	1.4	1.4	1.4	1.2	1.3	1.6	1.5	1.5	1.7	1.9
J01CF	Beta-lactamase resistant penicillins	5.7	5.8	5.9	5.7	6.4	6.6	6.8	6.7	7.1	8.1
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	12.8	13.9	15.1	14.5	16.2	16.5	16.0	15.8	15.0	14.8
J01DB -DE	Cephalosporins	7.0	7.4	8.4	8.4	8.8	10.1	10.2	11.1	12.1	13.4
J01DF	Monobactams	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01DH	Carbapenems	0.5	0.6	0.6	0.8	1.0	1.1	1.2	1.4	1.5	1.7
J01EA	Trimethoprim and derivatives	0.4	0.6	0.8	0.5	0.4	0.4	0.5	0.4	0.3	0.3
J01EC	Intermediate-acting sulfonamides	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.1	2.3	2.1	2.3	2.4	2.0	2.0	1.9	1.8	1.9
J01FA	Macrolides	2.3	2.8	2.5	2.8	2.7	2.6	2.7	2.9	2.8	2.6
J01FF	Lincosamides	1.8	1.9	2.0	2.1	2.1	2.4	2.3	2.3	2.2	2.3
J01GB	Aminoglycosides	2.2	2.6	2.5	2.6	3.9	4.2	4.1	3.9	3.3	3.5
J01MA	Fluoroquinolones	6.5	7.3	8.0	7.6	8.8	9.3	9.0	9.2	8.9	8.6
J01MB	Other quinolones	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
J01XA	Glycopeptides	0.6	0.8	0.7	1.0	1.1	1.3	1.3	1.3	1.4	1.5
J01XB	Polymyxins	0.1	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.2
J01XC	Steroid antibac-terials (fusidic acid)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
J01XD	Imidazole derivatives	1.7	1.5	1.7	1.8	1.7	1.8	1.9	2.2	2.3	2.6
J01XE	Nitrofuran derivatives	0.9	1.0	1.0	1.1	1.2	1.1	1.2	1.2	1.2	1.3
J01XX05	Methenamine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
J01	**Antibiotics for systemic use (total)**	**53.7**	**58.5**	**62.2**	**61.6**	**66.8**	**70.9**	**70.2**	**71.3**	**71.3**	**74.7**
	expressed in DDD/100 patiënt days										
J01	**Antibiotics for systemic use (total)**	**306.8**	**316.9**	**335.9**	**337.5**	**344.7**	**321.3**	**315.9**	**306.4**	**295.7**	**307.8**
	expressed in DDD/100 admissions										

* *From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

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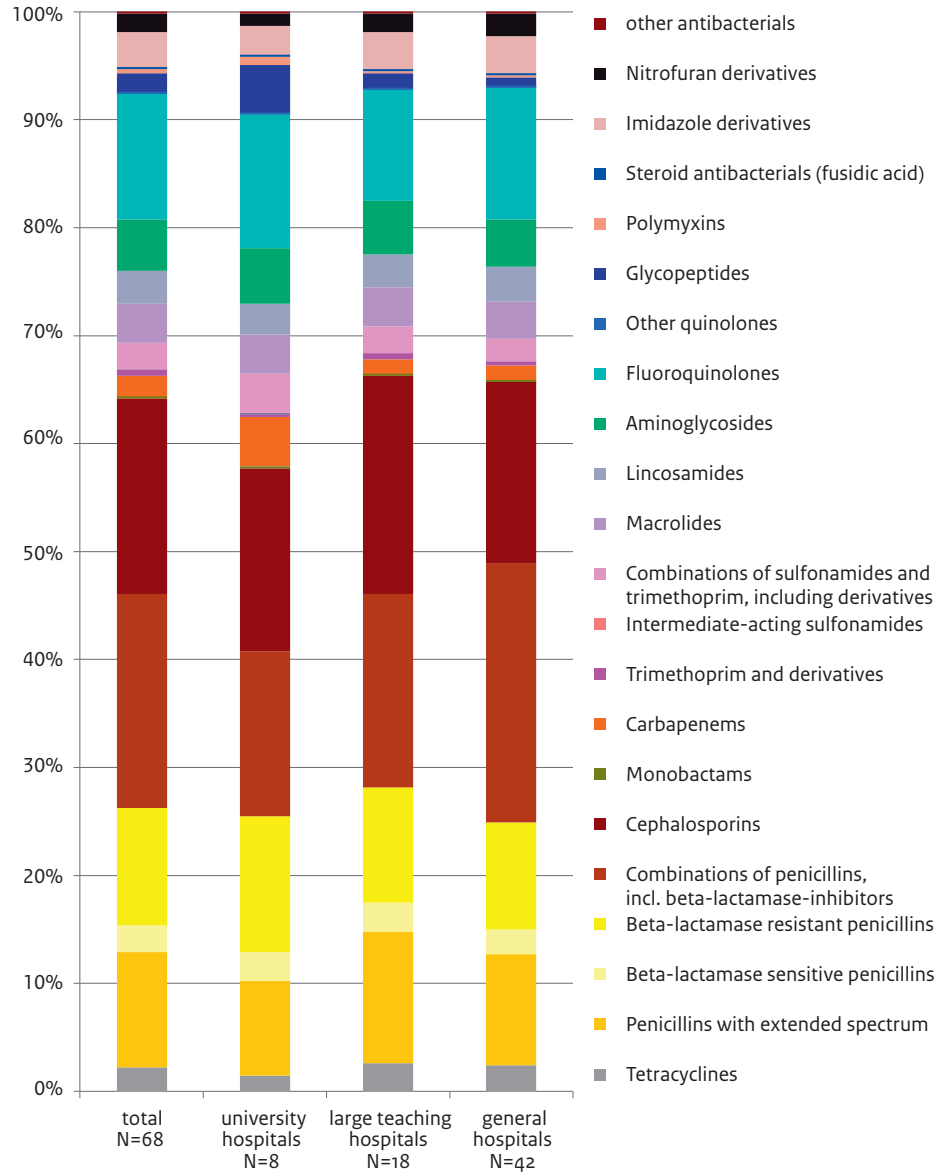
Figure 3.2 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2013 (Source:SWAB)



respectively 14.7% for second- and 15.3% for third-generation cephalosporins when measured in DDD/100 patient days. Use of second-generation cephalosporins consisted of: cefoxitin, cefuroxime, cefamandole and cefaclor, third-generation cephalosporins included use of: cefotaxime, ceftazidime,

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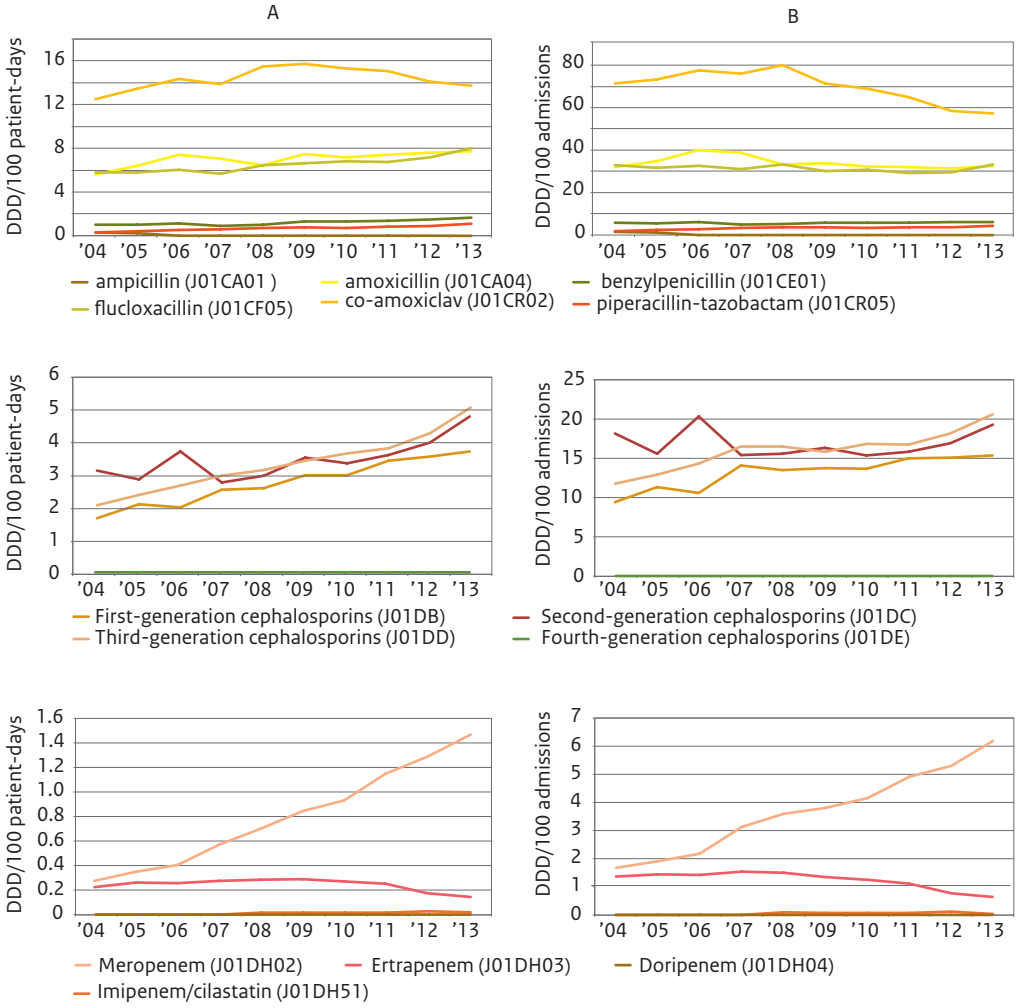
Figure 3.3 Use of beta-lactams in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2004-2013 (Source:SWAB).



ceftriaxone, cefixime and ceftibuten. University hospitals use much more third-generation cephalosporins than first- and second-generation ones, while in general hospitals, the use is evenly distributed among the three categories of cephalosporins (figure 3.5). However, in large teaching hospitals, use of second generation cephalosporins is increasing, while the first and third generation ones are decreasing.

Besides the cephalosporins there are some other antimicrobials with an increased use in 2013. Flucloxacillin shows a substantial increase to 7.9 DDD/100 patient-days. Meropenem use further

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Figure 3.4 Use of macrolides, aminoglycoside, fluoroquinolones and glycopeptides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2004-2013 (Source:SWAB).

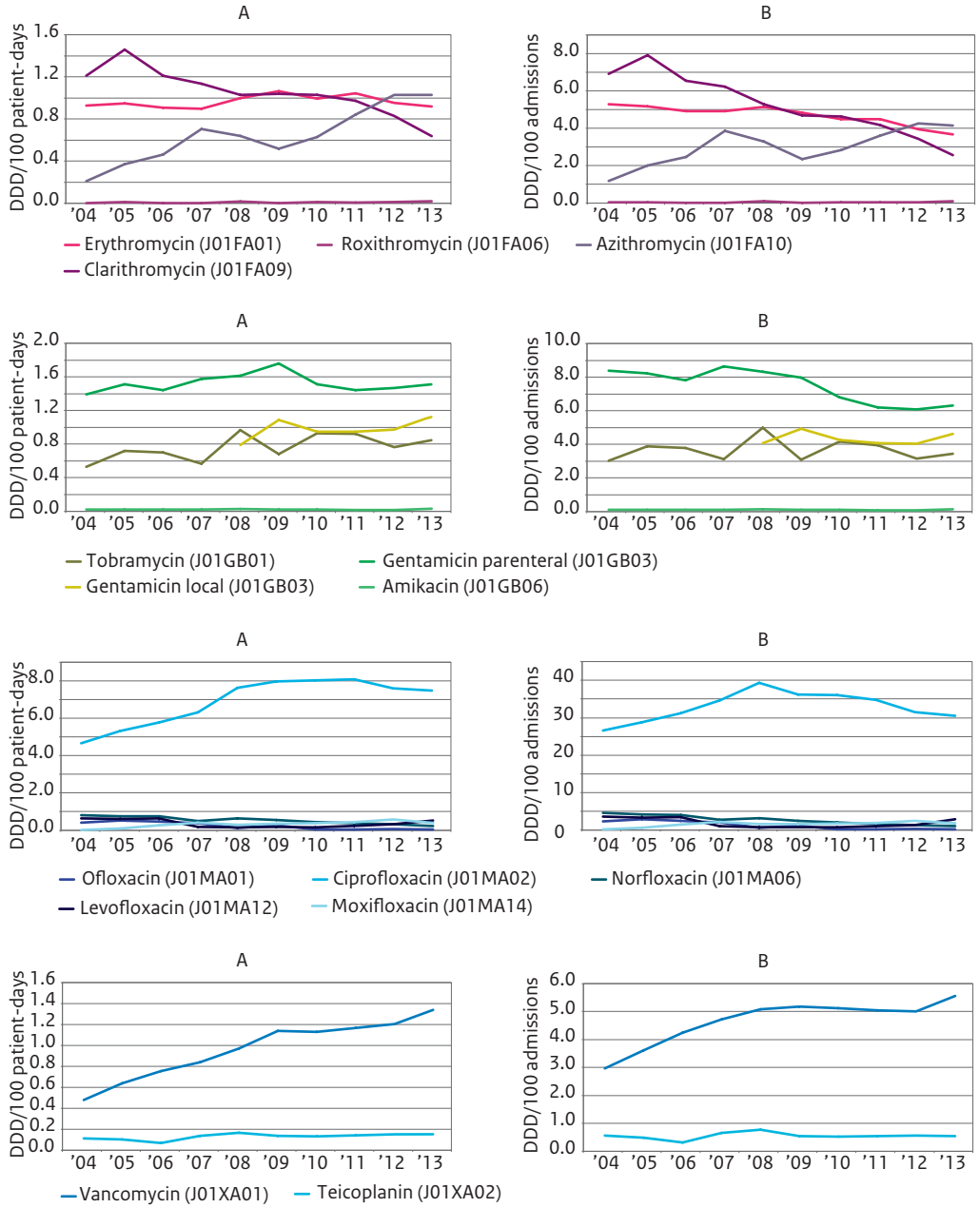

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Table 3.3 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1000 inhabitant-days), 2004-2013 (Source: SWAB).

ATC Group	Therapeutic group	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	0.025	0.027	0.027	0.025	0.023	0.025	0.027	0.026	0.024	0.022
J01CA	Penicillins with extended spectrum	0.093	0.106	0.113	0.110	0.101	0.111	0.110	0.103	0.100	0.099
J01CE	Beta-lactamase sensitive penicillins	0.019	0.021	0.022	0.020	0.019	0.023	0.023	0.020	0.023	0.023
J01CF	Beta-lactamase resistant penicillins	0.080	0.089	0.091	0.087	0.086	0.093	0.097	0.089	0.093	0.100
J01CR	Penicillins + beta-lactamase-inhibitors	0.212	0.231	0.239	0.233	0.229	0.241	0.256	0.223	0.211	0.199
J01DB-DE	Cephalosporins	0.103	0.121	0.127	0.124	0.118	0.137	0.147	0.145	0.158	0.164
J01DF	Monobactams	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J01DH	Carbapenems	0.006	0.008	0.009	0.010	0.011	0.014	0.015	0.018	0.019	0.020
J01EA	Trimethoprim and derivatives	0.008	0.009	0.009	0.009	0.007	0.007	0.009	0.006	0.005	0.004
J01EC	Intermediate-acting sulphonamides	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.000
J01EE	Sulphonamides + trimethoprim	0.032	0.035	0.034	0.033	0.029	0.030	0.030	0.026	0.024	0.024
J01FA	Macrolides	0.036	0.042	0.040	0.040	0.037	0.039	0.041	0.037	0.038	0.034
J01FF	Lincosamides	0.027	0.030	0.031	0.031	0.029	0.033	0.035	0.032	0.031	0.032
J01GB	Aminoglycosides	0.031	0.038	0.039	0.041	0.048	0.055	0.058	0.054	0.044	0.045
J01MA	Fluoroquinolones	0.104	0.115	0.121	0.124	0.139	0.129	0.138	0.127	0.124	0.116
J01MB	Other quinolones	0.002	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000
J01XB	Polymyxins	0.002	0.005	0.005	0.006	0.008	0.009	0.006	0.003	0.002	0.003
J01XE	Nitrofuran derivatives	0.014	0.017	0.016	0.018	0.016	0.017	0.018	0.015	0.018	0.016
J01XX05	Methenamine	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.000
J01XX08	Linezolid	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001
	other antibiotics	0.032	0.035	0.038	0.039	0.038	0.043	0.048	0.045	0.047	0.049
J01	**Antibiotics for systemic use (total)**	**0.827**	**0.931**	**0.965**	**0.952**	**0.941**	**1.008**	**1.061**	**0.971**	**0.963**	**0.951**

increased to 1.5 DDD/100 patient-days in 2013. University hospitals account for most of the meropenem use with 2.9 DDD/100 patient-days compared to 1.1 and 1.0 DDD/100 patient-days in large teaching and general hospitals respectively (figure 3.5). Finally use of aminoglycosides as well as glycopeptides overall showed a small increase in 2013. Large teaching and general hospitals show a higher use of gentamicin than university hospitals (figure 3.5), whereas glycopeptides were preferably used in university hospitals with 2.6 DDD/100 patient-days, compared to about 1 DDD/100 patient-days in large teaching and general hospitals.

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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, 2004-2013 (Source: SWAB)

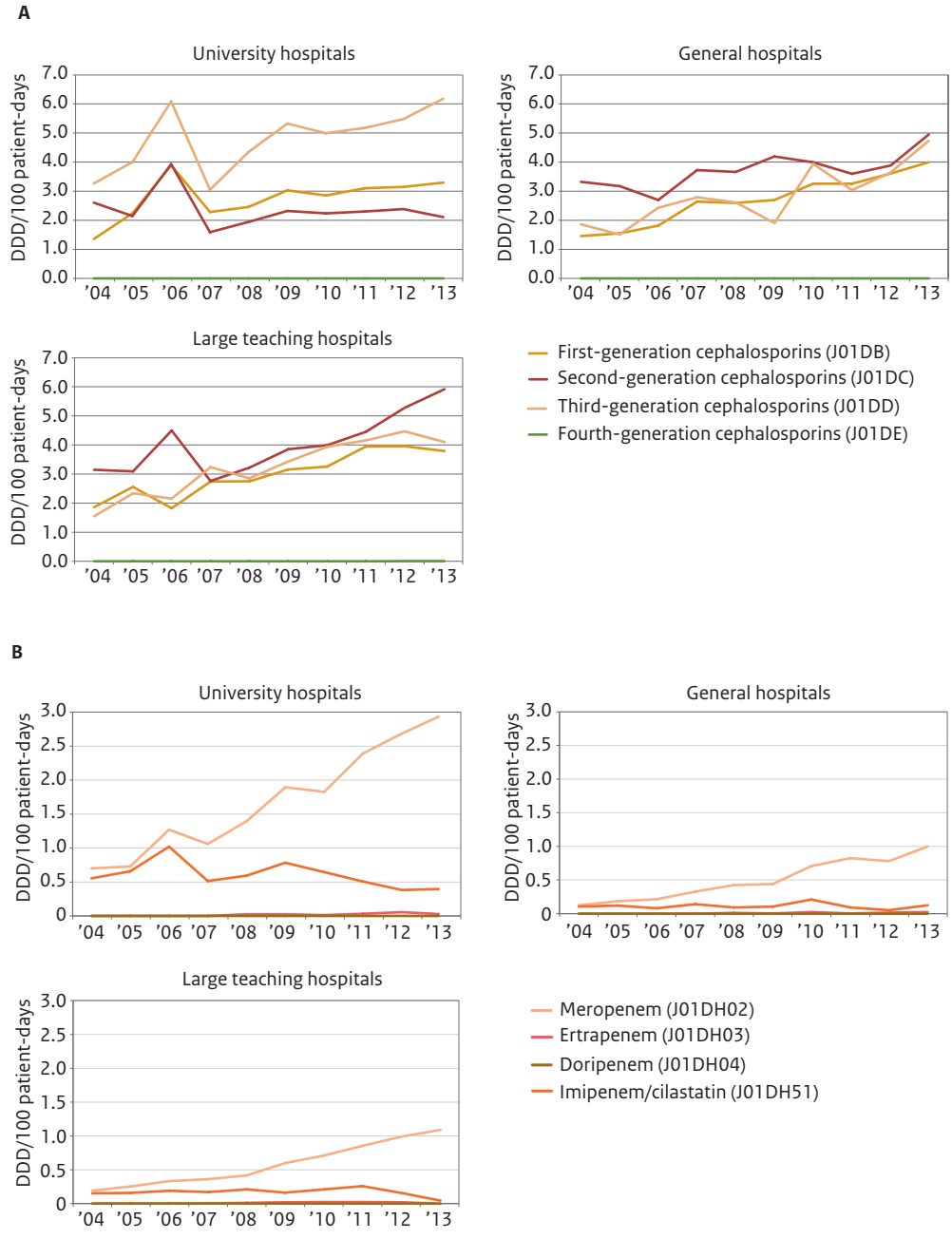

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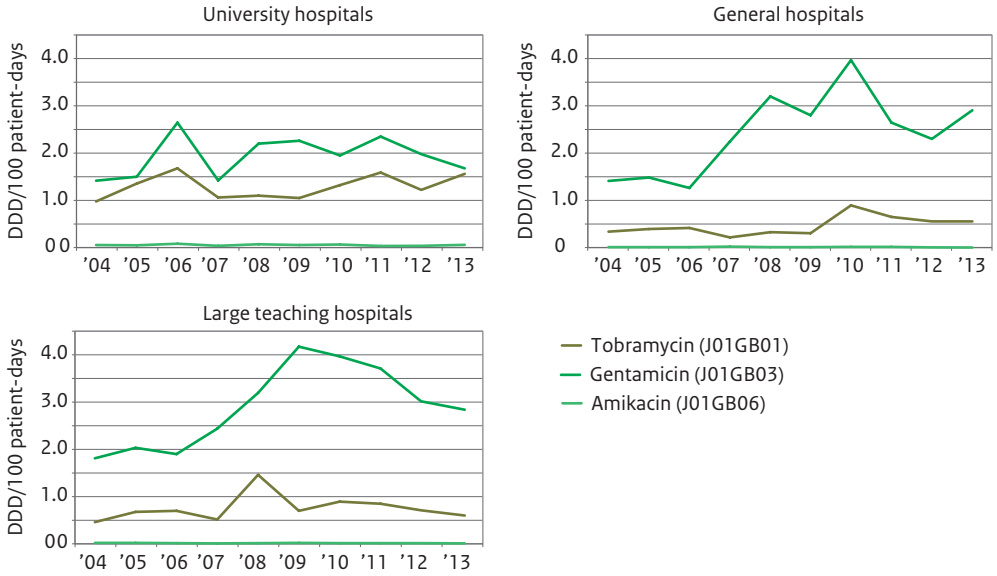
Figure 3.5 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2004-2013 (Source: SWAB)

C



D

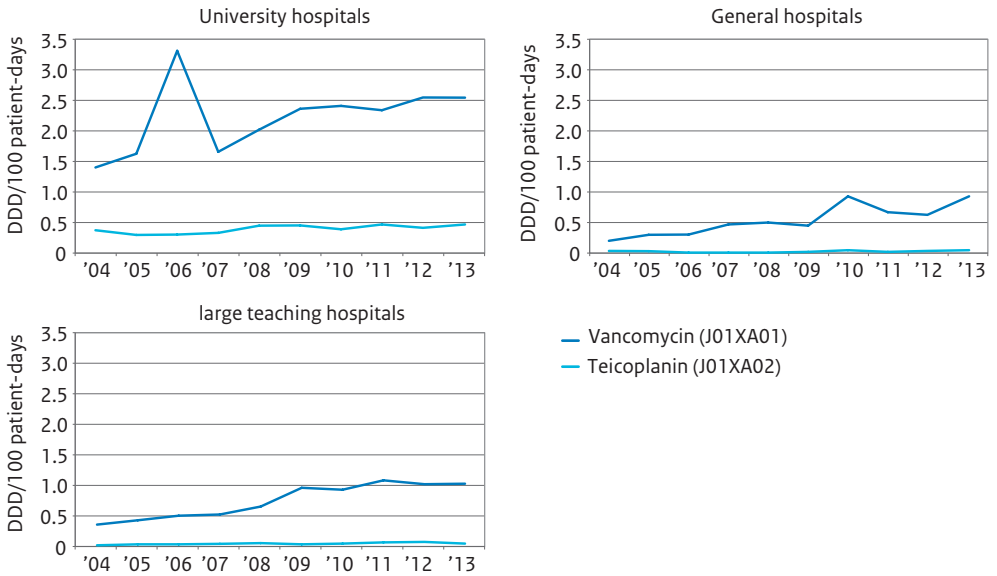


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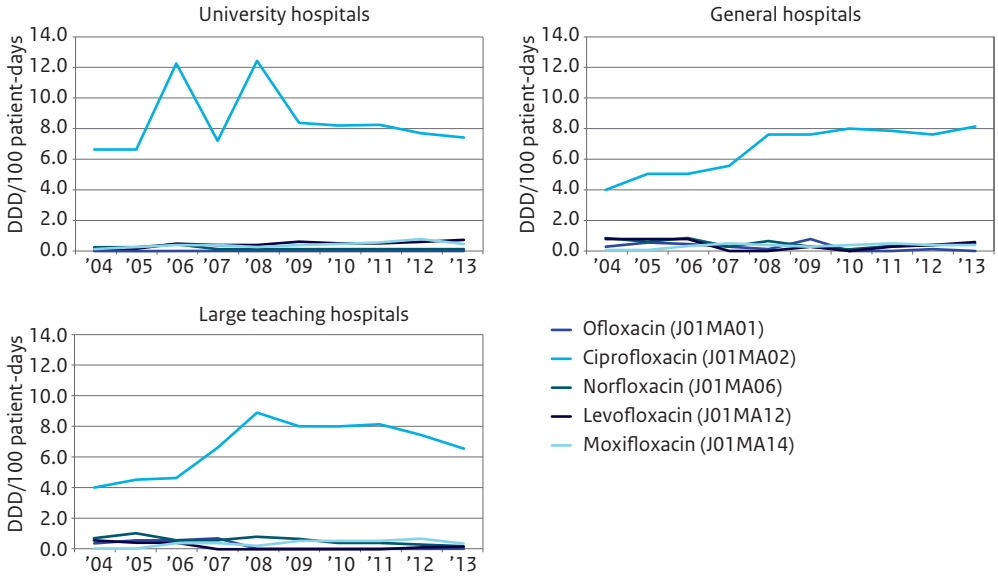
Figure 3.5 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2004-2013 (Source: SWAB)

E



A decrease in use in 2013 is seen for ciprofloxacin use, which decreased further by 1.8% compared to 2012, as well as the use of amoxicillin with clavulanic acid which declines from 14.1 in 2012 to 13.8 DDD/100 patient-days in 2013.

Over 75% of the antimycotics (J02), antimycobacterials (J04) and antivirals (J05) for systemic use were used in university hospitals. In table 3.4 use of J02, J04 and J05 in university hospitals is presented from 2007 until 2013, expressed in DDD/100 patient-days. The use of antimycotics increased in 2013 compared to 2012, mainly because of an increased use of amfothericin B. Use of antimycobacterials increased to 2.88 DDD/100 patient-days, caused by an increased use of rifampicin. Use of antivirals remained stable at 5.47 DDD/100 patient-days in 2013.

In 2014 PREZIES data were received from fifty one hospitals (twice as much as in 2013), including 12329 patients of which 3988 received antibiotics, with a total of 5302 prescriptions (2760 for community acquired infections, 681 for nosocomial infections, 674 for medical prophylaxis, 503 for surgical prophylaxis and 547 for other or unknown indications). Antibiotic use for these indications is depicted in figure 3.6. Most often used antibiotics were amoxicillin with clavulanic acid (18%), ciprofloxacin (11%) and cefuroxim (8%). Cefazolin was used in 54% cases of surgical prophylaxis. Use for medical prophylaxis was more diverse, ciprofloxacin was most often used (13%), followed by amoxicillin with clavulanic acid and trimethoprim/sulfamethoxazole.

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Table 3.4 Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2007-2013 (Source: SWAB).

ATC Group *	Therapeutic group	2007	2008	2009	2010	2011	2012	2013
J02AA01	Antibiotics (amphotericin B)	4.44	1.12	1.35	1.65	1.77	2.43	3.01
J02AB02	Imidazole derivatives (ketoconazole)	0.12	0.11	0.08	0.15	0.09	0.10	0.06
J02AC	Triazole derivatives	5.18	6.36	6.72	6.31	5.83	6.25	6.29
J02AX	Other antimycotics for systemic use	0.19	0.40	0.61	0.56	0.57	0.55	0.71
J02	**Antimycotics for systemic use (total)**	**9.93**	**7.98**	**8.77**	**8.66**	**8.26**	**9.33**	**10.06**
J04AA	Aminosalicylic acid and derivatives	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
J04AC	Hydrazides (mainly isoniazide)	0.39	0.29	0.40	0.34	0.30	0.40	0.57
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.00	0.01	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
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.00	0.01	0.02
J04BA	Drug for treatment of leprosy (dapson)	0.53	0.39	0.33	0.45	0.49	0.62	0.70
J04	**Antimycobacterials for systemic use (total)**	**2.74**	**2.33**	**2.35**	**2.58**	**2.62**	**2.57**	**2.88**
J05AB	Nucleosides excl. Reverse transcriptase inhibitors (J05AB)	1.72	2.00	2.22	2.02	2.18	2.24	2.33
J05AD	Phosphonic acid derivatives (J05AD)	0.06	0.11	0.13	0.10	0.10	0.15	0.12
J05AE	Protease inhibitors (J05AE)	0.70	0.92	0.75	0.78	0.55	0.81	0.63
J05AF	Nucleoside reverse transcriptase inhibitors (J05AF)	0.83	0.74	0.64	0.67	0.63	0.69	0.54
J05AG	Non-nucleoside reverse transcriptase inhibitors (J05AG)	0.20	0.25	0.23	0.22	0.14	0.18	0.16
J05AH	Neuraminidase inhibitors (J05AH)	0.02	0.05	n.a.#	0.21	0.42	0.19	0.49
J05AR	Antivirals for the treatment of HIV, combinations (J05AR)	0.33	0.52	0.55	0.76	0.69	0.91	0.89
J05AX	Other antivirals (J05AX)	0.00	0.06	0.06	0.15	0.17	0.24	0.29
J05	**Antivirals for systemic use (total)**	**3.86**	**4.65**	**4.59**	**4.91**	**4.89**	**5.41**	**5.47**

* *From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

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

Discussion

Compared with 2012 we seen an intensification of antibiotic use in hospitals and in individual patients, as antibiotics use increased by almost 5% when measured in DDD/100 patient-days and 4% when expressed in DDD/100 admissions.

There are marked shifts between different subgroups of antibiotics. Increased use of 3rd-generation-cephalosporins and meropenem is of particular interest, even though, in a European context, it's use is still low. As illustrated in the results there are marked differences between the three types of hospitals. Most remarkable is the difference in use of the cephalosporins. University hospitals have a higher use

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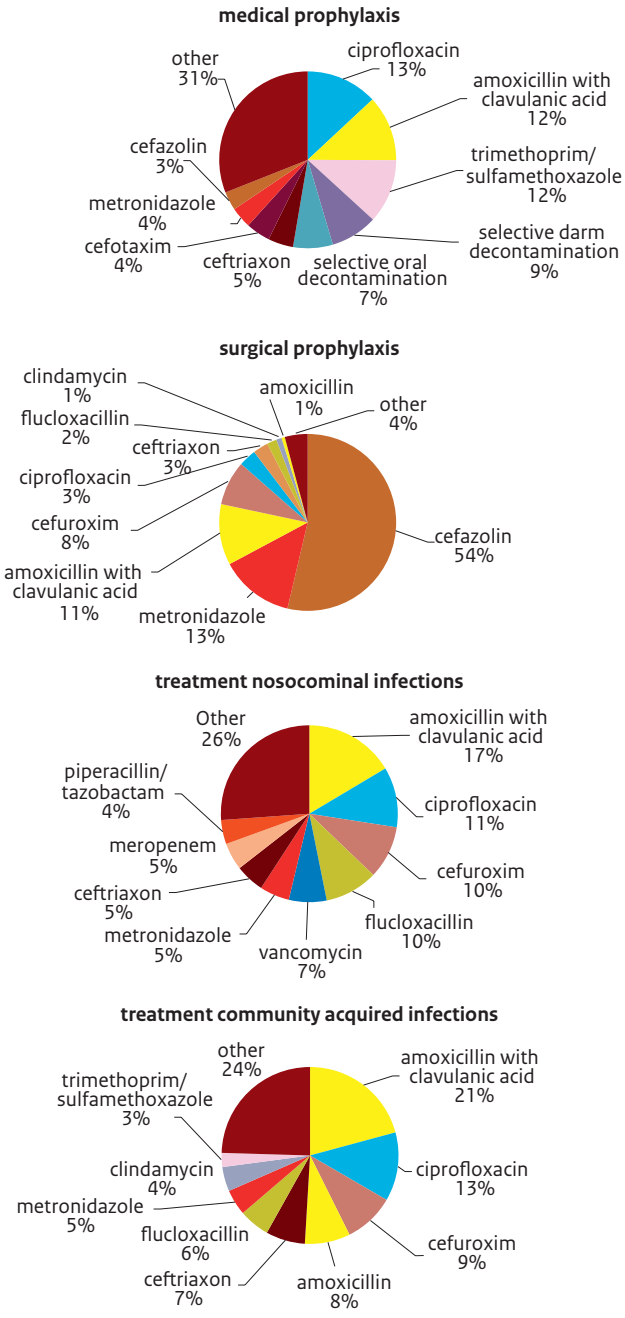
Figure 3.6 Distribution of the use of antibiotics for systemic use (J01) ; results of the point-prevalence studies 2014 (Source: PREZIES)



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of third-generation cephalosporins, whereas large teaching hospitals, use more second-generation cephalosporins. In general hospitals, use is evenly distributed between the three groups of cephalosporins. The high use of third-generation cephalosporins can partly 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.

The use of broad spectrum antibiotics deserves special attention. Meropenem use is still increasing, with much higher use in University hospitals compared to the other types of hospitals. The decrease in ciprofloxacin use is mainly caused by a decrease in large teaching hospitals. From a number of these hospitals, it is known that they initiated a campaign to limit quinolone use. Fluoroquinolone use now is highest in general hospitals, which is potentially worrisome.

Other remarkable changes are the increased use of metronidazol and rifampicine. The latter one probably for the treatment of bone and joint infections in combination with flucloxacillin. Metronidazol in combination with a second-generation cephalosporin is widely used as prophylactic regimen in large teaching hospitals.

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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. Data from 34 nursing homes were received. The size of these homes varied from 15 to 821 residents per home, with a mean of 250 residents. In total, the antibiotic use of 8499 residents was included. For each nursing home the amount of DDD/1000 residents/day was calculated, and their weighed mean was calculated.

In nursing homes a PREZIES prevalence study was performed according the same method as described in the intramural methods.

Results

This year we received data from 34 nursing homes, an increase compared with 25 in 2012. The use of antibiotics varied hugely for the different nursing homes with a minimum of 14 and a maximum of 131 DDD/1000 residents/day. The mean use was 65 DDD/1000 residents/day. Combinations of penicillins (mainly amoxicillin with clavulanic acid), with 18.9 DDD/1000 residents/day, nitrofurantoin derivates (13.7 DDD/1000 residents/day) and fluoroquinolones (7.9 DDD/1000 residents/day) were most frequently used (Table 3.5).

Figure 3.7 depicts antibiotics used in the PREZIES prevalence study in nursing homes. We received data from 45 nursing homes. A total of 5679 residents were participating, 1755 men and 4015 women of which 257 patients with an infection, with a total of 263 prescriptions. Prescriptions of an antibiotic used ten times or more are depicted in figure 3.7. Leader by far is nitrofurantoin (31% of the total antibiotic use), followed by amoxicillin with clavulanic acid and ciprofloxacin.

Discussion

Compared with previous years, more or less the same pattern of usage is seen. The most frequently used antibiotic is amoxicillin with clavulanic acid (29 %), followed by nitrofurantoin (21%) and fluoroquinolones (12%).

Notable is the relatively lower use of tetracyclines (11%) compared to the use in primary care. The high use of nitrofurantoin is not surprising, as urinary tract infections are common 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. Nursing homes provide a significant service and more information should be available in order to optimise antimicrobial use and limit the development of antimicrobial resistance.

PREZIES data on nursing homes are reported for the first time. The results of the point prevalence study show a somewhat different pattern of usage compared with the SWAB surveillance data, with nitrofurantoin as most frequently prescribed antibiotic. PREZIES data are based on prescriptions on an index day, whereas overall use is based on DDD's collected over 365 days.

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Figure 3.7 Distribution of the use of antibiotics for systemic use (J01) in nursing homes; results of the point-prevalence studies 2014 (Source: PREZIES)

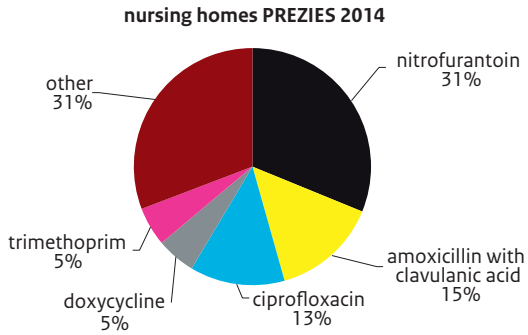

Table 3.5 Distribution of the use of antibiotics (J01) in nursing homes, expressed as DDD/1000 residents/day, 2011-2013 (Source: SWAB).

ATC Group	Therapeutic group	2011	2012	2013
J01AA	Tetracyclines	5.42	6.82	7.22
J01CA	Penicillins with extended spectrum	4.87	6.61	4.98
J01CE	Beta-lactamase sensitive penicillins	0.33	0.17	0.42
J01CF	Beta-lactamase resistant penicillins	2.53	3.72	1.60
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	18.55	18.07	18.90
J01DB -DE	Cephalosporins	0.71	1.28	1.10
J01DF	Monobactams	0.00	0.00	0.00
J01DH	Carbapenems	0.10	0.04	0.00
J01EA	Trimethoprim and derivatives	2.33	2.02	2.71
J01EC	Intermediate-acting sulfonamides	0.06	0.08	0.00
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	3.47	2.66	1.32
J01FA	Macrolides	2.15	2.39	2.41
J01FF	Lincosamides	3.73	4.48	2.17
J01GB	Aminoglycosides	0.12	0.12	0.01
J01MA	Fluoroquinolones	10.50	11.18	7.93
J01MB	Other quinolones	0.20	0.00	0.00
J01XA	Glycopeptides	0.10	0.08	0.07
J01XB	Polymyxins	0.37	0.39	0.01
J01XC	Steroid antibacterials (fusidic acid)	0.04	0.01	0.00
J01XD	Imidazole derivatives	0.07	0.14	0.02
J01XE	Nitrofuran derivatives	10.85	12.82	13.68
J01XX	other antibacterials	0.53	0.72	0.40
J01	**Antibiotics for systemic use (total)**	**67.02**	**73.83**	**64.97**

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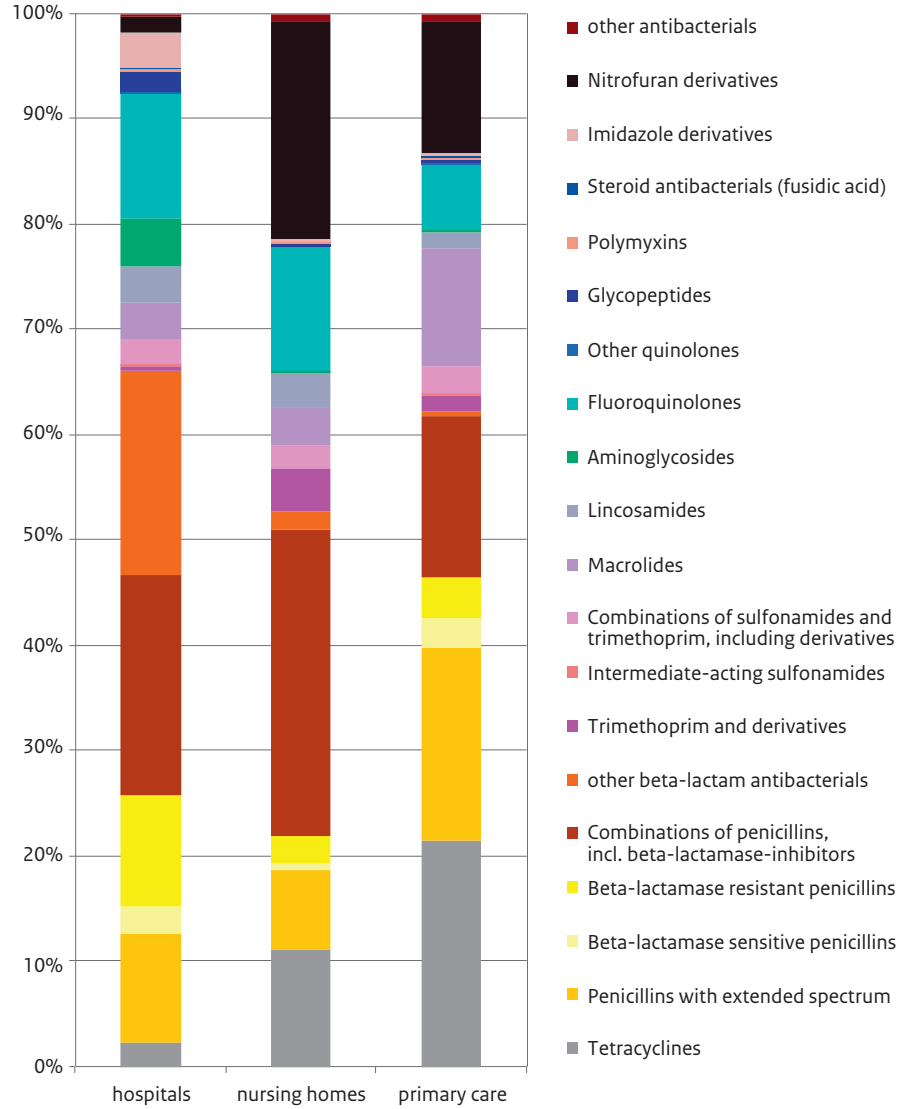
References

[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

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



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4 Surveillance of resistance

4.1 Methods and description of ISIS-AR data

4.1.1 Methods

The Infectious Disease Surveillance Information System for Antibiotic 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 Antibiotic Resistance (ISIS-AR). This surveillance system is a combined initiative of the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM), and is coordinated by the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) in Bilthoven. In 2014, ISIS-AR received data from 33 laboratories of which 21 laboratories had complete data over the five most recent years (2010 to 2014) over which we calculated time trends for the current report. To avoid bias in time trends due to incomplete data we used data from these 21 laboratories only for all analyses in the current report. Three of these laboratories were serving university hospitals, 16 laboratories were serving non-university hospitals and general practitioners and one laboratory was only serving general practitioners. We calculated resistance percentages and linear time trends over the five most recent years (2010 to 2014) for the most prevalent pathogens in combination with their main antimicrobial treatment options.

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) and 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,

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we conducted a separate analysis for blood isolates in in-patient 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 MICs from automated susceptibility test systems or gradient tests according to EUCAST 2014 breakpoints. 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 interpretable. However, for *H. influenzae, S. pneumoniae, M. catarrhalis, Enterococcus faecium* and *Enterococcus faecalis* less than 50% of the MICs could be interpreted when applying the EUCAST recommendations. Therefore the "S-I-R" interpretations, as reported by the 13 laboratories for which it was known that they used EUCAST recommendations in 2014, were included for calculating the percentage of resistant isolates.
Because no data on inducible clindamycin resistance tests were available, clindamycin resistance in *S. aureus* was calculated both using reinterpreted MIC-values, which do not show inducible resistance, and laboratory interpretation in which results of inducible resistance tests are taken into account. To be able to calculate time trends for the clindamycin resistance based on interpretation of the laboratories we accounted for change from CLSI to EUCAST recommendations by changing the interpretation from intermediate to resistant if the MIC-values were >0.5 mg/l. Both CLSI and EUCAST did not change breakpoints for clindamycin since 2010, which will therefore not cause a false time trend.

In some tables, data 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, and 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).

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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 2014, we calculated time trends over the five most recent years (2010 to 2014), 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 breakpoints (i.e. *E. coli, P. mirabilis, K. pneumoniae, E. cloacae, P. aeruginosa, Acinetobacter* spp. and *S. aureus* and coagulase-negative staphylococci including *S. epidermidis*). Two sided p-values <0.05 were considered statistically significant. For estimation of clinical relevancy the predicted resistances from the logistic model were used. If resistance in 2014 was below 10%, a change of ≥2.5% in the last 5 years was considered clinically relevant. If resistance in 2014 was above 10%, a change of ≥5% was considered clinically relevant. Statistically significant increasing trends that were 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, to facilitate the interpretation of time trends for pathogen-agent combinations with low resistance levels, the trends for the pathogen-agent combinations are shown in the figures if the percentage resistant isolates was between 0.5% and 30% in at least three years.

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4.1.2 Description of the ISIS-AR data

The current chapter shows some descriptive data of the data from the ISIS-AR antimicrobial resistance surveillance system. In figure 4.1.2.1 the distribution of laboratories over the country is shown 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 (for inclusion criteria see methods section). Therefore, laboratories included or excluded from analyses in the current report are shown in separate

Table 4.1.2.1 Characteristics of isolates in 2014 from 21 laboratories included in the analyses (laboratories that continuously reported to the ISIS-AR database from 2010 to 2014) and 13 laboratories excluded from the analyses (laboratories that started reporting later than 2010, or that did not continuously report until 2014)

	Included	Excluded
Total number of isolates	259984	132793
Mean number of isolates per laboratory	12380	10215
Pathogen		
E. coli	36	32
K. pneumoniae	5	5
E. cloacae	2	2
P. mirabilis	5	4
P. aeruginosa	5	4
Acinetobacter spp.	1	1
E. faecalis	5	7
E. faecium	1	1
S. aureus	11	12
CNS	5	6
S. pneumoniae	1	2
H. influenzae	2	3
M. catarrhalis	1	1
Other Enterobacteriaceae*	8	7
Other non-fermenters**	1	1
Other gram-positives	10	11
Sex of patient		
Male	39	41
Female	61	59

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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 postcode-4 area with categories based on quantiles of incidence. In table 4.1.2.1 some main descriptive data 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 data from included laboratories only are shown by pathogen. Finally, the age- distribution is shown in figure 4.1.2.3.

Table 4.1.2.1 (continued) Characteristics of isolates in 2014 from 21 laboratories included in the analyses (laboratories that continuously reported to the ISIS-AR database from 2010 to 2014) and 13 laboratories excluded from the analyses (laboratories that started reporting later than 2010, or that did not continuously report until 2014)

	Included	Excluded
Type of care		
GP	44	34
Outpatient departments	24	28
Inpatient departments (excl. Intensive Care Units)	27	34
Intensive Care Units	5	5
Age category of patient (y)		
0-4	4	4
5-18	6	5
19-64	38	39
>65	52	53
Isolate source		
Blood	5	5
Urine	59	54
Wound/Pus	14	17
Other sterile	15	14
Type of hospital		
Not applicable (GP) or missing data	44	35
General	22	32
Top clinical	24	33
University hospital	10	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.*

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Figure 4.1.2.1 Distribution of laboratories over the country by connection status

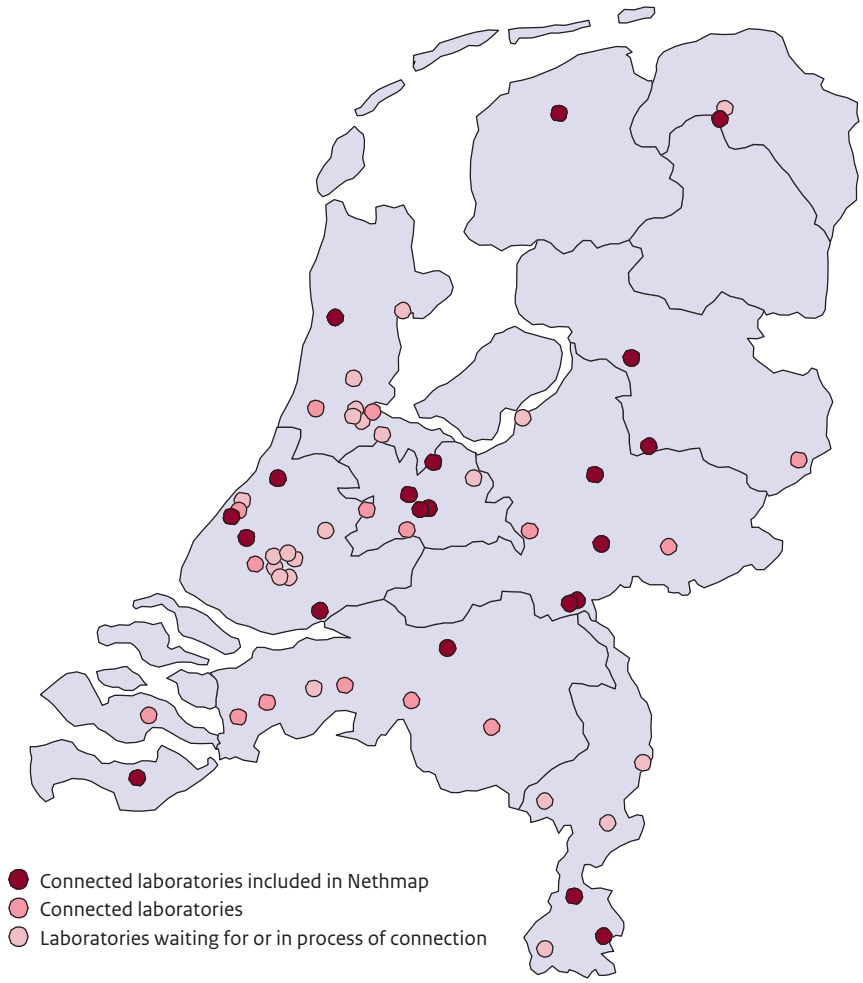

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Figure 4.1.2.2 Percentage of residents (%) for whom at least one isolate was included in the analyses for the current report by postcode-4 area

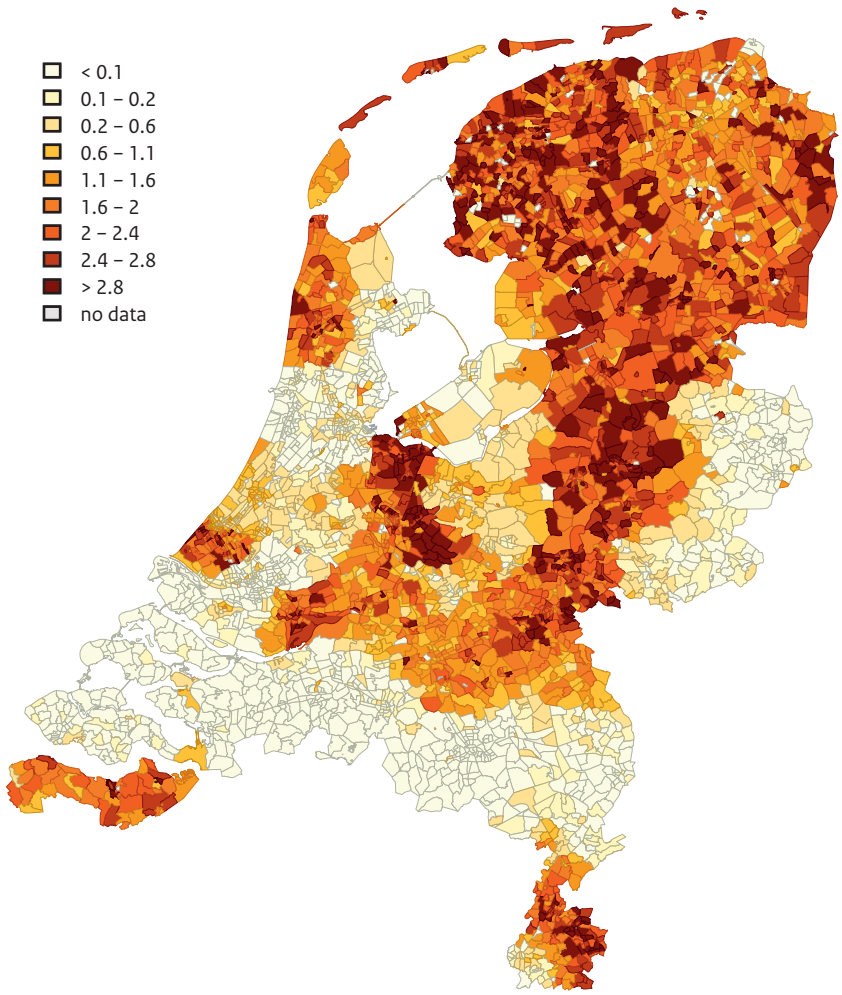

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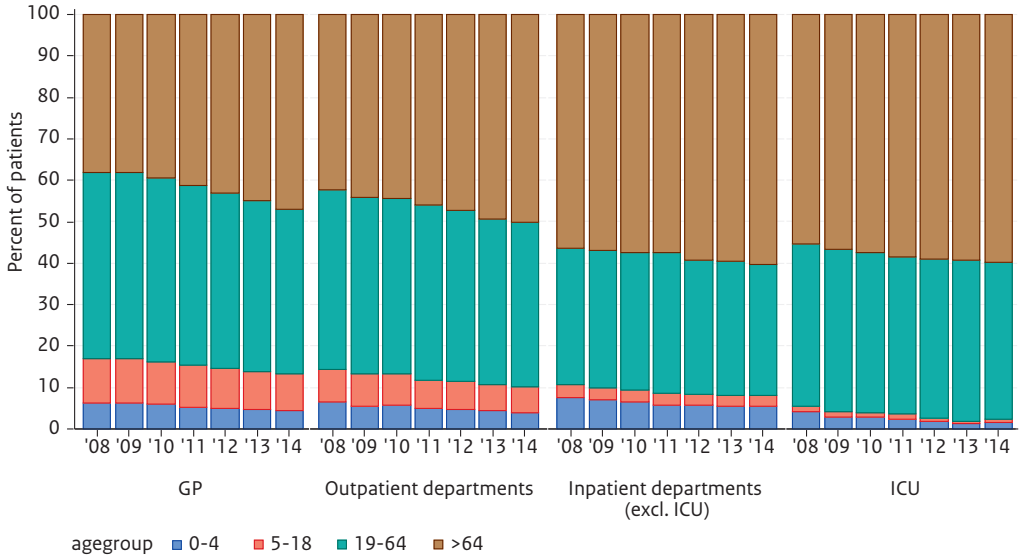
Figure 4.1.2.3 Distribution of age categories by year and institution type



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NethMap 2015

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Table 4.1.2.2 Characteristics of 209983 isolates in ISIS-AR in 2014, 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	94011	13010	5672	11996	12038	2963	13387	3013	29558	13615	3382	5720	1618
Sex of patient													
Male	27	33	51	40	53	47	50	51	51	49	54	53	53
Female	73	67	49	60	47	53	50	49	49	51	46	47	47
Type of care													
GP	61	50	33	50	33	53	37	8	26	21	10	13	12
Outpatient departments	16	21	25	22	32	25	26	11	42	15	32	45	40
Inpatient departments (excl. Intensive Care Units)	20	26	34	25	29	18	32	57	28	53	47	36	41
Intensive Care Units	3	4	8	3	6	4	4	24	4	11	10	7	7
Age category of patient (y)													
0-4	4	2	5	4	3	6	5	1	6	5	9	10	9
5-18	7	2	3	2	7	6	3	1	8	6	4	5	3
19-64	38	30	31	24	31	34	30	33	45	42	37	37	33
>65	51	67	61	70	59	53	62	65	41	46	50	49	55
Isolate source													
Blood	2	3	2	1	2	1	3	12	4	36	19	1	0
Urine	87	79	51	78	38	57	81	47	11	33	1	0	0
Wound/Pus	4	6	21	11	18	17	12	30	40	21	7	5	5
Other sterile	6	7	14	8	25	17	4	9	36	10	13	14	9
Type of hospital													
Not applicable (GP) or missing data	62	50	33	51	33	54	38	8	27	21	10	13	12
General	16	19	25	20	23	15	22	30	27	28	40	34	31
Top clinical	17	23	28	22	30	21	29	41	32	33	37	36	43
University hospital	6	9	14	7	13	11	12	21	14	18	12	17	14

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

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 the southern part of the Netherlands was relatively low (Figure 4.1.2.1).
- The regions of the laboratories included in Nethmap are reflected in the coverage data of Nethmap. The coverage in the southern part of the Netherlands, Zeeland, the areas around Rotterdam and Amsterdam, and the eastern part of the Netherlands was low. (Figure 4.1.2.2)
- 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 one laboratory that only serves general practitioners was included, the percentage of isolates from general practitioners was higher among the included laboratories than among the excluded laboratories (44 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, although slightly higher, we do not expect that the 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 in the GP population and outpatient departments is somewhat lower than in hospital departments (Figure 4.1.2.3). However, over the years the proportion of patients aged >65 years is increasing (38% in 2008 to 47% in 2014 for GP, 42-50% in outpatient departments, 56-60% in inpatient departments excluding intensive care units, and 55-59% in intensive care units).
- Enterobacteriaceae were more often isolated from female patients (e.g. 73% of *E. coli* in women vs. 27% in men, and 67% of *K. pneumoniae* in women vs. 33% in men), likely because women are more prone to urinary tract infections (Table 4.1.2.2). For the other pathogens, sex was more evenly distributed.
- 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 in patients from general practitioners and outpatient departments (58-78%, 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 64% respectively). Isolates of respiratory pathogens were evenly distributed over outpatient departments and inpatient departments (~40% each).
- All Enterobacteriaceae, *P. aeruginosa, Acinetobacter* spp. and *Enterococci* spp. were mainly isolated from urine (38-87%, depending on the pathogen), whereas *S. aureus* was mainly isolated from wound or pus (40%), and *H. influenzae, S. pneumoniae, M. catarrhalis* from the respiratory tract (59-85%, Table 4.1.2.2).

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4.2 Primary care

Surveillance data on resistance in patients attending a general practice (GP) are available from (1) the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) database (Chapter 4.2.1) and (2) the Extramural Resistance Surveillance (Chapter 4.2.2).

4.2.1 ISIS-AR

For the resistance analyses in GP patients 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 UTI or when there is no response to antimicrobial therapy. Isolates from women with complicated urinary tract infections, men, young children and persons that did not respond to the initial antimicrobial therapy are therefore overrepresented. As a result, the presented resistance levels are not representative for all patients with urinary tract infections or *S. aureus* wound and pus infections/ carriages presenting at the GP. Therefore, these patients are further referred to as 'selected general practitioner's patients'.

The distribution of pathogens in selected GP patients is shown in table 4.2.1.1 for pathogens isolated from urine samples and in table 4.2.1.2 for *S. aureus* isolated from wound and pus samples. The resistance levels in 2014 are shown in table 4.2.1.3 and table 4.2.1.4. Five-year trends in resistance are

Table 4.2.1.1 Distribution of isolated pathogens N (%) in clinical urine specimens from selected general practitioner's patients, presented per age category, ISIS-AR 2014

Pathogen	Age≤12 N(%)	Age>12 N(%)
E. coli	6205 (70)	51808 (56)
K. pneumoniae	120 (1)	6215 (7)
P. mirabilis	434 (5)	5302 (6)
P. aeruginosa	121 (1)	2179 (2)
S. aureus	87 (1)	1688 (2)
Other Enterobacteriaceae*	399 (5)	7933 (9)
Other non-fermenters**	157 (2)	1786 (2)
Enterococcus spp.	884 (10)	7904 (9)
Other gram-positives	453 (5)	7781 (8)

* *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.*

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presented in figure 4.2.1.1 and figure 4.2.1.2 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).

Table 4.2.1.2 Distribution of isolated pathogens N (%) in clinical wound and pus specimens from selected general practitioner's patients, presented per age category, ISIS-AR 2014

Pathogen	Age≤12 N(%)	Age>12 N(%)
S. aureus	317 (59)	2037 (50)
Other gram-positives	122 (23)	558 (14)
Enterobacteriaceae*	36 (7)	973 (24)
Other non-fermenters**	59 (11)	539 (13)

* *Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp., Morganella spp., Citrobacter spp., Serratia spp., Providencia spp.*
** *Acinetobacter spp., Pseudomonas spp., and Stenotrophomonas spp.*

Figure 4.2.1.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical urine isolates of E. *coli*, K. *pneumoniae*, P. *mirabilis* and P. *aeruginosa* from selected general practitioner's patients in ISIS-AR, presented per age category.

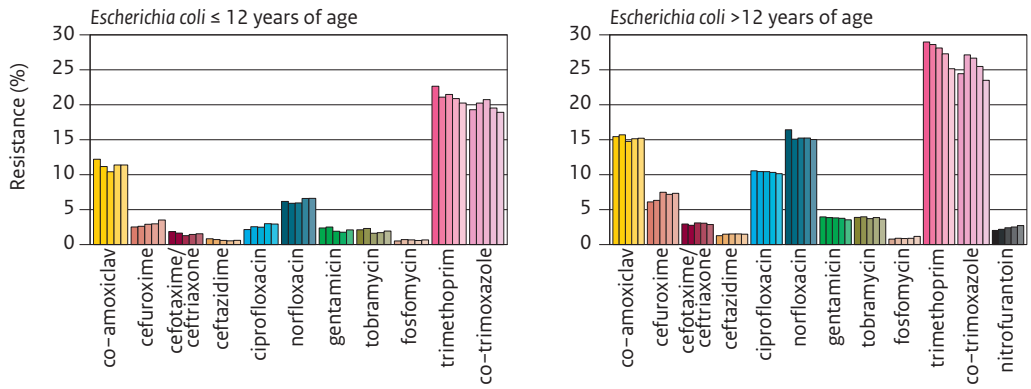

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Figure 4.2.1.1 (continued) Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from selected general practitioner's patients in ISIS-AR, presented per age category.

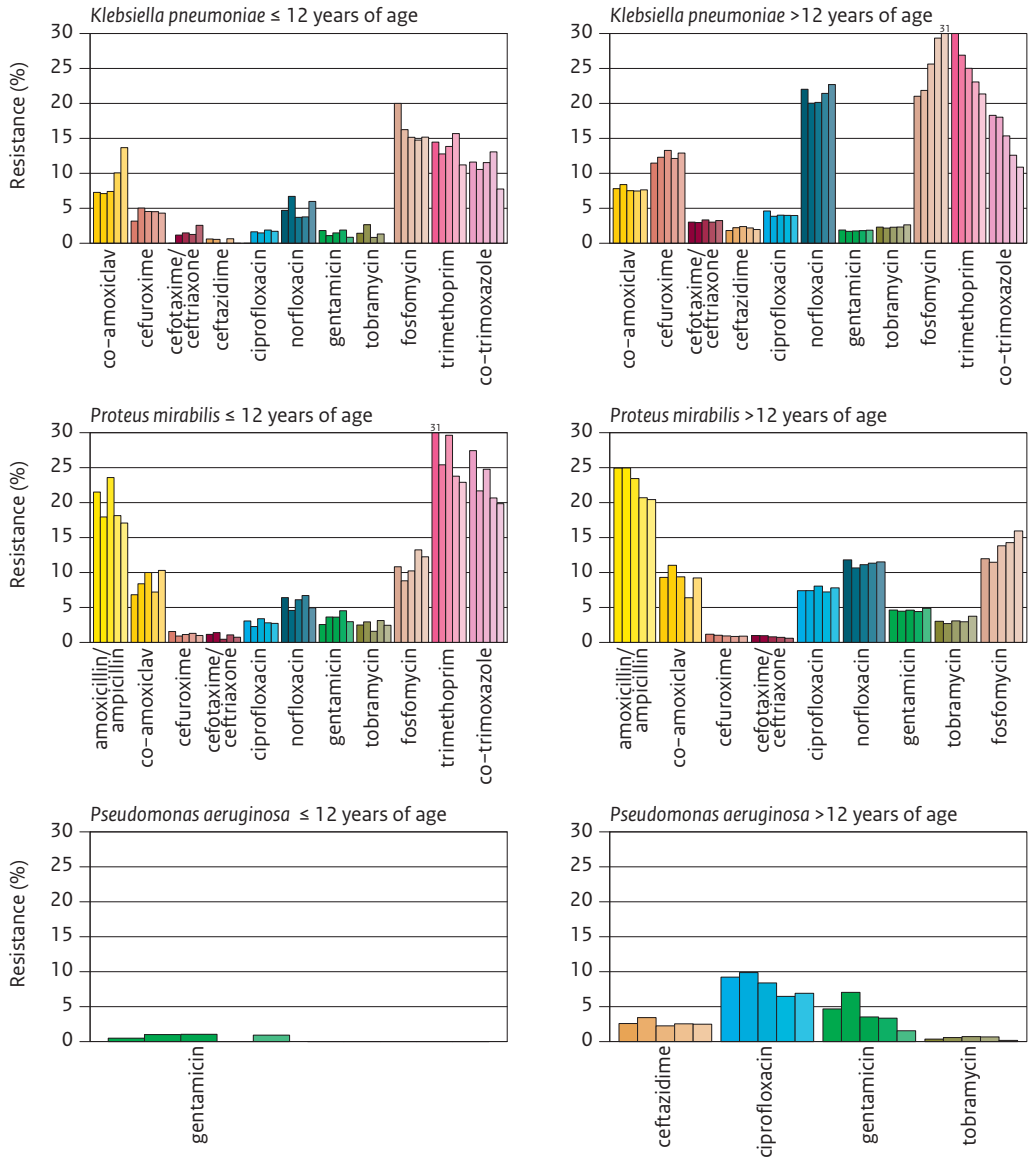

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Table 4.2.1.3 Resistance levels (%) of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* among clinical urine isolates from selected general practitioner's patients, presented per age category, ISIS-AR 2014

	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	64	5	73	3	74	3	79
Antibiotic								
amoxicillin/ampicillin	34	39	-	-	17	20	-	-
co-amoxiclav	11	15	14	8	10	9	-	-
cefuroxime	4	7	4	13	1	1	-	-
cefotaxime/ceftriaxone	2	3	3	3	1	1	-	-
ceftazidime	1	1	0	2	0	0	0	2
ciprofloxacin	3	10	2	4	3	8	1	7
norfloxacin	7	15	6	23	5	12	-	-
gentamicin	2	4	1	2	3	5	1	2
tobramycin	2	4	0	3	2	4	0	0
fosfomycin	1	1	15	31	12	16	-	-
trimethoprim	20	25	11	21	23	34	-	-
co-trimoxazole	19	23	8	11	20	28	-	-
nitrofurantoin	0	3	-	-	-	-	-	-
Multi-drug resistance								
HRMO*	2	4	3	4	1	2	-	-
multidrug-resistance**	0	3	1	1	0	1	-	-

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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*

* *Highly Resistant Microorganism (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*

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Table 4.2.1.4 Resistance levels (%) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients, ISIS-AR 2014

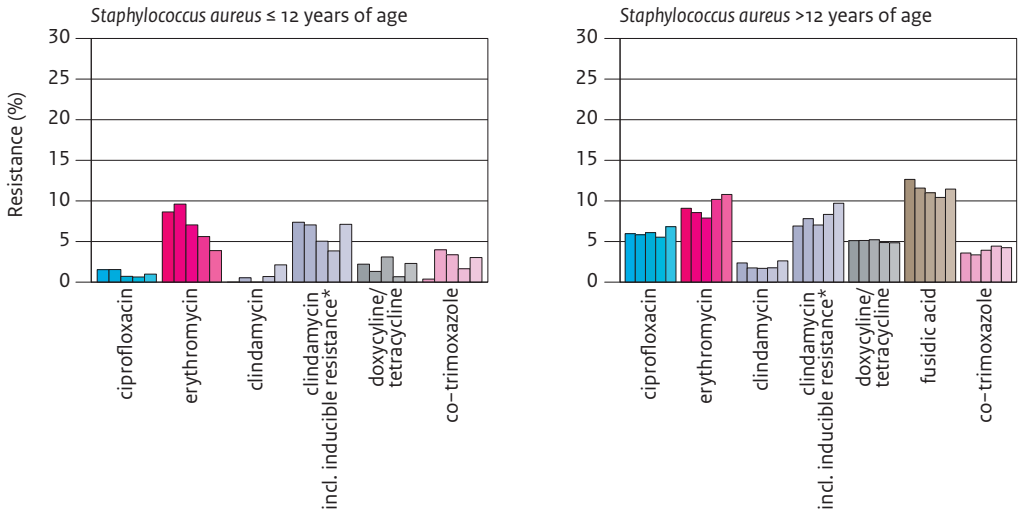
	S. aureus	
	age≤12	age>12
median age	4	57
Antibiotic		
ciprofloxacin*	1	7
erythromycin	4	11
clindamycin	2	3
clindamycin including inducible resistance**	7	10
doxycycline/tetracycline	2	5
fusidic acid	39	11
co-trimoxazole	3	4

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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 ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*

** *To estimate clindamycin resistance S-I-R interpretation of the laboratories was used (see methods for more detailed information).*

Figure 4.2.1.2 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical wound and pus isolates of *S. aureus* from selected general practitioner's patients in ISIS-AR, presented per age category.



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Key results

- In general, resistance levels in selected GP patients aged >12 years were higher than in patients aged ≤12 years, in particular for the fluoroquinolones. Only in *K. pneumoniae* resistance among selected GP patients ≤12 years was higher for co-amoxiclav (14% versus 8%), when compared with patients aged >12 years.

Enterobacteriaceae

- Resistance levels were below 8% for cefuroxime, cefotaxime/ceftriaxone, ceftazidime, gentamicin, and tobramycin for all Enterobacteriaceae, except for cefuroxime in *K. pneumoniae* in patients aged >12 (13%). Resistance levels were low for nitrofurantoin (≤3%) and fosfomycin (1%) in both age categories in *E. coli*. Resistance to co-amoxiclav remained below 10% in *K. pneumoniae* and *P.mirabilis* in patients aged >12 years
- In *K. pneumoniae* isolates of patients aged ≤12 years, the level of resistance to co-amoxiclav increased strongly and significantly, especially in the last two years, from 7% in 2010 to 14% in 2014.
- Higher levels of resistance were found for amoxicillin/ampicillin (≥17%), and trimethoprim (≥11%) in patients of all ages, and for co-trimoxazole (≥11%) and norfloxacin (≥12%) in patients aged >12 years.
- There was a statistically significant and clinically relevant decrease in resistance to amoxicillin/ampicillin in *P. mirabilis* in patients aged >12 years, although resistance was still high in 2014 (20%).
- Although remaining high, trimethoprim and co-trimoxazole resistance levels showed a strong decrease in *K. pneumoniae* in patients aged >12 years (from 30% in 2010 to 21% in 2014 for trimethoprim and from 18% in 2010 to 10% in 2014 for co-trimoxazole) and in *P. mirabilis* in patients aged ≤12 years (from 31% to 23% for trimethoprim and from 27% to 20% for co-trimoxazole). A statistically significant and clinically relevant increase from 21% in 2010 to 31% in 2014 in 2014 was seen for fosfomycin in patients aged >12 years in *K. pneumoniae*.
- 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 year in all Enterobacteriaceae (≤4%).

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 ciprofloxacin from 9% in 2010 to 7% in 2014 and for gentamycin from 5% in 2010 to 2% in 2014 in patients aged >12 years.

S. aureus

- Resistance levels for each of the tested agents were below 10% in patients aged ≤12 years, except for the resistance level of 39% for fusidic acid.
- Resistance was significantly decreasing from 9% in 2010 to 4% in 2014 for erythromycin in patients aged ≤12 years.
- In patients aged >12 years, resistance was 10% or higher for erythromycin, clindamycin including inducible resistance and fusidic acid.

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4.2.2 SERIN, Surveillance of Extramural Resistance in The Netherlands.

Antibiotic resistance among bacteria causing community acquired urinary tract infections (UTI) was determined for strains collected from female patients visiting GPs with symptoms of an acute uncomplicated urinary tract infection, i.e. strangury, dysuria, urinary frequency and urgency without fever, were eligible for inclusion. Excluded were patients with a catheter and those with urological or nephrological problems, diabetes mellitus or other immune compromising diseases. The GPs (42) participated in the national NIVEL network, which is representative for age, gender regional distribution and population density in the Netherlands.

A dip-slide was prepared from a fresh voided urine sample according to the manufacturer's instructions and sent to the National Institute for Public Health and the Environment (Centre for Infectious Disease Research , Diagnostics and Screening), Bilthoven. After arrival at the laboratory the dipslides were analyzed for bacterial growth and considered positive at 10e3 CFU/ml or more. Bacterial growth on the CLED were purified onto McConkey and ESBL agar plates. If 2 or more bacterial species were present the dipslides were excluded. Identification of the uropathogens was performed using standard biochemical methods.
Antibiotic susceptibility was performed using the disc diffusion method for amoxicillin, co-amoxyclav, trimethoprim, co-trimoxazole, nitrofurantoin, norfloxacin , ciprofloxacin and fosfomycin. Breakpoints for resistance were according to the EUCAST guidelines. For fosfomycin the CLSI guidelines were used as no EUCAST breakpoints were available. *Escherichia coli* ATCC 35218 and ATCC25922 were used as control strains.
Growth on the ESBL agarplate was tested for the production of an extended spectrum beta-lactamase(ESBL) by an combination disc diffusion method according to the guidelines of the Dutch Society for Medical Microbiology (NVMM).

The distribution of uropathogens found was: *Escherichia coli* (n=501), K*lebsiella pneumoniae* (n=26), *Klebsiella oxytoca* (n=10), no growth (30).
Table 4.2.2.1 shows the resistance percentages for *E. coli.* ESBL producing *E. coli* were observed in 2/501 isolates (= 0.4%). Both ESBL producing *E. coli* were from the oldest age group.

Table 4.2.2.1 Resistance (%) among *E. coli* from women with uncomplicated UTI in 2014

Age group, years	1-10 y	11-20 y	21-50 y	51-70 y	>70 y
(n)	**44**	**58**	**273**	**217**	**192**
***E. coli* (n)**	**24**	**31**	**149**	**155**	**142**
Amoxicillin	33	35	38	30	38
Co-amoxiclav	4	16	6	8	12
Ciprofloxacin	0	6.5	5	3	12
Nitrofurantoin	1	0	0	1	1
Trimethoprim	4	19	21	19	20
Co-trimoxazol	4	13	18	17	19
Fosfomycin	0	0	0	1	1

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Table 4.2.2.2 Resistance (%) of *E. coli* isolated from women with uncomplicated UTI in 2014 vs 2009

	2014 (n=501)	2009(n=489)	
Amoxicillin	35	34	
Co-amoxiclav	9	13	
Ciprofloxacin	6	3	
Nitrofurantoin	1	0	
Trimethoprim	20	19	
Co-trimoxazol	18	16	
Fosfomycin	1	0	

Key results

- The resistance data obtained from unselected *E. coli* , isolated from female patients attending their general practitioner in 2014 were similar to those found in 2009 (table 2).
- The prevalence of ESBL producing *E. coli* decreased from 1% in 2009 to 0.4 % in this study, however, the numbers are too low to draw meaningful conclusions.
- Resistance percentages in the different age groups were of the same magnitude, apart from the higher resistance (12%) to ciprofloxacin in the oldest age group. The ESBL producing isolates were also found in this age group.

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4.3 Hospital departments

Surveillance data on resistance in patients attending hospital departments are only available from the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) database. In the analyses for outpatient departments and inpatient departments (including intensive care units), the antimicrobial susceptibility results were based on blood, cerebrospinal fluid, lower respiratory tract, urine and wound isolates combined. Additionally, we conducted two separate analyses; 1) for blood isolates in inpatient hospital departments including ICU departments (chapter 4.3.4), and 2) for urinary isolates in urology departments (outpatient and inpatient departments, chapter 4.3.5).

4.3.1 Outpatient departments

Table 4.3.1.1 shows the distribution of pathogens isolated from clinical specimens (lower respiratory tract, urine, and wound) from patients attending outpatient departments. The resistance levels for outpatient department patients in 2014 are shown 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 presented in figures 4.3.1.1 and 4.3.1.2. From patients attending outpatient departments samples are taken more frequently than from GP patients. Therefore, bias due to selective sampling will be lower than in GP patients and we consider resistance percentages in this chapter a good reflection of resistance in outpatient departments.

Table 4.3.1.1 Distribution of isolated pathogens N (%) in clinical specimens from outpatient departments, ISIS-AR 2014

	Lower respiratory tract	Urine	Wound or Pus
Pathogen	**N(%)**	**N(%)**	**N(%)**
E. coli	442 (9)	15708 (44)	994 (7)
K. pneumoniae	191 (4)	2527 (7)	197 (1)
P. mirabilis	133 (3)	1894 (5)	579 (4)
P. aeruginosa	1044 (20)	1254 (4)	1001 (7)
E. faecalis	2 (0)	3262 (9)	409 (3)
S. aureus	1104 (22)	1073 (3)	6236 (44)
Other Enterobacteriaceae*	724 (14)	3718 (11)	1465 (10)
Other non-fermenters**	474 (9)	560 (2)	367 (3)
Other *Enterococcus* spp.	2 (0)	1049 (3)	176 (1)
Other gram-positives	978 (19)	4364 (12)	2889 (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.*

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Table 4.3.1.2 Resistance levels (%) among clinical isolates of *E. coli, K. pneumoniae, P. mirabilis and P. aeruginosa* from outpatient departments, ISIS-AR 2014

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	45	-	23	-
co-amoxiclav	19	9	11	-
piperacillin-tazobactam	5	5	0	6
cefuroxime	11	13	1	-
cefotaxime/ceftriaxone	5	5	1	-
ceftazidime	2	4	0	3
meropenem/imipenem	0	0	0	3
ciprofloxacin	17	6	10	8
norfloxacin	22	20	16	-
gentamicin	5	3	6	3
tobramycin	6	5	4	1
fosfomycin	1	29	15	-
trimethoprim	31	21	37	-
co-trimoxazole	28	13	28	-
nitrofurantoin	4	-	-	-
Empiric therapy combinations				
gentamicin + amoxicillin/ampicillin	5	-	5	-
gentamicin + co-amoxiclav	3	2	2	-
gentamicin + cefuroxime	2	2	0	-
gentamicin + cefotaxime/ceftriaxone	1	2	0	-
gentamicin + ceftazidime	1	1	0	0
Multi-drug resistance				
HRMO*	8	7	4	1
multidrug-resistance**	5	2	2	-

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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

* *Highly Resistant Microorganism (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*

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Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from outpatient departments in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.

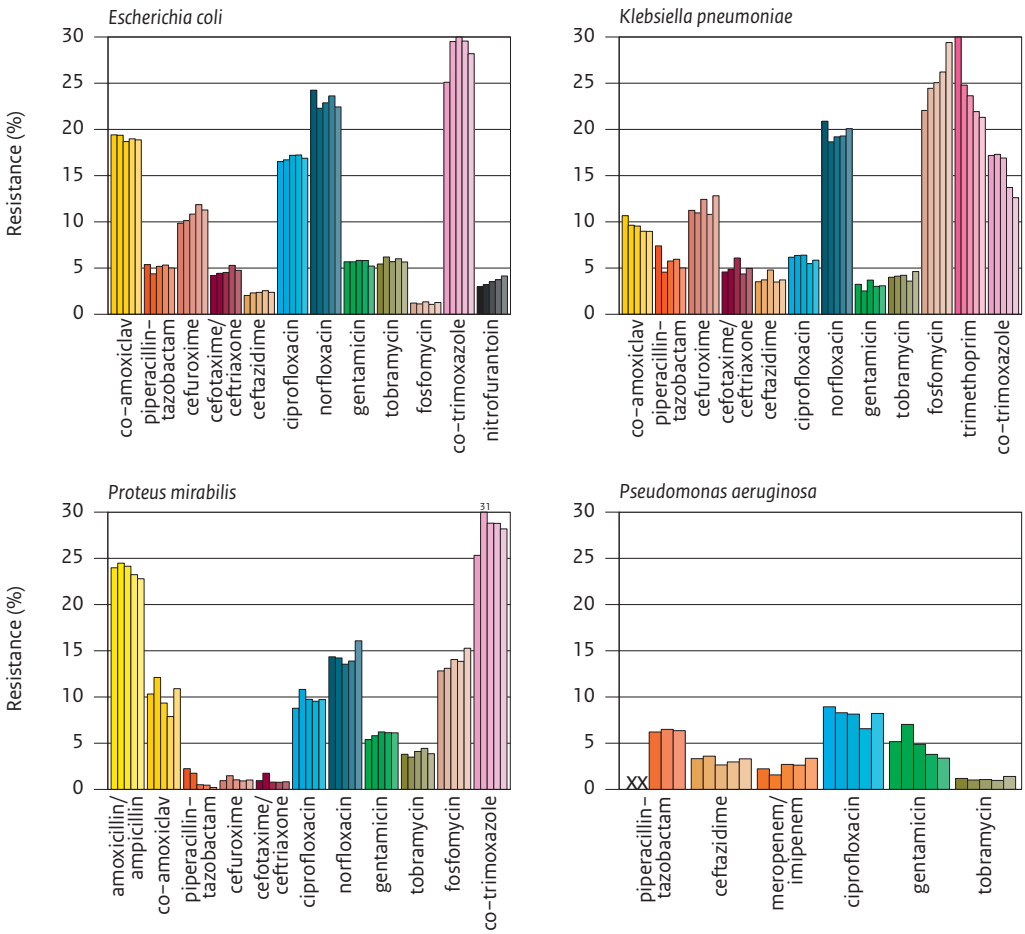

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Table 4.3.1.3 Resistance levels (%) among clinical isolates of *S. aureus* from outpatient departments, ISIS-AR 2014

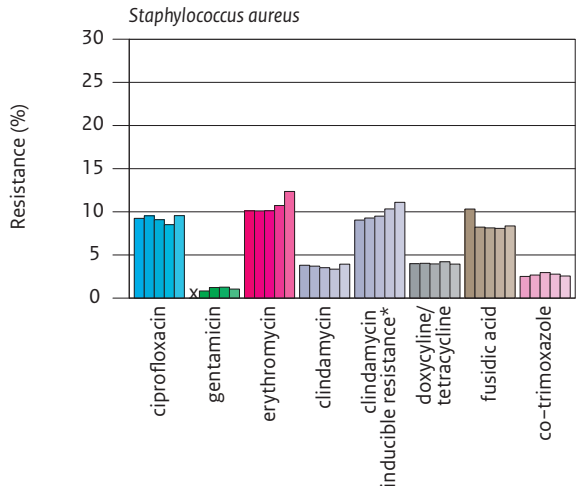
	S. aureus	
Antibiotic		
ciprofloxacin*	10	
gentamicin	1	
erythromycin	12	
clindamycin	4	
clindamycin including inducible resistance**	11	
doxycycline/tetracycline	4	
fusidic acid	8	
linezolid	0	
co-trimoxazole	3	
rifampicin	0	

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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 ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*
** *To estimate clindamycin resistance S-I-R interpretation of the laboratories was used (see methods for more detailed information).*

Figure 4.3.1.2 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *S. aureus* from outpatient departments in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Key results

Enterobacteriaceae

- Resistance levels for piperacillin/tazobactam (≤5%), cefotaxime/ceftriaxone (≤5%), ceftazidime (≤4%), imipenem/meropenem (<1%) and gentamicin and tobramycin (both ≤6%) were below 7% in all Enterobacteriaceae in 2014.
- Furthermore, resistance levels lower than 7% were found for nitrofurantoin (4%) in *E. coli*, for ciprofloxacin (6%) in *K. pneumoniae*, and for cephalosporins (≤1%) in *P. mirabilis*.
- Amoxicillin/ampicillin, trimethoprim, co-trimoxazole and norfloxacin resistance was higher than 12% for all Enterobacteriaceae. Additionally, resistance to co-amoxiclav (19%) and ciprofloxacin (17%) was high in E. coli.
- The percentage of HRMO was ≤8%, and the proportion of multidrug resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin combined, was ≤5%.
- In *K. pneumoniae*, fosfomycin resistance showed a significant and clinically relevant increasing trend from 22% to 29% between 2010 and 2014. On the other hand, resistance to trimethoprim (30% to 21%) and co-trimoxazole (17% to 13%) decreased in the last five years.

P. aeruginosa

- Resistance to each of the tested agents remained lower than 9%.
- Gentamicin resistance decreased significantly (and this was considered clinically relevant), especially in the last four years, from 7% in 2011 to 3% in 2014.

S. aureus

- Resistance to linezolid and rifampicin (both 0%) remained low.
- Resistance to each of the tested agents except clindamycin (including inducible resistance, 11%) and erythromycin (12%) was lower than 10% and remained stable over the last four to five years.

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4.3.2 Inpatient hospital departments (excl. ICU)

Table 4.3.2.1 shows 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). The resistance levels for inpatient hospital department patients in 2014 are shown in tables 4.3.2.2 (*E. coli, K. pneumoniae, E. cloacae, P. mirabilis, P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.3 (*Enterococcus* spp.) and 4.3.2.4 (*S. aureus*). Five-year trends in resistance are presented 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, the majority of infections is cultured for susceptibility testing. Therefore, bias due to selective culturing will be limited or non-existing.

Table 4.3.2.1 Distribution of isolated pathogens N (%) in clinical specimens from inpatient departments (excl. intensive care units), ISIS-AR 2014

	Blood or Cerebrospinal fluid	Lower respiratory tract	Urine	Wound or Pus
Pathogen	**N(%)**	**N(%)**	**N(%)**	**N(%)**
E. coli	3064 (24)	896 (13)	13691 (44)	2767 (15)
K. pneumoniae	479 (4)	363 (5)	2195 (7)	542 (3)
P. mirabilis	204 (2)	165 (2)	2247 (7)	614 (3)
E. cloacae	162 (1)	300 (4)	737 (2)	711 (4)
P. aeruginosa	243 (2)	1131 (17)	1568 (5)	1064 (6)
Acinetobacter spp.	39 (0)	90 (1)	166 (1)	149 (1)
E. faecalis	400 (3)	23 (0)	3208 (10)	1081 (6)
E. faecium	276 (2)	13 (0)	945 (3)	631 (3)
S. aureus	1317 (10)	1314 (19)	977 (3)	4787 (26)
CNS	4246 (33)	14 (0)	766 (2)	1935 (10)
Other Enterobacteriaceae*	551 (4)	975 (14)	2574 (8)	1433 (8)
Other non-fermenters**	24 (0)	424 (6)	138 (0)	185 (1)
Other gram-positives	1960 (15)	1104 (16)	1841 (6)	2639 (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.*

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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 inpatient departments (excl. intensive care units), ISIS-AR 2014

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	45	-	-	22	-	-
co-amoxiclav	20	11	-	12	-	-
piperacillin-tazobactam	5	7	-	0	7	-
cefuroxime	12	13	-	1	-	-
cefotaxime/ceftriaxone	5	7	-	1	-	-
ceftazidime	2	5	-	1	4	-
meropenem/imipenem	0	0	0	0	4	2
ciprofloxacin	13	6	4	8	7	7
gentamicin	5	4	4	6	3	5
tobramycin	5	6	5	5	1	6
co-trimoxazole	25	13	8	27	-	5
nitrofurantoin	3	-	-	-	-	-
Empiric therapy combinations						
gentamicin + amoxicillin/ ampicillin	4	-	-	5	-	-
gentamicin + co-amoxiclav	3	3	-	2	-	-
gentamicin + piperacillin-tazobactam	1	2	-	0	1	-
gentamicin + cefuroxime	2	3	-	0	-	-
gentamicin + cefotaxime/ ceftriaxone	1	3	-	0	-	-
gentamicin + ceftazidime	1	2	-	0	1	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	1	-
Multi-drug resistance						
HRMO*	7	8	2	4	1	2

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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*

* *Highly Resistant Microorganism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone 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.*

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Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from inpatient departments (excl. intensive care units) in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%

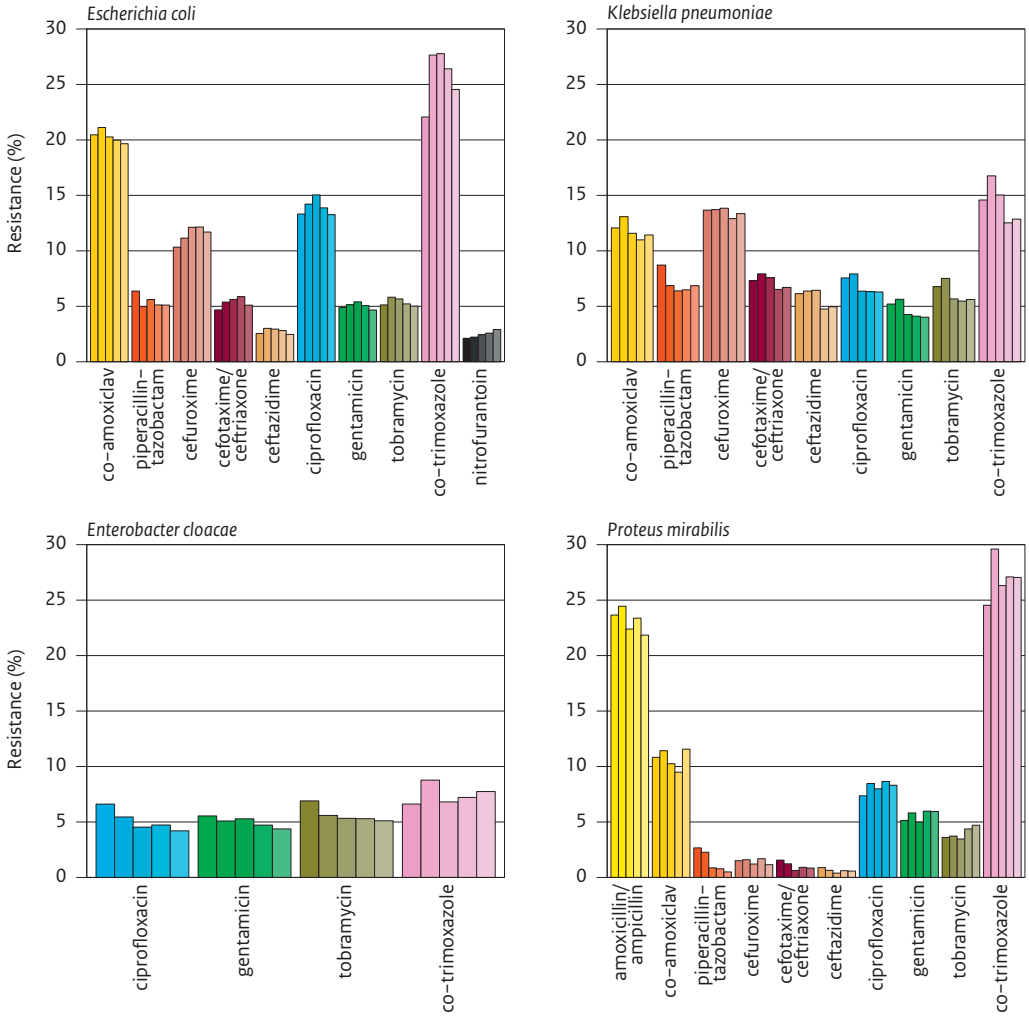

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Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from inpatient departments (excl. intensive care units) in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%

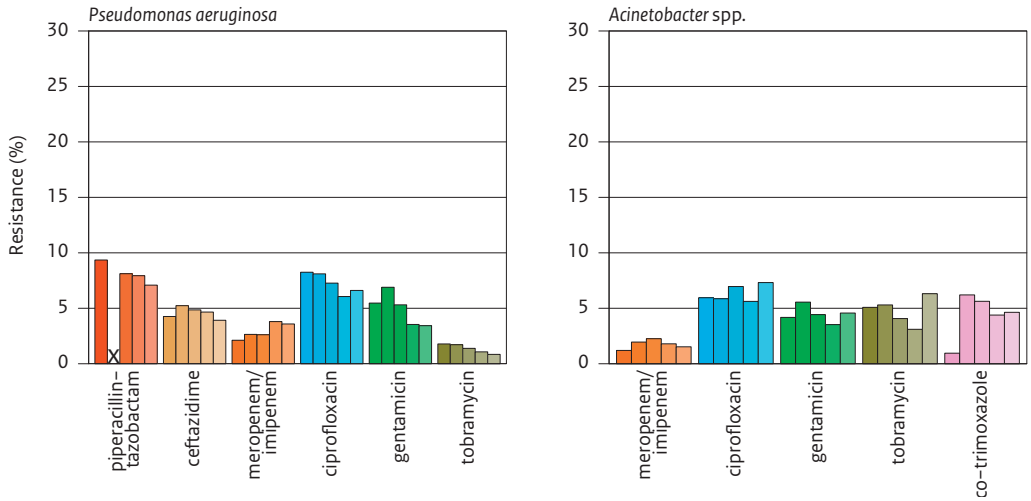

Table 4.3.2.3 Resistance levels (%) among clinical isolates of *E. faecalis* and *E. faecium* from inpatient departments (excl. intensive care units), ISIS-AR 2014

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

- *Resistance not calculated*

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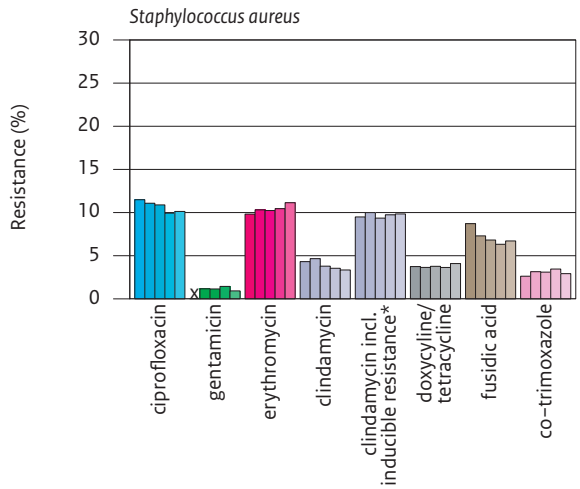
Table 4.3.2.4 Resistance levels (%) among clinical isolates of *S. aureus* from inpatient departments (excl. intensive care units), ISIS-AR 2014

	S. aureus	
Antibiotic		
ciprofloxacin*	10	
gentamicin	1	
erythromycin	11	
clindamycin	3	
clindamycin including inducible resistance**	10	
doxycycline/tetracycline	4	
fusidic acid	7	
linezolid	0	
co-trimoxazole	3	
rifampicin	0	

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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 ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*
*** To estimate clindamycin resistance S-I-R interpretation of the laboratories was used (see methods for more detailed information).*

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *S. aureus* from inpatient departments (excl. intensive care units) in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Key results

Enterobacteriaceae

- Overall, resistance to piperacillin/tazobactam (≤7%), cefotaxime/ceftriaxone (≤7%), ceftazidime (≤5%), imipenem/meropenem (0%), ciprofloxacin (≤8% except for *E.coli*), gentamicin and tobramycin (both ≤6%), and nitrofurantoin (*E. coli* only; 3%) remained below 9%.
- Resistance to amoxicillin/ampicillin remained high for *E. coli* and *P. mirabilis* (>20%).
- Resistance to co-amoxiclav and co-trimoxazole remained higher than 10%, except for co-trimoxazole resistance in *E. cloacae* (8%).
- For *K. pneumoniae*, resistance to each of the tested agents except co-trimoxazole remained relatively stable in the last 2-3 years.
- For *P. mirabilis* resistance to cephalosporins (1%) and piperacillin/tazobactam (0%) was rare.
- The percentage of HRMO was highest in *K. pneumoniae* (8%).

P. aeruginosa

- Resistance to each of the tested agents was below 8%.
- Imipenem/meropenem resistance increased from 2% in 2010 to 4% in 2014.
- For gentamicin, there was a significant and clinically relevant decreasing trend in resistance, especially in the last four years (from 7% in 2011 to 3% in 2014).

***Acinetobacter* spp.**

- Resistance to each of the tested agents remained below 8%.

***Enterococcus* spp.**

- Vancomycin resistance in *E. faecium* remained rare (0%).

S. aureus

- Resistance to gentamicin, co-trimoxazole and doxycycline/tetracycline was below 5% and remained stable over the last five years.
- Resistance to rifampicin and linezolid (both 0%) was still rare.

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4.3.3 Intensive Care Units

Table 4.3.3.1 shows 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. The resistance levels for ICU patients in 2014 are shown in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*), 4.3.3.3 (*Enterococcus* spp.) and 4.3.3.4 (*S. aureus* and coagulase negative *staphylococci*). Five-year trends in resistance are presented 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, pathogens from almost all infections are cultured for susceptibility testing. Bias due to selective culturing is therefore unlikely.

Table 4.3.3.1 Distribution of isolated pathogens N (%) in clinical specimens from intensive care units, ISIS-AR 2014

Pathogen	Blood or Cerebrospinal fluid N(%)	Lower respiratory tract N(%)	Urine N(%)	Wound or Pus N(%)
E. coli	268 (12)	429 (13)	661 (39)	566 (19)
K. pneumoniae	47 (2)	187 (6)	102 (6)	97 (3)
P. mirabilis	23 (1)	101 (3)	112 (7)	66 (2)
E. cloacae	34 (2)	212 (6)	41 (2)	120 (4)
P. aeruginosa	67 (3)	301 (9)	136 (8)	213 (7)
Acinetobacter spp.	8 (0)	47 (1)	10 (1)	18 (1)
E. faecalis	95 (4)	73 (2)	185 (11)	300 (10)
E. faecium	172 (8)	138 (4)	140 (8)	402 (14)
S. aureus	160 (7)	650 (20)	43 (3)	258 (9)
CNS	1020 (46)	38 (1)	53 (3)	321 (11)
Other Enterobacteriaceae*	86 (4)	571 (17)	152 (9)	261 (9)
Other non-fermenters**	10 (0)	180 (5)	7 (0)	39 (1)
Other gram-positives	236 (11)	406 (12)	64 (4)	268 (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.*

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Table 4.3.3.2 Resistance levels (%) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from intensive care units, ISIS-AR 2014

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	46	-	-	29	-
co-amoxiclav	19	16	-	13	-
piperacillin-tazobactam	6	9	-	0	14
cefuroxime	13	16	-	1	-
cefotaxime/ceftriaxone	6	10	-	2	-
ceftazidime	3	7	-	0	11
meropenem/imipenem	0	1	0	0	6
ciprofloxacin	13	10	7	9	8
gentamicin	5	7	8	6	4
tobramycin	5	10	9	3	3
co-trimoxazole	24	13	9	27	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	4	-	-	5	-
gentamicin + co-amoxiclav	3	6	-	2	-
gentamicin + piperacillin-tazobactam	1	3	-	0	2
gentamicin + cefuroxime	2	6	-	0	-
gentamicin + cefotaxime/ceftriaxone	2	5	-	0	-
gentamicin + ceftazidime	1	4	-	0	2
tobramycin + ceftazidime	-	-	-	-	2
tobramycin + ciprofloxacin	-	-	-	-	2
Multi-drug resistance					
HRMO*	8	11	4	5	4

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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*

* *Highly Resistant Microorganism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/ceftriaxone 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.*

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Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from intensive care units in ISIS-AR

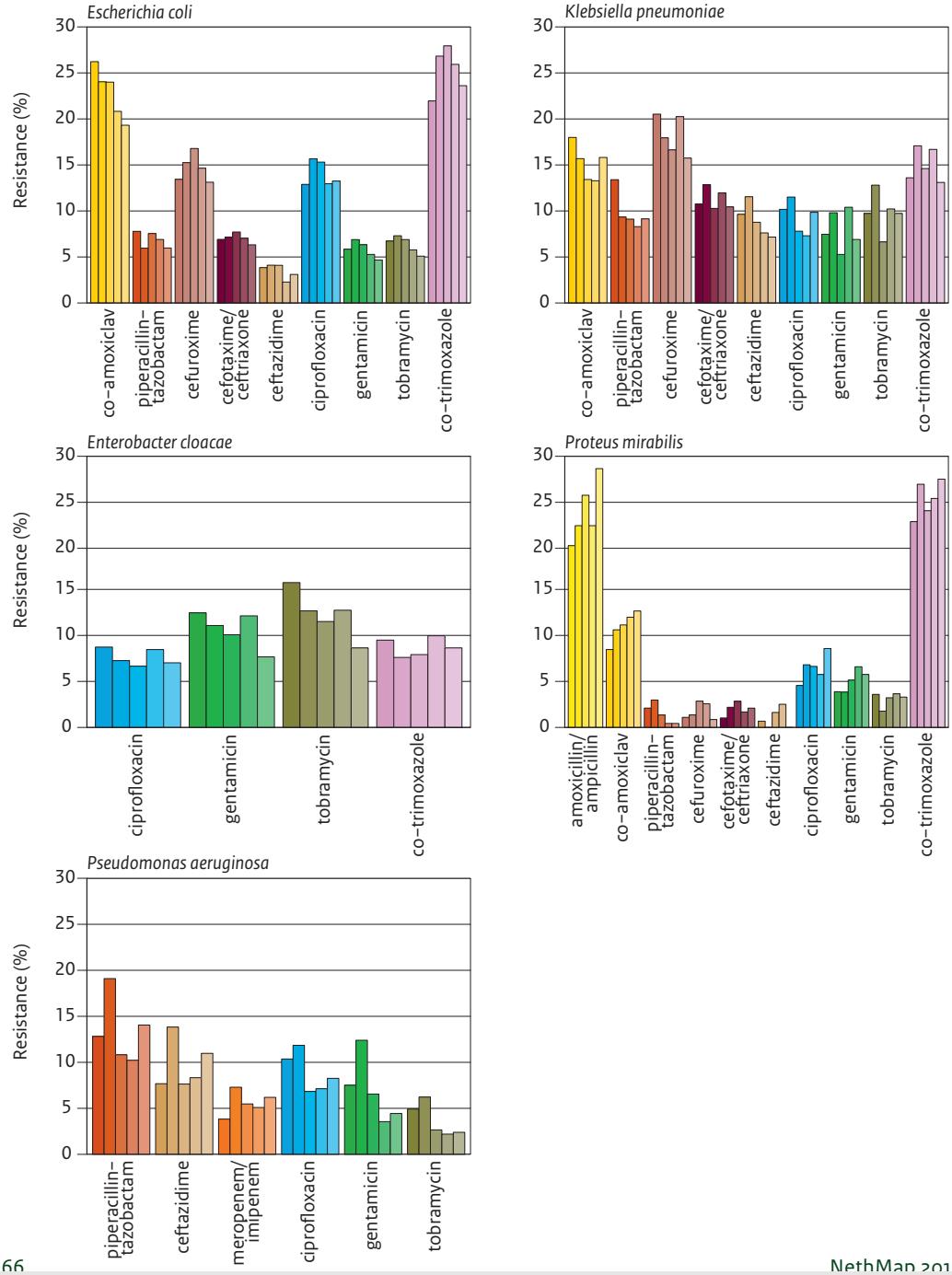

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Table 4.3.3.3 Resistance levels (%) among clinical isolates of *E. faecalis* and *E. faecium* from intensive care units, ISIS-AR 2014

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

- *Resistance not calculated*

Table 4.3.3.4 Resistance levels (%) among clinical isolates of *S. aureus* and coagulase negative staphylococci from intensive care units, ISIS-AR 2014

	S. aureus	*CNS*	
Antibiotic			
ciprofloxacin*	7	59	
gentamicin	1	50	
erythromycin	10	66	
clindamycin	2	46	
clindamycin including inducible resistance**	9	60	
doxycycline/tetracycline	5	27	
linezolid	0	1	
co-trimoxazole	2	49	
rifampicin	0	10	

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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)

CNS=Coagulase-negative staphylococci, including S. epidermidis

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

** *To estimate clindamycin resistance S-I-R interpretation of the laboratories was used (see methods for more detailed information).*

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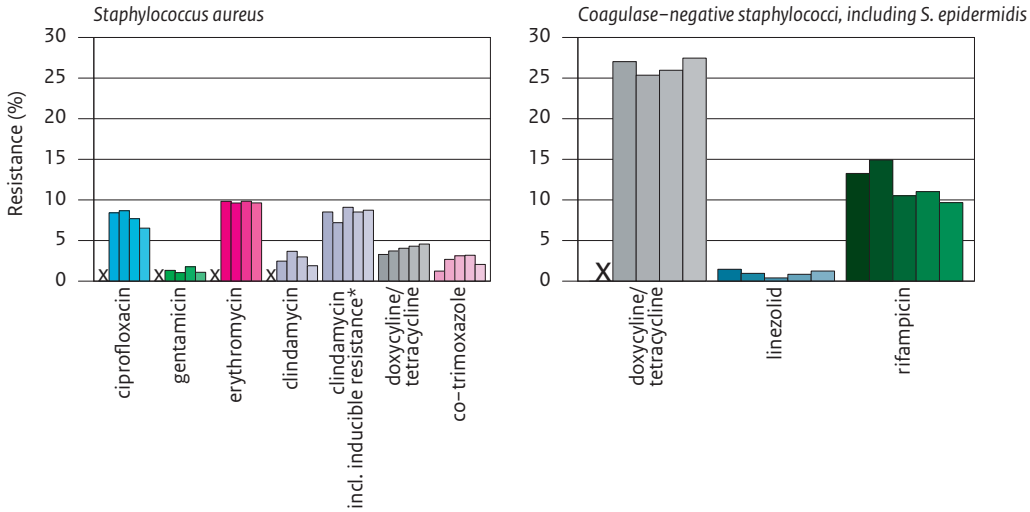
Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical isolates of *S. aureus* and *coagulase negative staphylococci* from intensive care units in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



Key results

Enterobacteriaceae

- Overall, resistance to piperacillin/tazobactam (≤9%), 3rd generation cephalosporins (≤10%), imipenem/meropenem (≤1%), gentamicin (≤8%), tobramycin (≤10%), the empiric therapy combinations, and HRMO (except for *K. pneumoniae*; 11%) remained ≤10%.
- Amoxicillin/ampicillin resistance was high (≥29%). For *P. mirabilis*, a statistically significant and clinically relevant increase in resistance was observed between 2010 and 2014 (20% to 29%).
- Resistance to co-amoxiclav, cefuroxime (except for *P. mirabilis*) and co-trimoxazole (except for *E. cloacae*) was ≥13%. However, in *E.coli*, co-amoxiclav resistance significantly decreased to a clinically relevant extent (from 26% in 2010 to 19% in 2014).
- In *K. pneumoniae*, piperacillin/tazobactam resistance in showed a significant and clinically relevant decrease from 14% in 2010 to 9% in 2014. Although there was no significant trend over 5 years, ceftazidime resistance decreased significantly in the most recent 4 years (2011-2014) from 11% to 7%.
- In *E. cloacae*, a significant and clinically relevant decrease was found for tobramycin resistance (from 16% in 2010 to 9% in 2014). Gentamicin resistance decreased as well (from 12% to 8% between 2010 and 2014), but this was not statistically significant.

P. aeruginosa

- There was a statistically significant and clinically relevant decrease in resistance to ciprofloxacin (10% to 8%), gentamicin (8% to 4%) and tobramycin (5% to 3%) between 2010 and 2014.

Enterococcus spp.

- Resistance to vancomycin in *E. faecium* remained rare (0%).

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S. aureus

- Resistance to each of the tested agents was lower than 10%.

Coagulase-negative staphylococci

- Apart from rifampicin and linezolid, resistance to each of the tested agents was high (>25%).
- Resistance to ciprofloxacin (64% to 59%), gentamicin (56% to 50%), erythromycin (71% to 66%) and rifampicin (15% to 10%) decreased from 2012 to 2014, whereas co-trimoxazole resistance increased from 38% in 2011 to 49% in 2014.

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

Table 4.3.4.1 shows the distribution of pathogens isolated from blood of patients admitted to inpatient departments (incl. intensive care units). The resistance levels for blood isolates are shown in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*), 4.4.4.3 (*Enterococcus* spp.), 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 the respective pathogens (except for *Enterococcus* spp.). 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 highly unlikely.

Table 4.3.4.1 Distribution of pathogens N (%) in clinical blood isolates from inpatient departments (incl. intensive care units), ISIS-AR 2014

Pathogen	Blood N(%)
E. coli	3296 (22)
K. pneumoniae	518 (3)
P. mirabilis	226 (2)
E. cloacae	190 (1)
P. aeruginosa	297 (2)
Acinetobacter spp.	45 (0)
E. faecalis	481 (3)
E. faecium	436 (3)
S. aureus	1428 (10)
CNS	5132 (35)
Other Enterobacteriaceae*	629 (4)
Other non-fermenters**	33 (0)
Other gram-positives	2147 (14)

* *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.*

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Table 4.3.4.2 Resistance levels (%) among clinical blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from inpatient departments (incl. intensive care units), ISIS-AR 2014

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	47	-	-	23	-
co-amoxiclav	20	10	-	12	-
piperacillin-tazobactam	5	6	-	1	9
cefuroxime	12	11	-	1	-
cefotaxime/ceftriaxone	6	5	-	1	-
ceftazidime	3	3	-	1	6
meropenem/imipenem	0	0	0	0	5
ciprofloxacin	15	5	9	12	6
gentamicin	5	4	6	7	2
tobramycin	6	5	6	5	0
co-trimoxazole	27	11	11	24	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	-	6	-
gentamicin + co-amoxiclav	3	3	-	4	-
gentamicin + piperacillin-tazobactam	0	2	-	0	1
gentamicin + cefuroxime	2	3	-	0	-
gentamicin + cefotaxime/ceftriaxone	2	2	-	0	-
gentamicin + ceftazidime	1	2	-	0	0
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	0
Multi-drug resistance					
HRMO*	8	6	3	5	2

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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*

* *Highly Resistant Microorganism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP); for all Enterobacteriaceae except E. cloacae as resistant to cefotaxim/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.*

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Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical blood isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from inpatient departments (incl. intensive care units) in ISIS-AR.

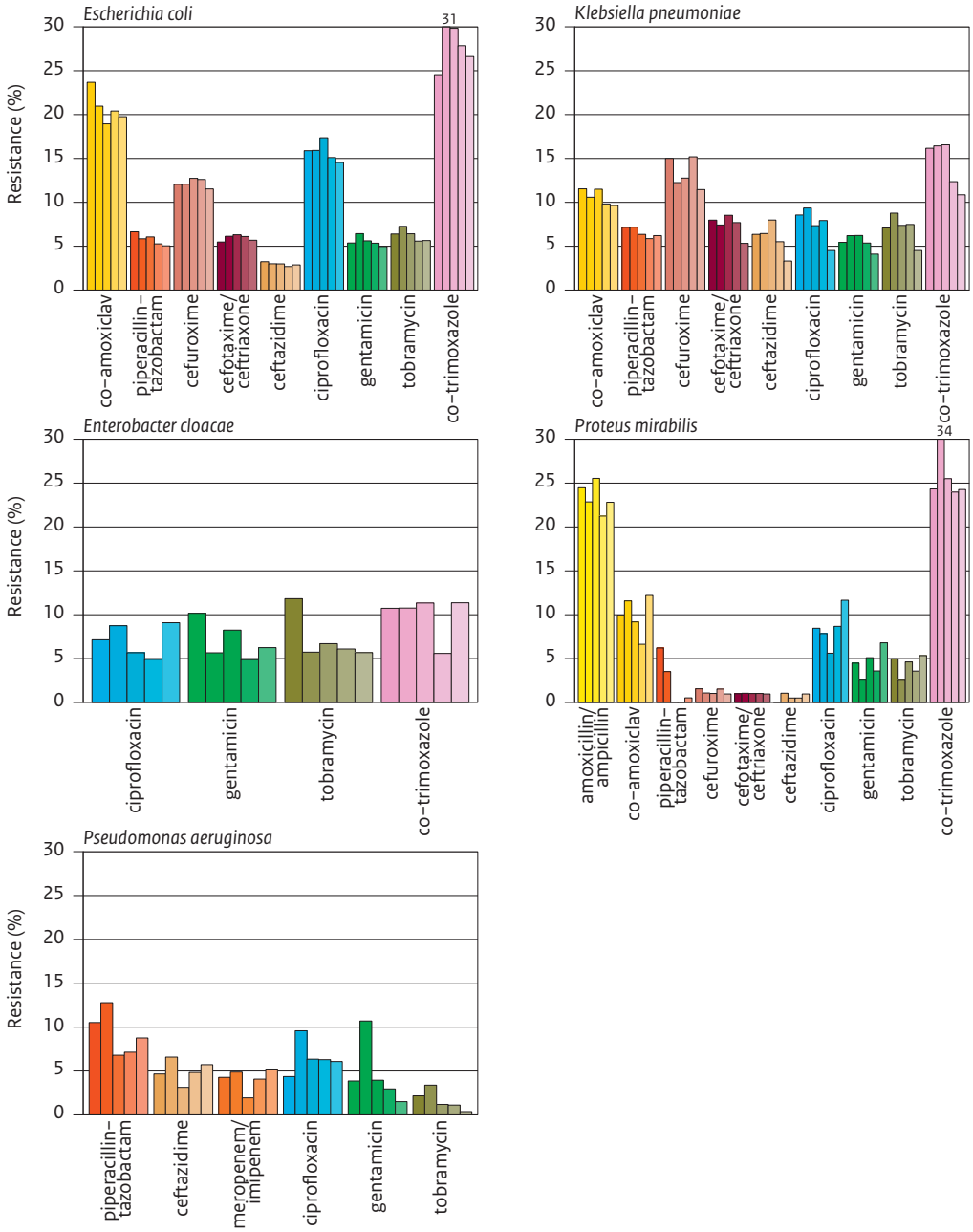

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Table 4.3.4.3 Resistance levels (%) among clinical blood isolates of *E. faecalis* and *E. faecium* from inpatient departments (incl. intensive care units), ISIS-AR 2014

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

- *Resistance not calculated*

Table 4.3.4.4 Resistance levels among clinical blood isolates of *S. aureus* from inpatient departments (incl. intensive care units), ISIS-AR 2014

	S. aureus	
Antibiotic		
ciprofloxacin*	9	
gentamicin	1	
erythromycin	10	
clindamycin	2	
clindamycin including inducible resistance**	9	
doxycycline/tetracycline	2	
linezolid	0	
co-trimoxazole	3	
rifampicin	0	

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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 ciprofloxacin is meant as class indicator for resistance against fluoroquinolones.*
** *To estimate clindamycin resistance S-I-R interpretation of the laboratories was used (see methods for more detailed information).*

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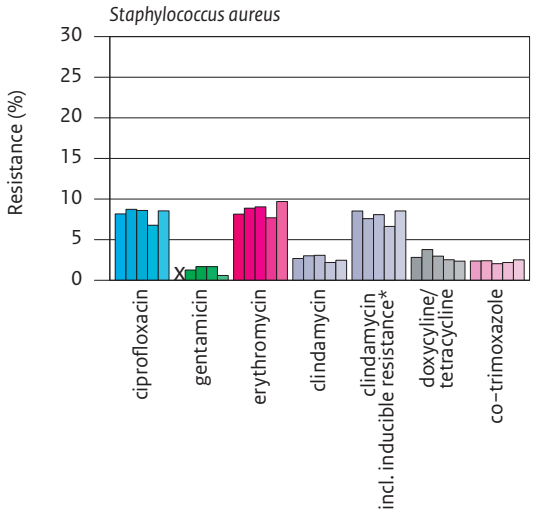
Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2010 to 2014) among clinical blood isolates of *S. aureus* from inpatient departments (incl. intensive care units) in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



Key results

Enterobacteriaceae and P. aeruginosa

- Resistance levels were similar to resistance levels in all materials combined, which are described in chapter 4.3.2 (inpatient departments excl. ICU) and 4.3.3 (ICU). Compared with those results, somewhat lower resistance levels were found for all agents in *K. pneumoniae*, such as for cefuroxime (11% in blood versus 13% in all materials). Somewhat higher resistance levels were found in *E. cloacae*, for co-trimoxazole (11% versus 9%) and ciprofloxacin (9% versus 5%) and in *P. mirabilis* for ciprofloxacin (12% versus 9%).
- Resistance to most agents was lower in 2014 in *K. pneumoniae* compared with previous years, especially for ceftazidime (from 6% to 3%), ciprofloxacin (from 9% to 5%) and co-trimoxazole (from 16% to 11%), which showed a statistically significant and clinically relevant decreasing trend between 2010 and 2014.
- Significant and relevant decreasing five-year trends were also seen for tobramycin resistance in *E. cloacae* (from 12% to 6%), piperacillin-tazobactam resistance in *P. mirabilis* (from 6% to 1%) and gentamicin resistance in *P. aeruginosa* (from 4% to 2%).
- Combined resistance to gentamicin + co-amoxiclav in *P. mirabilis* increased from 0% in 2010 to 4% in 2014, which was considered clinically relevant as well.
- HRMO resistance levels remained stable over time.

Enterococci and S. aureus

- Resistance levels in blood showed no difference compared with resistance levels in all materials.

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4.3.5 Urology services

Table 4.3.5.1 shows the distribution of pathogens in urine from urology outpatient departments (OPD) and urology inpatient departments (IPD). The resistance levels for the outpatient departments in 2014 are shown 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 are presented in figures 4.3.5.1 for the respective pathogens (except for *E. faecalis*).

Table 4.3.5.1 Distribution of isolated pathogens N (%) in clinical specimens from urology outpatient departments (OPD) and urology inpatient departments (IPD), ISIS-AR 2014

Pathogen	OPD N(%)	IPD N(%)	
E. coli	7653 (41)	1148 (30)	
K. pneumoniae	1325 (7)	234 (6)	
P. mirabilis	990 (5)	189 (5)	
P. aeruginosa	669 (4)	253 (7)	
E. faecalis	1970 (11)	482 (13)	
Other Enterobacteriaceae*	2220 (12)	601 (16)	
Other non-fermenters**	349 (2)	118 (3)	
Other *Enterococcus* spp.	491 (3)	186 (5)	
Other gram-positives	3028 (16)	587 (15)	

* *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.*

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Table 4.3.5.2 Resistance levels among urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments (OPD) and urology inpatient departments (IPD), ISIS-AR 2014

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	47	53	-	-	24	23	-	-
co-amoxiclav	19	21	8	14	11	13	-	-
piperacillin-tazobactam	5	6	5	10	-	0	5	8
cefuroxime	12	17	13	14	1	1	-	-
cefotaxime/ceftriaxone	5	8	5	10	1	1	-	-
ceftazidime	2	4	3	7	0	1	3	3
meropenem/imipenem	0	0	0	0	0	0	3	4
ciprofloxacin	21	30	6	9	11	15	9	11
gentamicin	6	9	3	7	7	9	3	4
tobramycin	6	10	4	8	4	8	1	0
co-trimoxazole	31	32	12	21	30	34	-	-
nitrofurantoin	5	4	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ ampicillin	6	8	-	-	6	6	-	-
gentamicin + co-amoxiclav	3	5	2	6	2	4	-	-
gentamicin + piperacillin-tazobactam	-	1	-	4	-	0	1	1
gentamicin + cefuroxime	2	4	2	5	0	0	-	-
gentamicin + cefotaxime/ ceftriaxone	1	2	2	4	0	0	-	-
gentamicin + ceftazidime	1	1	1	3	0	0	0	0
tobramycin + ceftazidime	-	-	-	-	-	-	1	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	1	0
Multi-drug resistance								
HRMO*	8	14	6	11	4	6	1	1
multidrug-resistance**	6	-	2	-	2	-	-	-

10	Significant and clinically relevant increasing trend since 2010
10	Significant and clinically relevant decreasing trend since 2010
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*

* *Highly Resistant Microorganism (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*

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Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2010 to 2014) among urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments and urology inpatient departments in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.

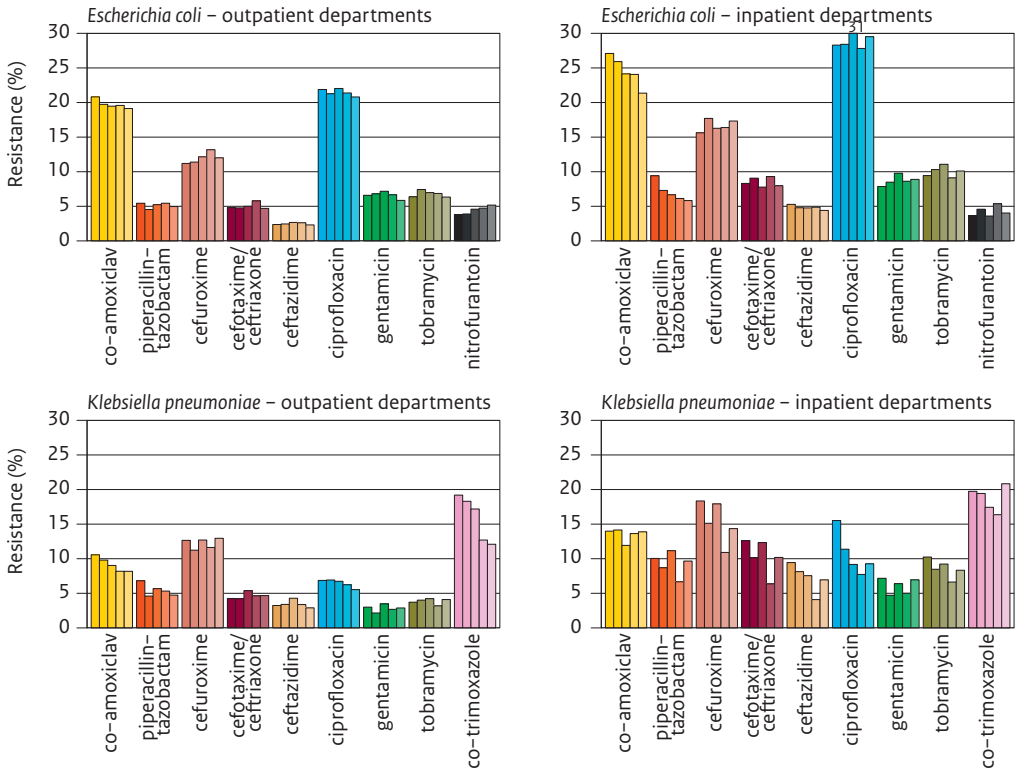

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Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2010 to 2014) among urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments and urology inpatient departments in ISIS-AR.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.

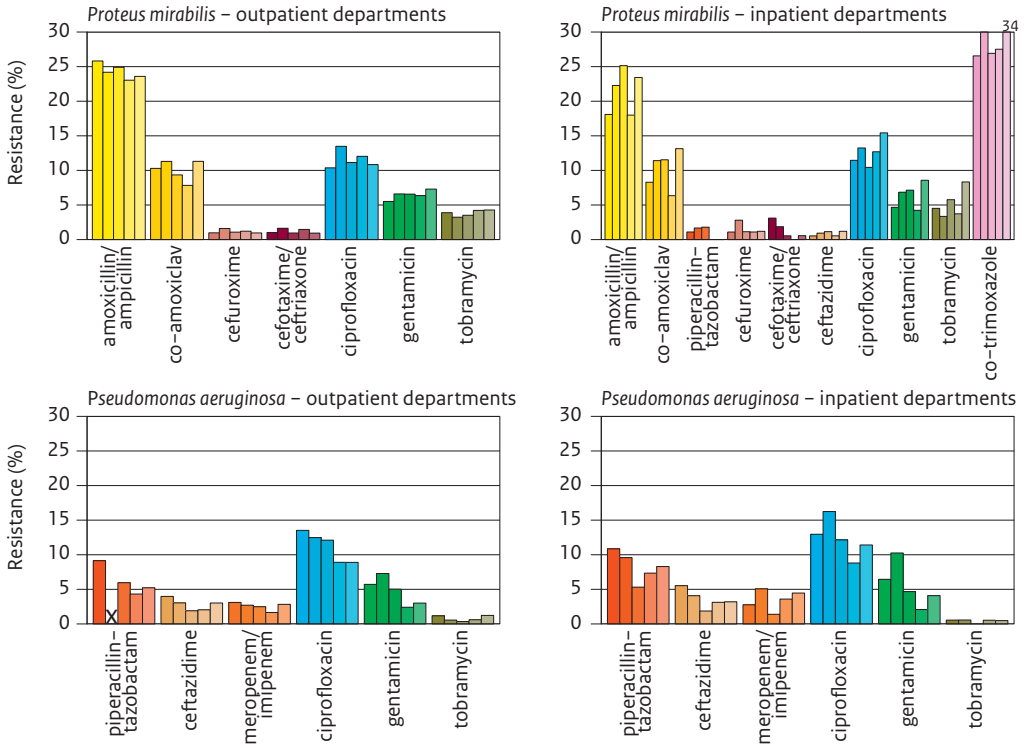

Table 4.3.5.3 Resistance levels among urinary isolates of *E. faecalis* from urology outpatient departments (OPD) and urology inpatient departments (IPD), ISIS-AR 2014

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

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Key results

Enterobacteriaceae

- In general, resistance to all tested agents was higher in patients from urology inpatient departments than in patients from urology outpatient departments.
- Low resistance levels were found for imipenem/meropenem (0%) and ceftazidime (≤7%) in all Enterobacteriaceae. Low resistance was also found for nitrofurantoin (≤5%) in *E. coli*, for cefotaxime/ceftriaxone (1%) and for cefuroxime (1%) in *P. mirabilis.*
- High levels of resistance were found for amoxicillin/ampicillin (≥23%), and co-trimoxazole (≥12%) in all Enterobacteriaceae and for co-amoxiclav (≥19%) and ciprofloxacin (≥21%) in *E. coli.* However, resistance to co-amoxiclav decreased from 27% in 2010 to 21% in 2014 in patients from inpatient departments.
- In *E.coli*, resistance to co-amoxiclav (27% to 21%) and piperacillin-tazobactam (from 9% to 6%) in patients from inpatient department decreased significantly and to a clinically relevant extent between 2010 and 2014.
- Significantly and relevantly decreasing five-year trends in resistance were also seen for *K. pneumoniae*: for co-amoxiclav (from 11% to 8%) and co-trimoxazole (from 19% to 12%) in patients from outpatient departments, and for ciprofloxacin in patients from inpatient departments (from 16% to 9%).
- Cefotaxime/ceftriaxone resistance in *P. mirabilis* significantly and relevantly decreased from 3% to 1% between 2010 and 2014 in patients from inpatient departments. Combined resistance to gentamicin + co-amoxiclav in inpatient departments increased from 1% in 2010 to 4% in 2014, which was considered clinically relevant as well.
- Multidrug resistance to co-trimoxazole, co-amoxiclav and ciprofloxacin combined, was ≤6% in all Enterobacteriaceae among patients from outpatient departments.
- HRMO levels ranged between 4% and 14% in all Enterobacteriaceae.

P. aeruginosa

- Resistance to all tested agents was below 8%, except for ciprofloxacin that had a resistance percentage of 11% in inpatient departments.
- Resistance to piperacillin-tazobactam (from 9% to 5%), ciprofloxacin (from 14% to 9%) and gentamicin (from 6% to 3%) showed a significant and relevant decrease in the last five years in outpatient departments, whereas for inpatient departments this was only the case for gentamicin (from 6% to 4%).
- The percentage HRMO remained low (≤1%).

***Enterococcus* spp.**

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

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4.3.6 Respiratory pathogens

For respiratory pathogens, resistance levels were calculated for hospitals only. The number of isolates from general practitioner's patients was too low to calculate resistance levels. Table 4.3.6.1 and table 4.3.6.2 show the distribution and resistance levels of pathogens isolated from patients admitted to hospital departments (including intensive care units). For *S. pneumoniae* and *H. influenzae* isolates less than 50% of the laboratories tested co-trimoxazole susceptibility. Therefore, co-trimoxazole resistance levels in were not presented for these pathogens.
In Dutch hospitals, pathogens from respiratory tract infections are routinely cultured when a lower respiratory tract infection is suspected. However, resistance levels in hospital patients may be higher than in the community, as hospital patients may 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 inpatient departments (incl. intensive care units) and outpatient departments, ISIS-AR 2014

	Blood or Cerebrospinal fluid	Lower respiratory tract
Pathogen	**N(%)**	**N(%)**
S. pneumoniae	664 (89)	1678 (25)
H. influenzae	77 (10)	3835 (58)
M. catarrhalis	5 (1)	1084 (16)

Table 4.3.6.2 Resistance levels (%) among isolated respiratory pathogens from clinical specimens from inpatient departments (incl. intensive care units) and outpatient departments, ISIS-AR 2014

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin	0	-	-
amoxicillin/ampicillin	-	18	-
co-amoxiclav	-	6	1
erythromycin	10	-	3
doxycyclin/tetracyclin	9	1	2
co-trimoxazole	-	-	3

- *Resistance not calculated*

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Key results

S. pneumoniae

- Resistance to penicillin (0%) was still rare.
- Resistance to erythromycin (10%) and doxycyclin (9%) was similar to previous years.

H. influenzae

- Resistance to amoxicillin (18%) remained high.
- Resistance to doxycyclin/tetracyclin (1%) remained low.

M. catarrhalis

- Resistance to each of the tested agents was lower than 4%. This is comparable with resistance in previous years.

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4.4 Highly resistant microorganisms

4.4.1 Carbapenem-Resistant Enterobacteriaceae

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE), particularly *Klebsiella pneumoniae* and *Escherichia coli*, are a growing worldwide public health threat. Because carbapenems represent a drug of last resort for treatment of many enterobacterial infections, 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 seen 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].

Many different carbapenemase-encoding genes and allelic variants thereof have been identified thus far. They are classified into two major molecular families based on their active sites: serine-carbapenemases with main representatives KPC (Ambler class A) and OXA-48 (Ambler class D), and metallo-carbapenemases (Ambler class B), of which NDM, VIM, and IMP are the most commonly detected members. The public health threat of the carbapanemase-producing *Enterobacteriaceae* (CPE) is compounded by the fact that these carbapenemase genes are often found on mobile genetic elements that simultaneously encode resistance to other antimicrobial groups, resulting in multi-drug resistance, and may facilitate carbapenemase genes to spread to epidemiologically successful clones through horizontal gene-transfer within and between species[1].

Up till now, in the Netherlands CPE are infrequently detected, mainly from patients transferred from a foreign hospital, and their spread is generally well controlled. The epidemiology of CPE in 2013 was classified as that of "sporadic hospital outbreaks"; defined as unrelated hospital outbreaks with independent, i.e. epidemiologically unrelated introduction or different strains, no autochthonous inter-institutional transmission reported[3]. Here we provide an overview of the current epidemiology of CRE and CPE based on ISIS-AR and isolates received at the RIVM for phenotypic and genotypic confirmation

Prevalence of CRE in The Netherlands

The ISIS-AR database (years 2013-2014) 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 2013 clinical breakpoints (MIC>2 mg/L), or ii) screen positive for meropenem (MIC>0.25mg/L) and/or imipenem (MIC>1 mg/L) as defined by NVMM (NVMM Guideline Laboratory detection of highly resistant microorganisms, version 2.0, 2012). Both screening and clinical isolates were included. Because ISIS-AR, does not routinely collect data on presence of carbapenemase genes for carbapenem-resistant *Enterobacteriacea*, each of the 34 participating laboratories was sent a spreadsheet with all selected isolates and was requested to provide any additional results from confirmatory testing of included isolates. In addition, we asked what procedures were used by the lab

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to confirm the carbapenemase production in *Enterobacteriaceae*. Twenty-four (71%) laboratories responded to the request.

Results of sequential testing of carbapenem susceptibility and genotypic/phenotypic testing of carbapenemase production, as prescribed by the NVMM[5], are presented in figure 4.4.1.1. Only one isolate per patient, i.e. the most resistant and most completely tested isolate, was included in the analysis. Both clinical and screening isolates were included. For the majority of isolates found non-susceptible (74.2%) and over half of isolates found screen-positive (54.7%) on automated testing, a gradient strip test result was available to confirm the result. In the majority of confirmed non-susceptible *E. coli* (70.0%) and *K. pneumoniae* (85.1%) molecular testing for presence of a carbapenemase-encoding gene was performed, but in less than half of confirmed screen-positive *E. coli* (42.5%) and *K. pneumoniae* (35.0%) a molecular test was performed.

The overall proportion of confirmed non-susceptible *E. coli* and *K. pneumoniae* was 0.01% and 0.15% respectively. A carbapenemase-encoding gene was found in 60.0% and 67.5% of non-susceptible *E. coli* and *K. pneumoniae* isolates, respectively. A carbapenemase encoding gene was found in 41.1% and 42.9% of screen-positive *E. coli* and *K. pneumoniae* isolates, respectively**.** The most common carbapenemase genes found were OXA-48 and NDM in *E. coli* and OXA-48 and KPC in *K. pneumoniae* (figure 4.4.1.1).

The high false-detection rate of around 88.8% and 44.7% of automatic testing for detecting carbapenem non-susceptibile *E. coli* and *K. pneumoniae*, respectively, is consistent with the fact that the positive predictive value of a diagnostic test is low in settings where the condition of interest is rare, even for tests with high sensitivity and specificity[6]. This observation underscores the necessity for confirmatory testing of unusual or rare resistance patterns to correct for the high number of spurious positive results on automated testing.

Epidemiology

In 2014, a total of 249 unique Enterobacteriaceae isolates from 226 patients were submitted to the RIVM by 41 laboratories for phenotypic and genotypic confirmation. In addition, clinical and epidemiological information related to these isolates is collected using a web-based questionnaire to gain insight into the main risk factors for colonization/infection with CRE in the Netherlands and the predominant classes and variants of carbapenemases produced by these strains.
At the RIVM, meropenem MIC was confirmed by E-test and carbapenemase-activity was assessed phenotypically by an in-house developed assay, the carbapenem-inactivation method (CIM)[7]. For genotypic confirmation of the presence of carbapenemases, a multiplex-PCR targeting genes encoding IMP, VIM, NDM, OXA-48 and KPC carbapenemases was performed.

In total, for 68 (27.3%) out of 249 isolates the presence of a carbapenemase-encoding gene was confirmed both phenotypically and genotypically. The predominant species among these 68 confirmed CPE's were *K. pneumoniae* and *E. coli*, and OXA-48 was the most frequently found carbapenemase (Table 4.4.1.1).

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For 42 of the 68 confirmed CPE isolates additional epidemiologic data, collected through questionnaire, was available. These data indicated that 27 (64.3%) isolates were detected through targeted screening; because a patient had a history of admission to a foreign hospital within the previous 2 months (n=19), had traveled abroad (n=4), was a known carrier (n=3) and for one such patient no risk factor was reported. Foreign hospital admissions were in Morocco (n=6), Turkey (n=2), Egypt (n=2), Brasil (n=1), Colombia (n=1), France (n=1), India (n=1), Italy (n=1), Kenia (n=1), Lebanon (n=1), Nigeria (n=1) and Spain (n=1). Fifteen (35.7%) isolates were unexpected findings; detected in samples taken on clinical indication (n=11) or during routine screening (e.g. as part of SDD protocol, n=4). Isolates detected in clinical samples were detected in urine (n=9), sputum (n=1), pleura drain (n=1) and rectum smear (n=1). For only 5 of 15 unexpected findings a risk factor could be identified retrospectively; history of admission to a foreign hospital within previous 2 months (Morocco and Egypt, n=2), a history of admission to a foreign hospital more than 2 months previous (Albania, n=1) or were from a country where CPE are known to be endemic (Morocco and Syria, n=2). For 10 of 15 unexpected findings no conclusion about the source could be deduced from the questionnaire. The majority (n=7) of these 10 unexpected and unexplained CPE were *K. pneumoniae* with an OXA-48 carbapenemase gene.

Conclusion

In conclusion, as in previous years in 2013-14 CRE were a rare occurrence in the Netherlands; only 0.01% of *E. coli* and 0.15% of *K. pneumoniae* were non-susceptible to carbapenems. The large part of isolates submitted to RIVM (64.3%) was detected through targeted screening. Importantly, a substantial number of isolates (10/42, 23.8%) were unexpected findings and, based on available data, could not be traced to a known risk factor. OXA-48 was the most prevalent carbapenemase detected, but NDM and KPC were also prevalent.

Table 4.4.1.1 Carbapenemases carried by the predominant Enterobacteriaceae submitted during 2014 as detected by PCR.

Species	KPC	OXA-48	NDM	VIM	Total
K. pneumoniae	7	28	4	1	40
E. coli	1	7	4	2	14
E. cloacae		2	2	1	5
C. freundii	1	1	1		3
Others		3	1	2	6
Total	9	41	12	6	68

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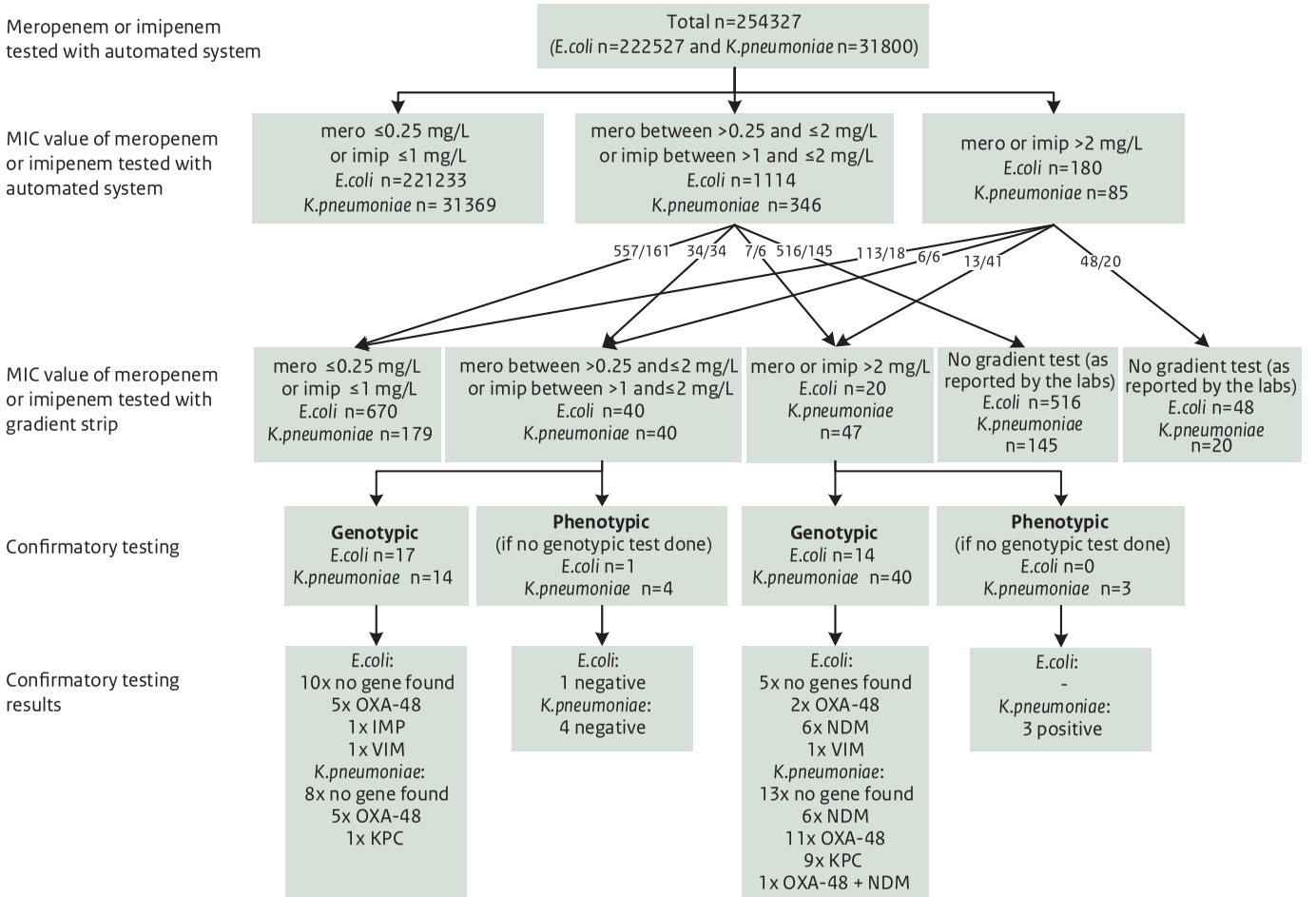
Figure 4.4.1.1 Results from sequential testing of carbapenem susceptibility and genotypic/phenotypic testing of carbapenemase production, according to NVMM Guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012), in 24 laboratories participating in ISIS-AR.



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4.4.2 Vancomycin Resistant *Enterococci* in Dutch hospitals

Incidence of VRE in the Netherlands

Table 4.4.2.1 shows the incidence of VRE in various hospital departments in the Netherlands based on ISIS-AR. The highest percentage was found in Intensive Care Units, amounting to 0.4% of isolates.

Epidemiology

In 2014 VRE outbreaks were reported in 14 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, 31 hospital outbreaks with VRE have been reported in the Netherlands. Since the UMC Utrecht started to offer molecular diagnostics on clinical VRE-isolates, which started in May 2012, 37 hospitals have sent 515 VRE to the UMC Utrecht (status of March 20th 2015). These represented 263 strains carrying the *vanA* gene cluster, 249 the *vanB* gene cluster, 1 strain carried both the *vanA* and the *vanB* gene cluster and two isolates carried the *vanD* gene cluster. Of these 515 VRE, 469 were typed by Multi Locus Sequence Typing MLST. This revealed a total of 31 different Sequence Types , suggesting that at least 31 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, 14 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 frequent STs include ST117 (21 hospitals), ST203 (15 hospitals), ST18 (13 hospitals), ST80 (7 hospitals).

Prognosis

To enhance the resolution of the current *E. faecium* MLST scheme, which is currently being used to study the molecular epidemiology of VRE in hospitals in the Netherlands, the UMC Utrecht has developed and is currently evaluating a standardized core genome allele-based typing scheme, or core genome MLST (cgMLST) scheme. In this scheme the allelic variation in 1423 core genes is indexed, instead of the seven genes in classical MLST. This cgMLST scheme was constructed using 40 *E. faecium* strains from an international collection that represented all three *E. faecium* clades (LeBreton *et al.*, mBio 2013) and all major BAPS groups (Willems *et al.*, mBio 2012). Current evaluation involves the performance analyses of this scheme using 99 *E. faecium* strains from five well-defined VRE outbreaks from three countries as well 71 epidemiologically unrelated strains. It is to be expected that this scheme and accompanying database will be available and fully operational second half of 2015.

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

Type of department	Number of isolates tested for all relevant antibiotic classes	Absolute number of VRE*	Percentage VRE*
GP	187	0	0
Outpatient departments	366	1	0.3
Inpatient departments excluding Intensive Care Units	1348	3	0.2
Intensive Care Units	512	2	0.4

**VRE is defined as resistant to amoxicillin/ampicillin and vancomycin.*
Numbers based on a selection of 21 laboratories
The first Enterococcus faecium isolate per patient was selected
Based on interpretation of the laboratories

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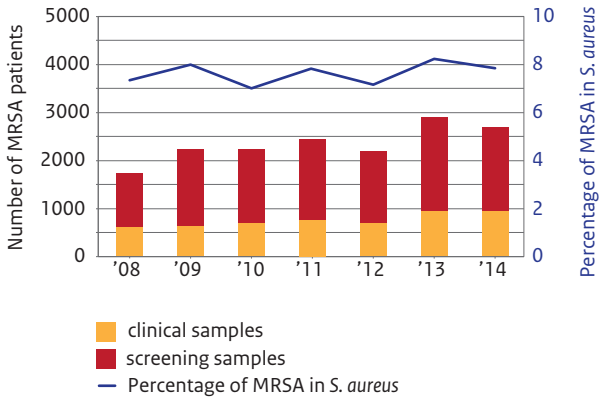
4.4.3 Methicillin resistant *Staphylococcus aureus*

The Netherlands has maintained low levels of methicillin-resistant *Staphylococcus aureus* (MRSA), despite high MRSA levels in surrounding countries. This is, for a large part, due to the thorough 'search and destroy' containment program[1], which identifies potentially colonized and infected persons in order to implement measures to prevent transmission.

We identified *Staphylococcus aureus* isolates and MRSA isolates in the ISIS-AR database. ISIS-AR received data from 33 laboratories in 2014. We selected the most resistant *S. aureus* and the most invasive isolate per patient. If both a screening and a clinical sample were available, we selected the clinical sample. The results were compared with those in previous years.
In 2014 in ISIS-AR, there were 50,210 unique patients with at least one *Staphylococcus aureus* isolate. For the majority of patients a clinical sample was available (n=46,920; 93%), and screening samples only were available from 3290 patients (7%).
In 2014, the percentage of methicillin resistance was 2% in S. *aureus* isolates from clinical samples (894 out of 46,920) and 53% in isolates from screening samples (1751 out of 3290) in 2014. Most clinical samples were wound or pus samples (54%), while most screening samples were throat, nose and perineum samples (70%) (table 4.4.3.1). Thirty-one patients had MRSA isolated from blood or cerebrospinal fluid. MRSA isolates were found in patients from general practices and hospital departments, but were mostly found in patients from outpatient departments (~40%).

The number of patients with an MRSA isolate over time is shown in figure 4.4.3.1. Here, we observe a small increase in the number of patients with either a clinical sample or a screening sample over the years, but the number of laboratories used in the analysis each year increased over time. Thus, the increase in absolute numbers can be explained by the increase in laboratories that joined ISIS-AR over time. Another explanation is the emergence of livestock-associated MRSA since 2003[2] and the inclusion

Figure 4.4.3.1 The number of MRSA patients detected by screening samples or clinical samples per year.



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of the risk factor 'pig farmers' in the MRSA guidelines in 2005[1], after which screening of this risk group increased[3]. However, the percentage of MRSA in *S. aureus* was stable over time from the year 2008 to 2014. This suggests that the increase in absolute numbers of MRSA over time is more due to the increase in the number of laboratories joining ISIS-AR, as more laboratories in ISIS-AR would result in the same level of MRSA, whereas more screening of MRSA would lead to a higher MRSA level.

The coverage of ISIS-AR for this analysis was 55%; 32 out of 58 laboratories in the Netherlands. For hospitals, we estimate a higher coverage of around 65-70%. If the number of MRSA patients found in ISIS-AR in 2014 is corrected with a factor of 1.4-1.5 (65-70%) the total number of MRSA patients in The Netherlands in 2014 is estimated at 3779-4069 patients.

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Table 4.4.3.1 Characteristics of methicillin resistant *Staphylococcus aureus* (MRSA) isolates from unique patients (selection of the most resistant *S. aureus* and the most invasive isolate per patient and a selection of clinical samples over screening samples).

	Clinical samples	Screening samples
MRSA	894	1751
Materials		
Blood or cerebrospinal fluid	31 (3%)	0 (0%)
Lower respiratory tract	101 (11%)	7 (0%)
Urine	112 (13%)	12 (1%)
Wound or Pus	487 (54%)	479 (27%)
Throat, nose and perineum	141 (16%)	1223 (70%)
Other materials	22 (2%)	30 (2%)
Site		
General practices	200 (22%)	503 (29%)
Outpatient departments	355 (40%)	679 (39%)
Inpatient departments	256 (29%)	304 (17%)
Other sites	83 (9%)	265 (15%)

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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. The emergence of multidrug-resistant (MDR) *P. aeruginosa* is a problem of global concern, and currently there are reports of hospital outbreaks of MDR *P. aeruginosa* from countries around the world, including The Netherlands. *P. aeruginosa* may become MDR due to the simultaneous acquisition of several resistance genes that are clustered in integrons through horizontal gene tranfer. 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 positive *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. However, sometimes bla_{VIM} is carried by plasmids and most other carbapenemase encoding genes in *P. aeruginosa* and other Gram-negatives are virtually always 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 nosocomial infections. Of latter group, worldwide, the most frequently found species associated with hospital infections is *Acinetobacter baumannii*. However, the numbers of infections due to MDR-*Pseudomonas* spp. and MDR-*Acinetobacter* spp. in The Netherlands is not known yet. As other non-fermenter species may not only cause disease by themselves, but also serve as a source for resistance genes such as the carbapenemase-encoding genes, they have been included in this chapter.

Prevalence in The Netherlands

In 2014, multi-resistant *P. aeruginosa*, as defined by the working group of infection prevention (WIP) in their guideline "Highly resistant microorganisms (HRMO)", was detected in 1.36 percent of all isolates the in- and out-hospital patients (table 4.4.4.1). The frequency of HMRO in out-hospital patients was comparable to that in in-hospital patients. This means that patients carrying these bugs may transfer them into the community. However, until now, no niche or even source in the community has been identified. Of all patients cultured with carbapenem resistant *P. aeruginosa*, 21.6 % were cared for at the ICU. Carbapenem-resistance was present in 72% of HRMO-*P. aeruginosa*. In other words, in case of an HRMO, the chance that this was (partly) due to carbapenem resistance was very high. However, the mechanism of resistance is not registered in the surveillance and can be either due to carbapenemase activity or alteration of outer membrane porins and increased efflux pump activity. In patients with CF, the resistance mechanism is hardly ever by carbapenemase production, suggesting reduced cell wall permeability remains the key resistance mechanism in CF isolates. To understand and prevent infections by HRMO *P. aeruginosa*, the mechanisms of resistance is important to know and effort should be made to obtain more detailed information on HRMO *P. aeruginosa*. In surveillance, both epidemiology and genetic variations should be addressed to learn and to predict epidemiology and to be able to prevent by interventions further increase of HRMO *P. aeruginosa*.

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Epidemiology

Since 2010 the RIVM performs surveillance of carbapenemase-producing Enterobacteriaceae (CPE). Medical microbiology laboratories (MMLs) are asked to send there Enterobacteriaceae isolates with a MIC for meropenem ≥ 0.25 µg/ml. This surveillance is far from complete and biased as only a fraction of the isolates found in the MMLs, is sent to the RIVM and the motives for sending the strains are diverse. Surprisingly, the majority of the isolates are non-fermenters rather than Enterobacteriaceae. For this reason these data cannot be used to infer prevalence or accurate distribution of carbapenemase producing Gram-negatives in the Netherlands.

Isolates were analyzed phenotypically for carbapenemase production by the CIM assay. In addition, the presence of genes encoding for the carbapenemases was assessed by a multiplex PCR that detects the bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM} and bla_{IMP} genes. There is a clear association between MIC, carbapenemase activity and presence of carbapenemase encoding genes (Table 4.4.4.2). A considerable number of the non-fermenter isolates produced carbapenemase, but no gene was identified. The majority of these isolates were *Pseudomonas* spp. other than *P. aeruginosa* and *Acinetobacter baumannii* isolates.

Of all 441 non-fermenter isolates the vast majority (76%) were *P. aeruginosa* and 22% of these *P. aeruginosa* carried a carbapenemase encoding gene (Table 4.4.4.3). Of latter isolates 88% carried the bla_{VIM} gene. There was a single *Pseudomonas monteilii* isolate that carried two carbapenemase encoding genes: bla_{VIM} and bla_{IMP}. The only PCR-positive *A. baumannii* isolate carried a bla_{NDM} gene. National data from ISIS-AR (table 4.4.4.4) shows up until now a very low prevalence of carbapenem resistent *Acinetobacter* spp.

Prognosis

The number of HRMO-*P. aeruginosa* will likely increase in near future. Not only due to increasing import of HRMO in general, but also because patients have increasingly more severe underlying diseases adding to the complexity of their care. The risk factors for *P. aeruginosa* acquisition are use of carbapenems, of other antibiotic and of medical devices. As patients in the hospital will get more medical devices (days) and carbapenem usage is increasing due to treatment of infections with HRMO

Table 4.4.4.1 Number of multidrug resistant *P. aeruginosa* in the Netherlands as reported in the ISIS-AR surveillance database in 2014 (first isolate per person only).

Type of departement	No. of isolates	No. of MDR *P. aeruginosa** (%)	No. of MDR *P. aeruginosa* resistant to carbapenems (%)
GP	2270	0 (0)	
Out-patient departements	3251	37 (1.1)	27 (73)
In-patient departements excluding ICUs	3722	43 (1.2)	31 (72)
ICUs	582	23 (4)	16 (70)

* *Multidrug resistant (MDR) P. aeruginosa is defined as resistant to >=3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

Numbers are based on a selection of 21 laboratories

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(ESBL) Enterobacteriaceae, the number of HRMO-*P. aeruginosa* infections will increase as well. Furthermore, when an HRMO *P. aeruginosa* has been present in the hospital, there is a risk that this microorganism may contaminate and finally persist in bathrooms or sinks. Efforts to eradicate *P. aeruginosa* from these potential sources are up until now disappointing. This may eventually lead to uncontrollable and ongoing transmission in the hospital and other health care centers.

Table 4.4.4.2 Assessment of carbapenemase activity (CIM) and carbapenemase encoding genes (PCR) among non-fermenter isolates submitted to the RIVM in 2014 (first isolate per patient only).

Meropenem MIC	n	No. of CIM-pos. (%)	No. of PCR-pos. (%)
≤ 0.25 µg/ml	10	3 (30)[1]	0 (0)
>0.25 - ≤ 2 µg/ml	58	5 (8.6)	4 (6.9)
>2 µg/ml	373	110 (29.5)	73 (19.6)
All	441	118 (26.8)	76 (17.2)[1]

[1] Three *Aeromonas* species were CIM-positive, but no gene was detected
[2] One *P. monteilii* isolate was PCR-positive for both bla_{IMP} and bla_{VIM}

Table 4.4.4.3 Carbapenemase encoding genes in the non-fermenter isolates submitted during 2014 as detected by PCR (first isolate per patient only).

	PCR detection of carbapenemase encoding gene					
Species	bla_{VIM}	bla_{VIM} + bla_{IMP}	bla_{IMP}	bla_{NDM}	No gene detected	Total
Pseudomonas aeruginosa	65		7	2	262	336
Pseudomonas spp.		1			51	52
Acinetobacter baumannii				1	27	28
Acinetobacter spp.					8	8
Other non-fermenters					17	17
Total	**65**	**1**	**7**	**3**	**365**	**441**

Table 4.4.4.4 Number of multidrug resistant *Acinetobacter* spp. in the Netherlands as reported in the ISIS-AR surveillance database in 2014 (first isolate per person only).

Type of department	No. of isolates	No. of MDR* Acinetobacter spp.(%)
GP	1113	0 (0)
Out-patient departments	615	1 (0.2)
In-patient departments excluding ICUs	426	4 (0.9)
ICUs	73	1 (1.4)

* *MDR Acinetobacter spp. is defined as resistant to meropenem/imipenem and ciprofloxacine and gentamicine and/or tobramycine. Numbers are based on a selection of 21 laboratories*

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4.4.5 Extended spectrum Beta-lactamase producing bacteria

Extended spectrum Beta-lactamase (ESBL) producing Gram-negative bacteria have become a concern over the years in various countries. Initially ESBL/AmpC-producing organisms were associated with hospitals and institutional care in humans, but they are now increasingly found in the community, in food-producing animals and the environment[1].

In previous Nethmap reports we showed an increasing trend of ESBL-producing bacteria in hospitalized patients and patients consulting their general practitioner (GP). To anticipate on the potential threat of ESBPL producing bacteria, several studies have tried to determine the prevalence of ESBL carriage in the Dutch community. A large nationwide study in 2006 among 22 laboratories including 1880 strains showed 5.8% phenotypically confirmed positive ESBL[2]. Subsequent studies in 2011-2013 showed percentages between 5.1% and 8.2% in the general population and up to 10.1% of ESBL-producing Enterobacteriaceae in Dutch primary care patients with gastrointestinal complaints. All these suggest an increase in prevalence of ESBL in the Dutch population in the last decade[3-6]. Depending on the population under study several risk factors have been reported: nursing home residency, prior hospitalization and/or antibiotic use, having ESBL-positive family members, contact with companion animals and travelling. In 2012-2014 a study was performed by Bunt et al. to assess ESBL/AmpC prevalence in Dutch households[7]. Using a questionnaire, a large survey and fecal samples, they found a prevalence of ESBL in young children of 4.2%. They also found a significant association between day care attendance and ESBL/AmpC carriage in both children and parents, and having a high likelyhood of sharing the same genotypes. The most common genotype found was CTX-M15 (one-third of individuals).

In summary, the overall prevalence of ESBLs in the general population at present appears to be below 10%. Routes of transmission between animals, humans, and the environment and the relative contribution of each source, remain to be elucidated.

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

The Signaling Consultation of Hospital acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded in 2012. The purpose of the Signaling Consultation is the prevention or mitigation of large-scale outbreaks in hospitals through early recognition. The SO-ZI/AMR assesses the risk of the outbreak to public health and may advise a hospital to request external expertise. The SO-ZI/AMR also monitors the course of the outbreak. Based on this risk assessment and course, outbreaks are categorized in phases. Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to the SO-ZI/AMR.

In 2014, a total of 55 new outbreaks were reported by 37 healthcare institutions (4 nursing homes and 33 hospitals). Most of these outbreaks (48) ended in 2014, which means that the causative bacteria and the source were identified, and that transmission to other patients was stopped. None of the outbreaks were considered uncontrollable or a direct threat to public health.

Most of the outbreaks (44) were reported because of the potential closure of (a part of) the healthcare institution. The outbreaks lasted on average 117 days, with a range of 22 days to 714 days, until the outbreaks were considered under control. The outbreak which lasted 714 days started already in 2012 and is closed in 2014.
In case an outbreak lasts more than 2 months it will be designated a phase 2 outbreak. In 2014, 7 outbreaks were placed in phase 2. In total there were 404 patients involved in these outbreaks with a range from 1 to 50 per outbreak. Of these 404 patients, 158 had signs of an infection with a range between 1 and 49 patients per outbreak.

It took an average of 61 days, with a range between 0 and 670 days, before an outbreak was reported to the SO-ZI/AMR. There were 4 institutions that had a request for help, 2 with an outbreak of pseudomonas, 1 with norovirus and 1 outbreak with VRE.

Most outbreaks were related to *Staphylococcus aureus* (MRSA, resistant against Methicillin), enterococci (VRE, resistant against Vancomycin) and *Pseudomonas aeruginosa*. Outbreaks of other bacteria or viruses were notified sporadically.

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Table 4.4.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2014.

	2014 n=55 n (%)
Kind of microorganisme (resistance mechanism)*	
Staphylococcus aureus (MRSA)	19 (34)
Enterococcus faecium (VRE)	14 (25)
Enterococcus faecium (ARE)	-
Klebsiella pneumonia (ESBL)	1 (2)
Klebsiella pneumoniae (CPE)	-
Escherichia coli (ESBL)	2 (4)
Enterobacter cloaecae	4 (7)
Citrobacter freundii	-
Pseudomonas aeruginosa	7 (13)
Clostridium difficile	1 (2)
Acinetobacter	1 (2)
Astrovirus	-
Norovirus	4 (7)
Measles	1 (2)
Unknown	1 (2)
Reason of reporting	
(Threatened) closure	44 (80)
Ongoing transmission	9 (16)
Unknown	2 (4)
Highest level phase	
Phase 1	48 (87)
Phase 2	7 (13)
Phase 3	0 (0)
Phase 4	0 (0)
Phase 5	0 (0)
Number of patiënt: (range)	404 (1-50)
Number of patient with signs of an infection: (range)	158 (0-49)
Duration outbreak in day's from reporting date until closing the outbreak (phase 0): (range)	117 (22-714)
Duration in days between detection of the first patient and the reporting day to the SO-ZI/AMR: (range)	61 (0-670)
Request for help	4 (7)

* *MRSA=meticilline resistance Staphylococcus aureus; VRE=vancomycine resistance enterokokken; ARE=amoxicilline resistance enterokok; ESBL=extended-spectrum beta-lactamase; CPE=carbapenemase producing enterobacteriaceae*

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4.5 Resistance in specific pathogens

4.5.1 *Neisseria meningitidis*

Lodewijk Spanjaard en Arie vd Ende

From 1994-2014 a total of 4767 strains from cerebrospinal fluid (CSF) and 3014 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).

- Penicillin resistance (MIC >0.25 mg/l) was occasionally found until 2006, in 2013 in one strain from CSF and one from blood. In 2014 no penicillin-resistant strains were received.
- The number of strains moderately susceptible to penicillin (MIC 0.125-0.25 mg/l) was 1-5% until 2009, increased to 42% for blood isolates and 35% for CSF isolates in 2012, and decreased subsequently to 12% (5/42) and 16% (5/31) respectively in 2014 (figure 4.5.1.1).
- In 2014, a total of 9 moderately susceptible strains from blood and/or CSF belonged to serogroup B, and one to serogroup Y.
- No resistance to ceftriaxone or rifampicin was found.
- 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 (0.06 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. Therefore, the *penA* gene of the isolates was sequenced.
- Alterations in the *penA* gene, associated with non-susceptibility to penicillin, were detected in 8 (11%) of the 73 strains. These alterations occurred predominantly in phenotypically non-susceptible strains but also in some strains with MIC = 0.06 mg/l (table 4.5.1.1).
- Apparently, E-test with EUCAST criteria yields more strains (14%; 2013: 21%) non-susceptible to penicillin than *penA* genotyping does (11%; 2013: 10%) and both methods do not agree completely.
- One or more of the following reasons may be involved: 1) other factors than *penA* gene alterations also confer non-susceptibility to penicillin; 2) a considerable number of minor interpretation errors occurs because the susceptible/moderately susceptible breakpoint lies at the peak of the susceptibility distribution; 3) this EUCAST breakpoint is too low and should be repositioned at 0.25 mg/l.

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Conclusions

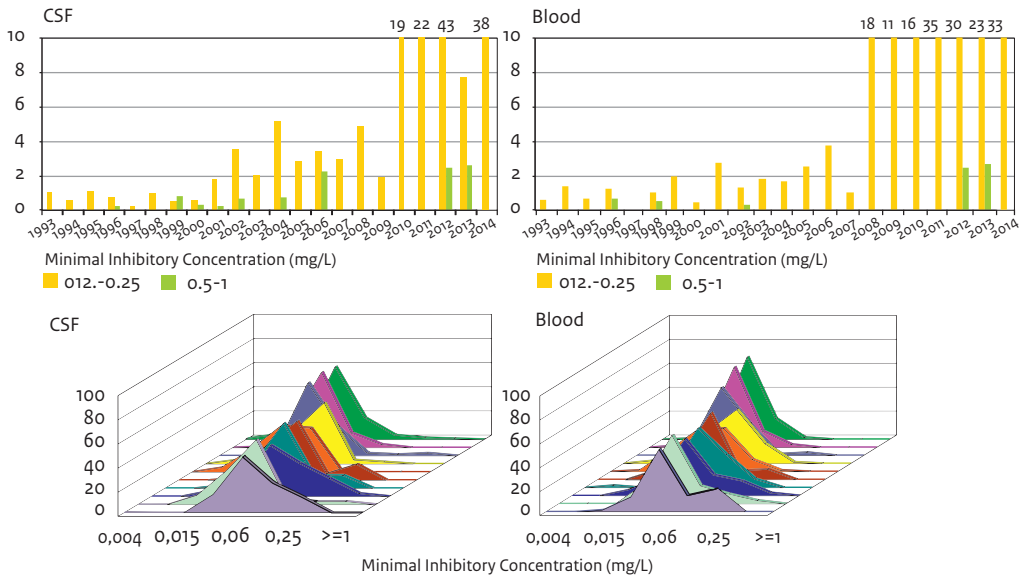
1. Penicillin resistance sporadic (two strains in 2013, zero in 2014).
2. Increase of strains moderately susceptible to penicillin with a peak in 2012; the clinical relevance of this observation is matter of discussion.
3. Alterations in the *penA* gene are present in about 10%.
4. Resistance to ceftriaxone not found; resistance to rifampicin sporadic (one strain in 2013).

Table 4.5.1.1 Alterations in the *penA* gene and penicillin susceptibility in *Neisseria meningitidis* (2014)

Altered *penA* gene*	Number (%) of strains with penicillin MIC:			
	<0.06 mg/l	0.06 mg/l	0.12 mg/l	0.25 mg/l
Yes	0 (0)	3 (9)	2 (28)	3 (100)
No	25	35	5	0
Total	25	38	7	3

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

Figure 4.5.1.1 Trends in penicilin resistance and MIC distributions of penicilin for *Neisseria meningitidis* from CSF



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4.5.2 *Neisseria gonorrhoeae*

Sanne Hofstraat, Alje van Dam, Birgit van Benthem

The national project Gonococcal Resistance to Antimicrobials Surveillance (GRAS) started in 2006, collecting epidemiological data on gonorrhoea and resistance patterns of isolated strains from STI (Sexual Transmitted Infections) centres. 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 10500 isolates was performed by E-test for penicillin, tetracyclin, ciprofloxacin and cefotaxime; in 2011, ceftriaxone, azithromycin and spectinomycin were added to the panel and testing for penicillin and tetracyclin became optional. In 2014, testing for spectinomycin was also made optional. Resistance levels were calculated using the EUCAST breakpoints for resistance.

Results

- Resistance to tetracyclin (33%) and ciprofloxacin (34%) decreased since 2009, but ciprofloxacin remained stable since last year. Resistance to cefotaxime (3%) and azithromycin (8%) increased slightly since 2013 and resistance to penicillin (8%) decreased somewhat since last year. (Figure 4.5.2.1)
- No resistance was found for ceftriaxone and spectinomycin. (Figure 4.5.2.2)
- Cefotaxime resistance in 2014 was highest among heterosexual women (5%), patients who had sexual contact with commercial sex workers in the last 6 months (8%), and patients from Turkish (5.7%), Eastern European (6.3%) or Moroccan (9.8%) origin.
- MIC distributions of cefotaxime and ceftriaxone were both highly skewed to the right. (Figure 4.5.2.3 a&b)

Conclusions

1. Continuing trend to fewer cultures, now close to 40%.
2. Slight increase in cefotaxim resistance to 3% and azithromycin 8%
3. No resistance to ceftriaxone and spectinomycin

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

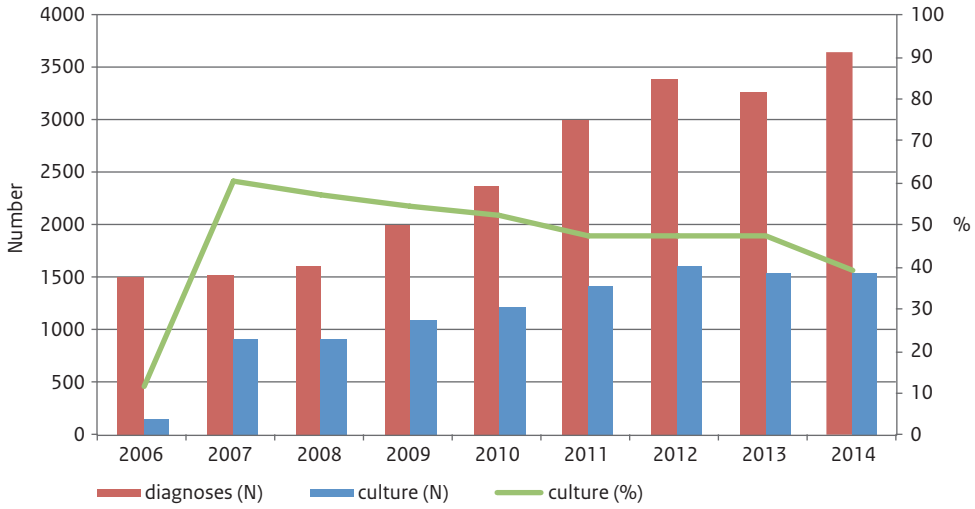
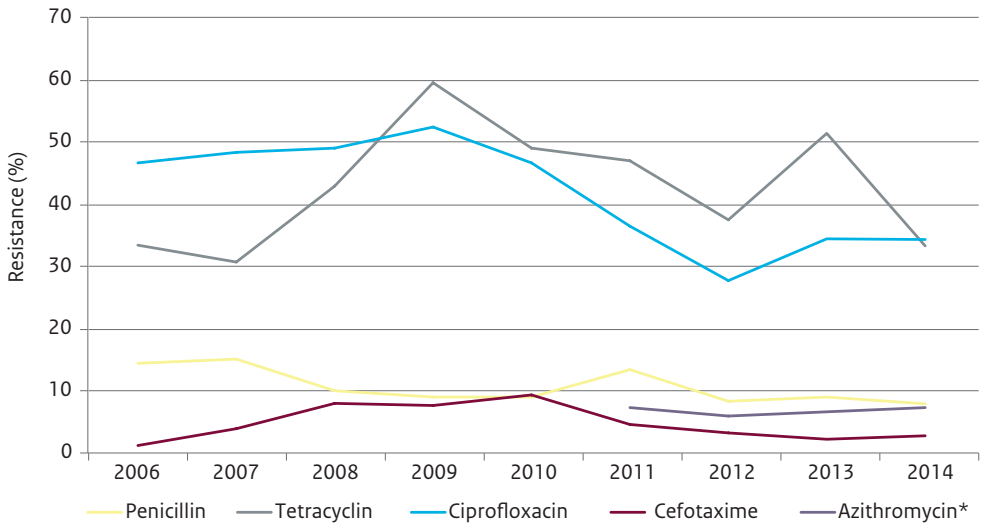


Figure 4.5.2.2 Trends in antibiotic resistance among *Neisseria gonorrhoeae* (N=10,500)



* *Ceftriaxone, azithromycin and spectinomycin were added to the panel in 2011 and testing for penicillin, tetracycline and spectinomycin became optional.*
No resistance was found for ceftriaxone and spectinomycin.

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

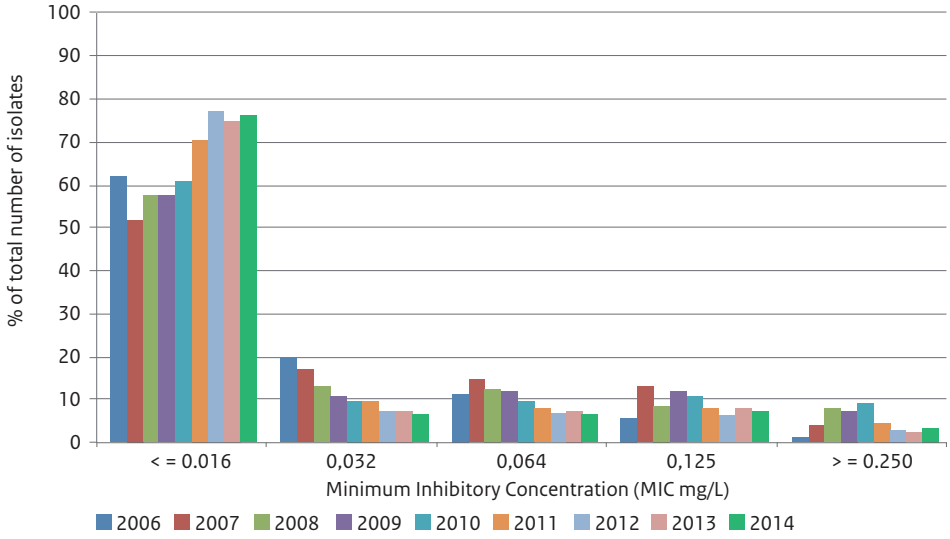
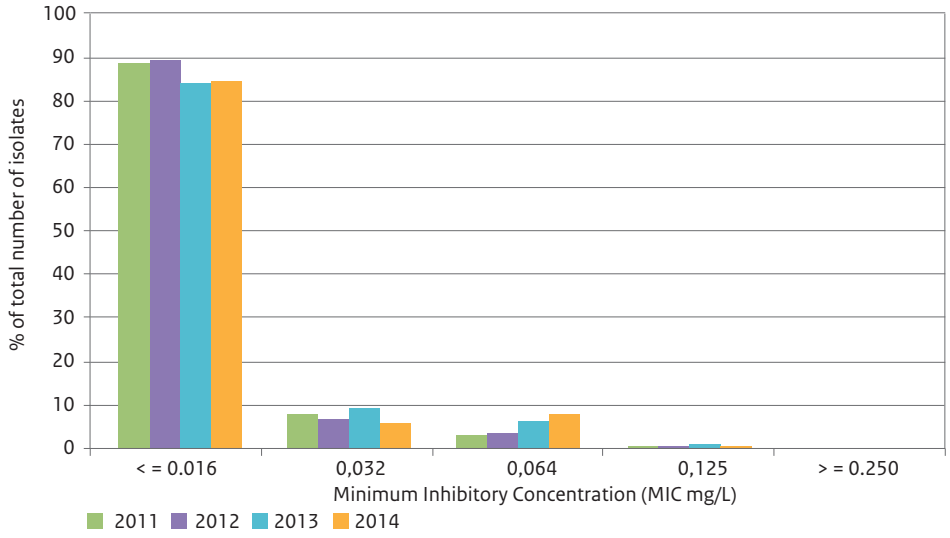


Figure 4.5.2.3b MIC distributions of cefotaxime and ceftriaxone for *Neisseria gonorrhoeae*



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4.5.3 *Mycobacterium tuberculosis*

Miranda Kamst and Dick van Soolingen

As of 2011, not all strains are sent to the RIVM for susceptibility testing.
Around 25 % of these tests are now performed at peripheral laboratories. We assume that the results of the tests performed elsewhere invariably represent sensitive tuberculosis strains, as otherwise we would have been requested to verify the results and test additional drugs. The presented data is preliminary because strains and results are still received because of the slow growth of mycobacteria.

Results

- In 2014, 548 *M. tuberculosis* complex isolates were received for epidemiological typing. Drug susceptibility testing at the RIVM was done for 399 strains.
- Since 2010, the number of *M. tuberculosis* strains received yearly gradually decreased from 784 in 2010 to 548 in 2014.
- Until 2010, INH resistance increased to 9.0%, since 2011 it decreased to 6.6% in 2014. From 2012 until 2014 the INH resistance is stable. (figure 4.5.3.1)
- Rifampicin resistance decreased from 3.1 % in 2013 to 1.3% in 2014.
- Resistance to ethambutol remained low, fluctuating in the period 1997 to 2014 between 0.2% and 1.6%. In 2014, resistance amounted to 0.7%.
- Multidrug (MDR) resistant tuberculosis (MDR-TB), defined as at least resistance to INH and rifampicin, was found in 1.1 % of the isolates in 2014, and this was a significant reduction in comparison to the 2.8 % in 2013. XDR-TB was not diagnosed in 2014. (figure 4.5.3.2)

Conclusions

1. Resistance to INH remained stable over the last 3 years.
2. MDR-TB increased to 2.8 % in 2013, but decreased to 1.1 % in 2014.

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Figure 4.5.3.1 Trends in antibiotic resistance TB

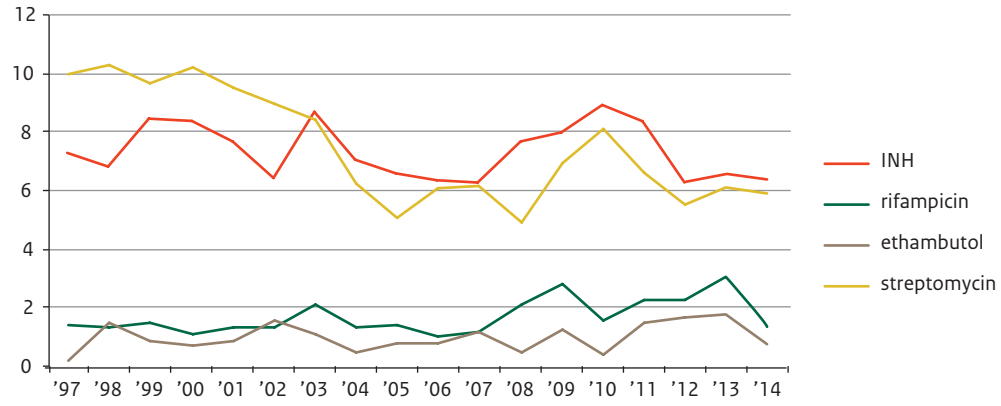
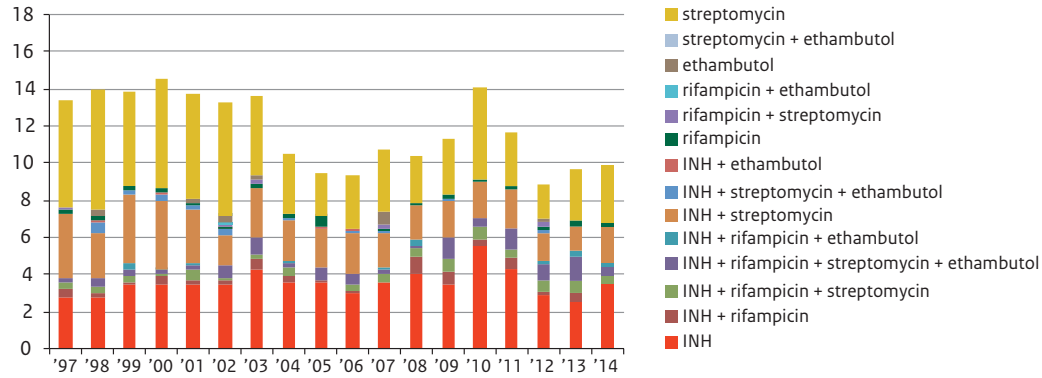


Figure 4.5.3.2 Trends in combined resistance TB



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4.5.4 Resistance to influenza antiviral drugs

Adam Meijer

Surveillance for resistance

In the Netherlands the susceptibility of influenza viruses for the M2 ion channel blockers (M2B) amantadine and rimantadine and the neuraminidase enzyme inhibitors (NAI) oseltamivir and zanamivir are 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 (IC_{50}). In the absence of known NAI resistance amino acid substitutions detected by genotypic assays, determination of the IC_{50} is the only way to determine the NAI susceptibility of an influenza virus. The major marker for M2B resistance is the M2 S31N amino acid substitution.

Results

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 2013/2014 season not reported in the 2014 NETHMAP report are highlighted here. The NIC received an A(H1N1)pdm09 positive specimen that was collected from a patient in March 2014, which appeared to comprise the NA H275Y oseltamivir 'highly reduced inhibition' amino acid substitution. Specimens of two patients with A(H3N2) infection, collection dates both in July 2014, showed a mixture of NA 292R and NA 292K amino acid composition; R292K being associated with highly reduced inhibition by oseltamivir and zanamivir. None of the A(H1N1)pdm09, A(H3N2) and B influenza viruses analysed so far for the 2014/2015 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, rendering the M2B useless for influenza antiviral therapy and prophylaxis.

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Table 4.5.4.1 (Highly) reduced inhibition of influenza viruses by NAIs and M2Bs in the Netherlands, 2005/2006 - 2014/2015[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[11]	0/709	33/33 (100%)	NA	NA	0/84	5/5 (100%)	0/4

1 *Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; M2B = M2 ion channel blocker; 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 IC_{50} for oseltamivir and mild outlier IC_{50} for zanamivir.*

3 *Both viruses with reduced inhibition had outlier IC_{50} 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 IC_{50} 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 IC_{50} for oseltamivir and a 5-fold increased IC_{50} 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 H25Y amino acid substitution. No patient characteristics or viral exposure data available.*

11 *Preliminary data.*

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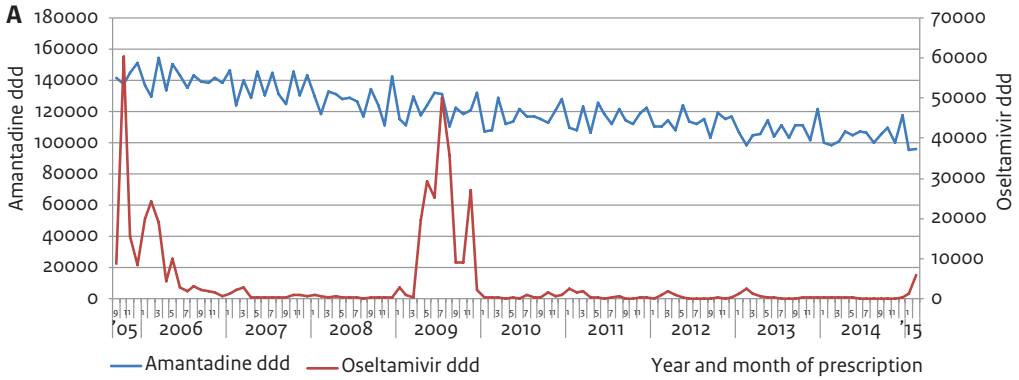
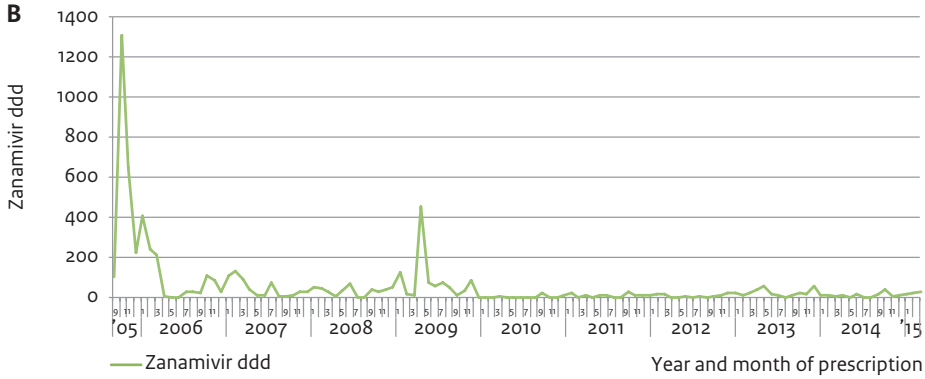
Figure 4.5.4.1 Prescriptions of amantadine and oseltamivir (A) and zanamivir (B). Shown are the Defined Daily Doses (ddd) cumulated by month. Prescriptions of oseltamivir and zanamivir are linked to the seasonal epidemiology of influenza virus infections.





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4.5.5 Resistance among human anaerobic pathogens

Linda Veloo and Arie Jan van Winkelhoff

Anaerobic bacteria isolated from clinical materials obtained from patients at the University Center of Groningen were identified using Matrix Assisted Laser Desorption time-of-flight Mass Spectrometry (MALDI-TOF MS) and their antibiotic profile for amoxicillin, amoxicillin with clavulanic acid (only gram-negatives), clindamycin and metronidazole was determined using Etest (BioMerieux, l'Étoile, France). The percentage resistance was assessed using EUCAST breakpoints.
Differences in antibiotic profiles between the different genera is described. Difference in antibiotic resistance between the most encountered species (n≥10) in the *Bacteroides fragilis* group and gram-positive anaerobic cocci (GPAC) was also assessed.

Gram-negative anaerobic bacteria

Amoxicillin resistance was observed in the *B. fragilis* group (93%), *Prevotella* sp. (51%), *Parabacteroides* sp. (55%) and *Veillonella* sp. (22%). *Veillonella* strains resistant to amoxicillin also showed resistance to amoxicillin with clavulanic acid indicating that the resistance is probably not due to beta-lactamase production (data not shown). Amoxicillin resistance among *Parabacteroides* sp., *Prevotella* sp. and species in the *B. fragilis* group is most often due to beta-lactamases production.
Resistance to clindamycin was observed in the *B. fragilis* group, and the genera *Campylobacter* and *Parabacteroides* (20-25%). The resistance differs between the different *B. fragilis* species: *Bacteroides ovatus* showed no clindamycin resistance while resistance was found in *Bacteroides fragilis* (26%) and *Bacteroides thetaiotaomicron* (22%). No clindamycin resistance was observed in *Fusobacterium* and *Veillonella*.

The percentage resistance of gram-negative anaerobic bacteria for amoxicillin, co-amoxiclav and clindamycin observed in 2014 is similar as in previous years[1]. The MIC distribution for amoxicillin and clindamycin is shown is figure 4.5.5.1 and figure 4.5.5.2.
In 2014 we reported on two metronidazole resistant *Prevotella bivia* strains. This year, no metronidazole resistance among *Prevotella* was observed. However, two *B. fragilis* strains were found resistant. No further metronidazole resistance was encountered among the gram-negative anaerobes.

Gram-positive anaerobic bacteria

Amoxicillin resistance was observed in the genus *Clostridium* (14%), but not in other gram-positive anaerobic bacteria. Clindamycin resistance was encountered in GPAC (18%), *Actinomyces* (11%) and *Propionibacterium* (3%), but was not observed in the genus *Clostridium*. Species in the group of GPAC show a difference in clindamycin resistance. Strains belonging to *Finegoldia magna* and *Peptoniphilus harei* showed clindamycin resistance, 26% and 33% respectively. No resistance was observed for *Parvimonas micra* and *Peptostreptococcus anaerobius*. These observations are in line with findings in previous years [1].
Metronidazole resistance was not encountered among gram-positive anaerobic bacteria.

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Figure 4.5.5.1 The MIC distribution of amoxicillin for gram-negative anaerobic bacteria.

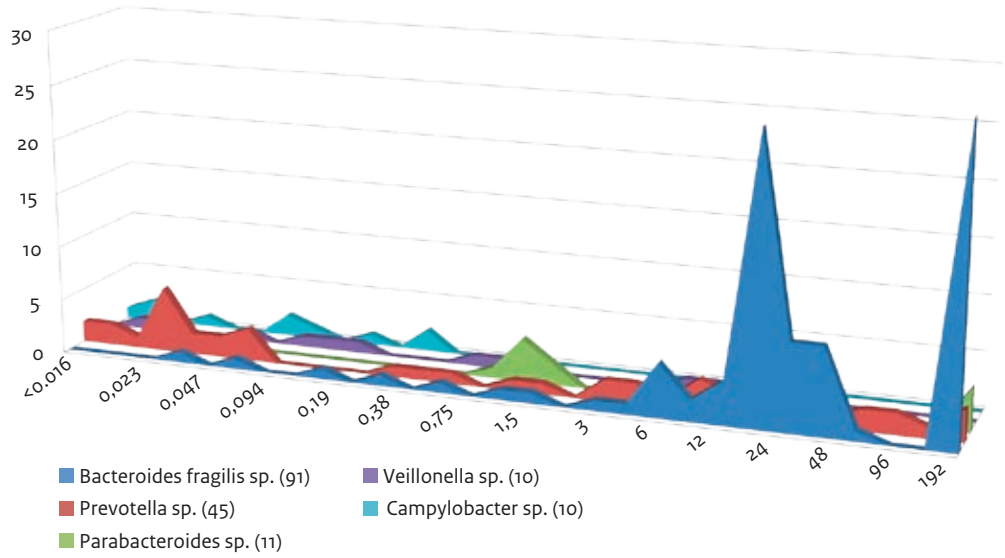
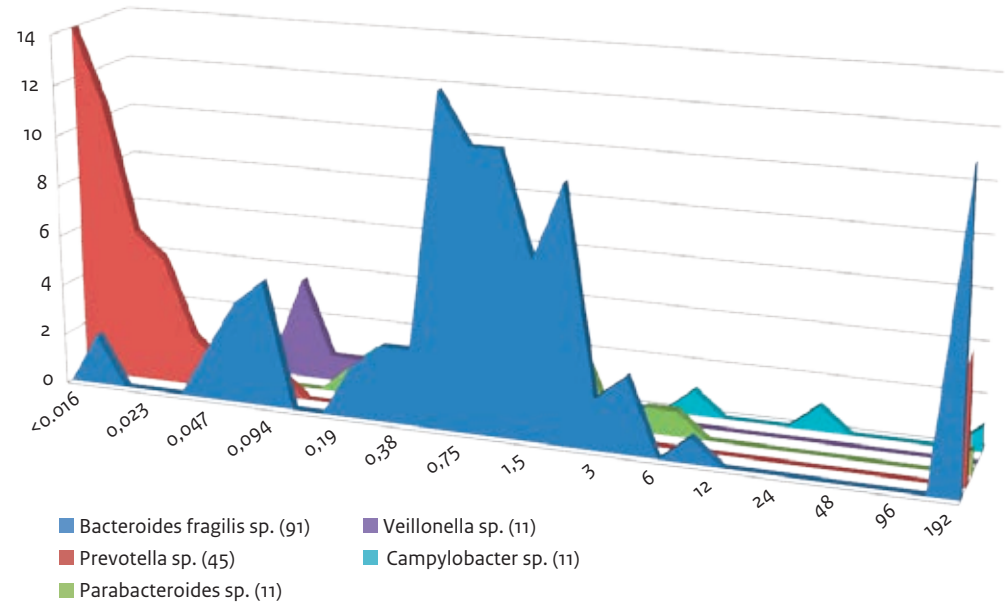


Figure 4.5.5.2 The MIC distribution of clindamycin for gram-negative anaerobic bacteria.



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4.5.6 *Clostridium difficile*

Sofie van Dorp, Celine Harmanus, Ingrid Sanders, Daan Notermans, Sabine de Greeff, Ed Kuijper

Epidemiology

The National Reference Laboratory for *C. difficile* operates since the emergence of the PCR ribotype 027 strain in the Netherlands in 2005. The emergence of ribotype 027 in Canada, the United States, and Europe was found to be associated to fluoroquinolone resistance of two distinct lineages.[1] The Netherlands managed to reduce the transmission of ribotype 027 in 2006.[2] Three years later, a representative sentinel surveillance program was initiated and is currently applied in twenty-two acute care hospitals in the Netherlands. Faeces samples or isolates of all included patients are characterised by PCR ribotyping.

In the period May 2013-May 2014, ribotype 027 was found in 3% of these isolates. The most frequently encountered PCR ribotypes were 014/020 (14%), 078/126 (13%), and 001 (8%). Compared to the previous years, the prevalence of ribotype 001 decreased (2010-2011, 20%; 2011-2012, 17%; 2012-2013,14%). No important new or emerging ribotypes were observed.[3] In the same period, the Reference Laboratory also received faeces samples and isolates from healthcare institutes that did not participate in the sentinel surveillance program; 32% of these 161 *C. difficile* isolates were ribotype 027. Five outbreaks associated with ribotype 027 and two outbreaks with *C. difficile* ribotype 001 were detected.[3]

Resistance

Susceptibility testing is not part of the routine activities of the Reference Laboratory for *C. difficile*, but is performed when resistance is suspected. None of the tested isolates was found to be resistant to the therapeutic drugs metronidazole and vancomycin, using CLSI/EUCAST cut-off levels[4;5].

The Netherlands also participated in a Pan-European longitudinal surveillance study of antibiotic resistance, coordinated by Leeds Teaching Hospitals Trust.[6] Almost thousand isolates from 22 European countries were investigated; no resistance to the therapeutic drugs metronidazole, vancomycin, or fidaxomycin was found[6]. Hundred randomly selected *C. difficile* isolates (time period 2011-2013) from the sentinel surveillance program in the Netherlands were included in this study. Results of the isolates from the Netherlands are summarised in table 4.5.6.1.

Conclusions

1. The prevalence of ribotype 027 was stable at 3% according to the sentinel surveillance data, however, vigilance is needed due to the occurrence of ribotype 027 in other healthcare facilities.
2. There are no indications for clinical relevant resistance to metronidazole, vancomycin, and fidaxomycin.
3. There is a high rate of resistance to clindamycin in ribotypes 001.

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Table 4.5.6.1 MIC_{50} (mg/L) for several antibiotic agents of hundred isolates from in the sentinel surveillance program (isolated in 2011-2013) stratified per ribotype, as investigated by Freeman *et al*. One isolate was not testable. [a] The range is not reported if all isolates had an equal MIC. In the Netherlands, no resistance or reduced susceptibility was found to metronidazole, vancomycin or fidaxomicin.

	MIC_{50} (range)								
Ribotype (no. of isolates tested)	**Metronidazole**	**Vancomycin**	**Fidaxomicin**	**Rifampicin**	**Moxifloxacin**	**Clindamycin**	**Imipenem**	**Chloram-phenicol**	**Tigecycline**
001/072 (11)	0.5 (<0.125-2)	0.5 (0.125-1)	0.016 (0.004-0.03)	0.001 (<0.001-0.004)	32 (2-32)	>64[a]	4 (2-8)	16 (4-32)	0.03 (<0.03-0.06)
002 (7)	<0.125 (<0.125-0.5)	1 (0.5-2)	0.06 (0.03-0.125)	0.002 (0.002-0.004)	2 (1-2)	8 (2-16)	4 (2-4)	4 (4-8)	0.03 (0.03-0.06)
005 (7)	0.25 (<0.125-0.25)	1 (0.5-1)	0.06 (0.03-0.125)	0.002 (0.002-0.004)	2 (1-2)	8 (1->64)	4 (2-4)	4 (2-4)	0.03 (0.03-0.06)
014/020 (12)	0.25 (<0.125-2)	0.5 (0.5-1)	0.06 (0.016-0.125)	0.002 (<0.001-0.004)	2 (1-16)	8 (2-32)	4 (2-16)	4 (4-8)	0.06 (<0.03-0.06)
027 (4)	1 (0.5-2)	3 (2-4)	0.06 (0.06-0.125)	>16 (0.002->16)	16[a]	>64 (16->64)	6 (4-8)	16 (4-16)	0.03 (0.03-0.06)
078/126 (11)	<0.125 (<0.125-0.5)	0.5 (0.5-2)	0.03 (0.016-0.125)	0.002 (<0.001->16)	1 (1-16)	2 (2-8)	2 (2-8)	4 (2-8)	0.03 (<0.03-0.06)
Other types (47)	0.25 (<0.125-2)	0.5 (0.5-4)	0.06 (0.016-0.25)	0.002 (<0.001->16)	2 (0.5-32)	8 (<0.125->64)	4 (2-8)	4 (2-32)	0.03 (<0.03-0.06)

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4.5.7 Azole resistance in *Aspergillus fumigatus*

Paul Verweij on behalf of Jacques Meis, Bart Rijnders, Karin van Dijk, Ed Kuijper, Jan Arends, Pieter-Jan Haas and Bram Lestrade.

Introduction

The saprophytic mold *Aspergillus fumigatus* is abundantly present in our environment and is known to cause a spectrum of fungal diseases in humans and animals. The clinical syndromes range from allergic aspergillosis to acute invasive disease. Invasive aspergillosis carries a significant mortality in high risk patient groups, including patients with leukemia, hematopoietic stem cell or solid organ transplantation and in critically ill patients. Antifungal azoles, including itraconazole, voriconazole, posaconazole, and the new azole isavuconazole, are the cornerstone of prevention and treatment of aspergillus diseases.

In the past decade resistance to azoles has emerged as a new clinical problem. Although resistance may develop during azole therapy, primarily in patients with chronic lung diseases, a cavity harboring *A. fumigatus* and on azole therapy, but the main burden of resistance is through resistance selection in the environment. The clinical characteristics of this route of resistance is that any patient can present with any aspergillus disease caused by an azole-resistant isolate. The majority of these patients are azole-naïve and surveillance studies indicate that the mortality rate is very high, i.e. 88% in culture-positive patients. The resistance is caused by a limited number of resistance mechanisms associated with the *Cyp51A*-gene, including TR_{34}/L98H, TR_{53}, and TR_{46}/Y121F/T289A.

The azole resistance surveillance is performed using an agar-based screening plate on which *A. fumigatus* from primary culture is subcultured. If the aspergillus isolate is able to grow on the agar supplemented with an azole, the probability of resistance is very high. These isolates are further characterized for azole resistance phenotype and genotype in the Radboudumc. The total number of isolates that is screened is registered in the laboratory information systems of the participating centers and is used to claculate the frequency of resistance. In 2015 the frequency of resistance was calculated for 5 UMCs as we were also able to include the data from the VuMC for 2013 and 2014.

Results

In 2014 azole resistance was observed in all UMCs with a frequency varying between 3.8% and 13.3% of patients with a positive *A. fumigatus* culture (Table 4.5.7.1). The highest frequency was observed in the LUMC, similar to 2013, although the frequency of resistance in 2014 was lower compared to 2013 (13.3% compared with 19.2%). The frequency of resistance clearly varies between the different UMCs, although the reason for this observation remains unknown. The overall frequency of resistance in 2014 was 7.2% compared to 7.8% in 2013. The resistance phenotype of the *A. fumigatus* isolates was determined using the EUCAST reference method. Overall, 83.6% of the isolates were resistant to itraconazole, 91.8% was resistant to voriconazole and 86.3% resistant to posaconazole.

Analysis of the underlying mutations indicates a major role of the environmental route of resistance selection. In total 73 *A. fumigatus* isolates were analyzed for the presence of mutations in the Cyp51A-gene. In 36 isolates (49.3%) the TR_{34}/L98H resistance mechanism was found, while 29 isolates

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(39.7%) harbored the TR_{46}/Y121F/T289A resistance mechanism. This might indicate an increasing trend of the TR_{46}/Y121F/T289A resistance mechanism, which is characterized by high-level resistance to voriconazole, the recommended first choice treatment option. Overall, resistance mechanisms of environmental origin we found in 89% of azole-resistant *A. fumigatus* isolates.

Discussion

Analysis of the underlying diseases of the patients with positive azole-resistant *A. fumigatus* cultures indicates that there is not a clear risk group. Azole resistant isolates were recovered from patients with Cystic Fibrosis, critically ill patients, patients with hematological malignancy or cancer, and patients with chronic lung diseases. We believe that since azole-susceptible and azole-resistant *A. fumigatus* conidia are present in the ambient air, individuals will be exposed to both. It might be that azole therapy will cause selection of azole-resistant *A. fumigatus* in the lung and thus facilitate azole-resistant disease, but this has not be proven. Evidence is increasing that the frequency for azole resistance might vary for different patient groups. A very high frequency of azole resistance in culture positive critically ill patients was noted in the LUMC, with 10 of 38 (26%) patients with a positive *A. fumigatus* culture harboring an azole-resistance mechanism [1]. In the same study period, 24 (14%) azole-resistant *A. fumigatus* isolates were cultured from 170 patients hospitalized at other departments (p= 0.06). A second study performed in UMCU also indicates high resistance rates in high risk patients [2]. Over a three years period (2011 to 2013) 105 *A. fumigatus* primary cultures from 105 patients were analyzed for azole resistance. The frequency of patients with voriconazole-resistant isolates in this three year period was 16.2% (24.6% in hematology and 4.5% in the ICU). A third study in two transplant centers in Germany analyzed the frequency of azole resistance in 762 HSCT patients [3]. In 27 patients with a positive *A. fumigatus* culture, 8 (29.6%) were found to have azole-resistant invasive aspergillosis, of which 7 patients died. Further studies are needed to identify risk factors for azole-resistant aspergillus disease for different patient groups.

Our current surveillance relies on positive *A. fumigatus* cultures, but the frequency and implications of azole resistance in culture-negative patients remains unknown. The frequency of positive cultures is very low in patients with hematological malignancy. In the previously mentioned German study a positive culture was obtained in only 27 of 762 (3.5%) HSCT patients[3]. A recent audit in the Hematology Department of the Radboudumc indicated that approximately 11% of patients have a positive culture (unpublished observations). It is believed that monitoring of galactomannan and early CT scan allow early diagnosis, with often negative cultures from BAL. The consequence is that culture-negative aspergillus disease represents the majority of cases, but is not captured in the surveillance. Therefore we aim to include molecular tests for detection of aspergillus and resistance in the near future. Preliminary studies indicate that resistance can be detected in BAL from culture-negative patients[4].

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Conclusions

1. Azole resistance varied between 3.8% and 13.3%
2. The overall frequency of resistance in 2014 was 7.2% compared to 7.8% in 2013.
3. Resistance mechanisms of environmental origin were found in 89% of azole-resistant *A. fumigatus* isolates

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Table 4.5.7.1 Overview of number of *A. fumigatus* culture-positive patients and frequency of azole resistance in 5 UMCs in 2013 and 2014.

	2013		2014	
	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)
LUMC	99	19 (19.2)	113	15 (13.3)
Radboudumc	123	6 (4.9)	143	7 (4.9)
UMCG	194	16 (8.2)	191	18 (9.4)
VuMC	113	8 (7.1)	104	9 (8.7)
Total	**760**	**58 (7.8)**	**814**	**59 (7.2)**

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MARAN 2015

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2014

June 2015

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Colophon

This report is published under the acronym MARAN-2015 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-2015 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-2015 is published in a combined back-to-back report with NETHMAP-2015. 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-2015 can be ordered from the secretariat of CVI-Lelystad, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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1 Summary

Antibiotic Usage

In 2014 the sales of antimicrobial veterinary medicinal product (207 tonnes) decreased by 4.4%, compared to 2013 (217 tonnes). The total sales decreased from 2009, the index year as defined by the Ministry of Economic Affairs, to 2014 by 58.1%. This means that the policy objective for 2015, a 70% reduction compared to 2009, will be a challenge. Compared to 2007, the year with highest sales (565 tonnes), the decrease in sales is 63%.

Decreases in sales were recorded for tetracyclines, aminoglycosides and polymyxins. Increases in sales were noted for amphenicols, 1st and 2nd generation cephalosporins, macrolides, penicillins and 1st generation quinolones. Although reductions in use in livestock of 3rd and 4th generation cephalosporins and fluoroquinolones were realized, total sales of both groups increased by 4.3% and 2.4%, respectively. The increase for these groups was due to usage in animal species which are at present not monitored such as companion animals and small livestock sectors (rabbits). In most livestock sectors reductions in antibiotic use levelled off in comparison with 2013, except for poultry and dairy cattle. In poultry antibiotic use increased again in 2014, probably as a result of changes in prescription patterns. In dairy cattle a substantial reduction in use was noted and a shift in antibiotic use from 3rd and 2nd choice to 1st choice antibiotics, particularly in dry cow treatment.

Antimicrobial resistance

In 2014 *S.* Typhimurium (16%) in combination with its monophasic variant: *S. enterica subspecies enterica* 1,4,[5],12:i:- (20%), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (23%) 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, but was also found (but less predominant) in cattle and poultry. *S.* Enteritidis was mainly present in poultry and more specifically in laying hens and contaminated eggs. Also travel was a risk factor for acquiring a *Salmonella* infection. In pigs, *S.* Typhimurium and its monophasic variant dominated followed by *S.* Derby. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. *S.* Paratyphi B var. Java was again the most predominant serovar in poultry. In 2013

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and 2014 *S.* Heidelberg was frequently isolated from poultry sources. This was mainly due to contaminated poultry meat imported from Brazil, which differed from the *S.* Heidelberg isolates that resulted in human cases in 2014.
Highest resistance levels were observed for *S.* Heidelberg, the monophasic *S.* Typhimurium 1,4,[5],12:i:- and *S.* Paratyphi B var. Java, and to a lesser extent in *S.* Typhimurium and *S.* Infantis. Resistance to the (fluoro)quinolones were mainly observed in *S.* Enteritidis, *S.* Typhimurium or *S.* Chester predominantly derived from humans and *S.* Heidelberg, *S.* Infantis and *S.* Java associated with poultry. Resistance levels in *S.* Typhimurium isolates from human samples have increased over the years until 2010 after which a constant tendency to decrease was observed until 2013. In 2014 resistance levels for almost all antimicrobials tested stabilized.
In 2014 the resistance rates seem to have stabilized in *C. jejuni* from broilers and poultry meat. The highest resistance levels of *C. jejuni* in poultry were observed for tetracycline and the quinolones ciprofloxacin and nalidixic acid, which were substantially lower in isolates from laying hens. Macrolide resistance was not detected in *C. coli* from pig meat. Ciprofloxacin resistance was at a high level in poultry and still rising in *Campylobacter* spp. causing infections in human patients, which is a concern for public health. However, resistance to erythromycin, the first choice antibiotic in human infections, was still low. For *C. jejuni* from human patients, resistance levels were higher for all three antimicrobials tested for travel related infections compared to domestically acquired campylobacteriosis.

Over the last decade, STEC isolates show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim. Resistance to the quinolones (ciprofloxacin and nalidixic acid) decreased from 4.2% in 2013 to 2.4% in 2014. As in the former four years, no ESBL-producing isolates were detected.

Among indicator *E. coli* from animals and meat, resistance to ampicillin, tetracyclines, sulfonamides and trimethoprim was 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. In most animal species the resistance levels of indicator *E. coli* from faecal samples stabilized in 2014. This may reflect the use patterns of antibiotics in the different livestock species. In isolates from broiler meat, beef and pork, resistance showed a tendency to decrease. In veal the trends are variable due to low numbers annually examined. Resistance to third-generation cephalosporins was low in most animal species, most likely the result of the stringent limitations in usage of cephalosporins in food producing animals. Although resistance to fluoroquinolones decreased, it was still commonly present in indicator *E. coli* from poultry sources and to a lesser extent from white veal calves.

In 2014 only enterococci from pigs 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 every three years instead of annually. In slaughter pigs, highest resistance levels were observed for tetracycline (71.1% in *E. faecalis* and 81.2% in *E. faecium*), erythromycin (39.5% in *E. faecalis* and 19.4% in *E. faecium*). In *E. faecium,* additional high levels of resistance were observed for quinu/dalfopristin (86.7%), and to a lesser extent to ampicillin (18.2%).

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Isolation rates of *E. faecalis* and *E. faecium* differ between faeces and meat. 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. Vancomycin resistant enterococci were not detected in pigs in 2014.

The decrease in cefotaxime resistant *E. coli* from 2008 – 2013, has levelled off in 2014. The prevalence of livestock being positive for ESBL/AmpC producing *E. coli* in the faeces was 67% in broilers, 34% in laying hens, 18% in slaughter pigs, 23% in white veal calves, 14% in rosé veal calves and 9% in dairy cows. Similarly to prevalence in faeces, poultry meat was most frequently contaminated (67%), which was a bit lower than found in former years (83% in 2013 and 73% in 2012). Fifty one percent of turkey meat was found positive (in 2013 this was 35%) while in beef and pork the prevalence of confirmed ESBLs was low and comparable to 2013. ESBL/AmpC prevalence in processed meat products was higher compared to raw meat. Cross-contamination during processing of the meat might explain these differences. The dominant human ESBL-gene ($bla_{CTX\text{-}M\text{-}15}$) was more frequently found in animals or their products. This is an unwanted development that warrants extra attention in the surveillance in food-animal sources. In 2014 in 1601 faecal samples from broilers, veal calves, slaughter pigs and dairy cows no carbapenemase-producing *Enterobacteriaceae* were detected.

The prevalence of ESBL-producing *Salmonella* in 2014 was 2.1%, almost half the amount of 2013 (4%). In isolates from human sources a variety of ESBL-genes were found: $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}65}$. These isolates were all highly multidrug resistant, which could affect the success of a therapy in infected humans. No resistance was detected against the last resort antibiotic class: the carbapenems (meropenem).

Conclusions

It can be concluded that antibiotic sales for animals have decreased substantially from 2007 to 2013. In 2014 the reduction in use levelled off in most animal species except for poultry and dairy cattle. In poultry the use increased while in dairy cattle a substantial decrease in use was observed. This usage pattern was reflected in the resistance data of 2014. Resistance levels stabilized in 2014 in bacterial organisms sampled from all animal species, including occurrence of cefotaxime resistant ESBL-suspected *E. coli* in the gut of broilers. However, the proportion of poultry meat products contaminated with ESBLs showed a tendency to decrease in 2014 compared to previous years. These findings indicate that reductions in the total quantity of antibiotics used in the Netherlands and in 3rd and 4th generation cephalosporins were associated with a reduction of the general levels of antimicrobial resistance and the levels of ESBLs to a certain extent. These associations are indicative of a direct causal association between usage of antibiotics and antimicrobial resistance. The current levelling off in antibiotic use was directly followed by a stabilization of resistance levels. This may warrant a re-evaluation of the current targets for antibiotic use in relation to targets for antimicrobial resistance in animals and food thereof.

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2 Usage of antimicrobials in animal husbandry in the Netherlands

Usage of antimicrobials in the Netherlands is monitored through sales data, provided by FIDIN, and active monitoring in major livestock farming sectors (pigs, broilers, turkey, veal calves, dairy- and other cattle) on the basis of delivery records, performed by the Netherlands Veterinary Medicines Authority (SDa). The SDa provides extensive reports on the developments of antimicrobial medicines usage in the Netherlands, has defined benchmark thresholds (signalling and action) for each production sector to enable benchmarking within sectors, thus stimulating awareness and supporting reduction goals (SDa 2015).

2.1 Total sales of veterinary antimicrobial veterinary medicinal products in the Netherlands 2014

2.1.1 Analysis of sales data

FIDIN, the federation of the Netherlands veterinary pharmaceutical industry, provided sales data of all antimicrobial veterinary medicinal products on package level sold in the Netherlands in 2014, 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. The usage in the monitored sectors, on the basis of delivery records, covered 91.8% of the sales.

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. The sales figures from 1999 to 2008 were recalculated and corrected 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 substance mass (excluding mass of salts and esters) is calculated,

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including (unlike ESVAC reports) topical applications like ointments, eye drops and sprays. Sales data in this report gives information about the total sales for all animals, not per animal species.

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, between 2.5-2.6 million tons. This indicates that the reported reduction in sales of antimicrobials are indicative of true reductions 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	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Piglets (less than 20 kg)	3.896	4.300	4.170	4.470	4.680	4.555	4.809	4.649	4.797	4.993	4.920	5.115
Sows	1.052	1.125	1.100	1.050	1.060	1.025	1.100	1.098	1.106	1.081	1.095	1.106
Fattening pigs	5.818	5.715	5.730	5.700	5.970	6.155	6.199	6.459	6.200	4.189	4.209	4.087
Other pigs	1.883	1.865	1.900	1.660	1.960	2.050	2.100	2.040	2.021	1.841	1.789	1.765
Turkeys	1.112	1.238	1.245	1.140	1.232	1044	1060	1036	990	827	841	794
Broilers	42.991	43.854	45.525	42.529	44.487	50.270	52.323	54.367	57.811	43.912	44.242	47.020
Other poultry	37.129	42.922	48.695	50.666	49.992	47.914	46.383	48.218	40.442	52.356	54.345	56.924
Veal calves	748	775	813	824	860	913	886	921	906	908	925	921
Cattle	2.986	2.984	2.933	2.849	2.960	3.083	3.112	3.039	2.993	3.045	3.064	3.230
Sheep	1.476	1.700	1.725	1.755	1.715	1.545	1.091	1.211	1.113	1.093	1.074	1.070

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 in 2014 showed a further reduction of antimicrobial veterinary medicinal products. Due to a mismatch of product numbers, the sales of *2013* had to be adjusted for tetracycline sales with an additional 7.4 tonnes. This resulted in total sales of 217 tonnes in 2013. In 2014 total sales were 207 tonnes, a reduction of 4.4%. Total sales decreased by 58.1% over the years 2009-2014. Sales of 1st and 2nd generation cephalosporins and other antimicrobials increased due to underreporting of companion animal products in previous years (not corrected because data are unavailable).

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Figure ABuse01 Antimicrobial veterinary medicinal product sales from 1999-2014 in kg (thousands).

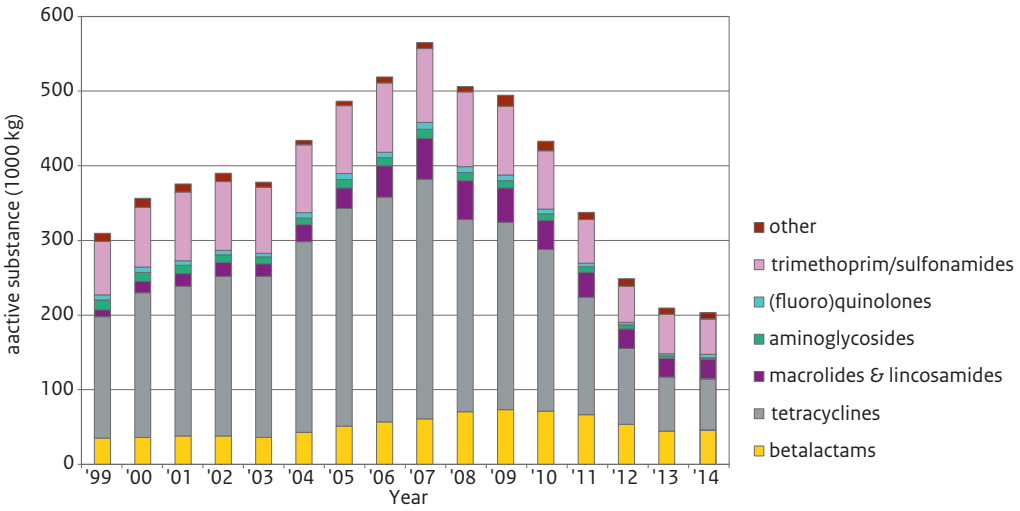

Table ABuse02 Antimicrobial veterinary medicinal product sales from 1999-2014 in kg (thousands) (FIDIN, 2014).

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14
betalactams	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69
macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28
aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	8,6	7,3	5,8	3,4	1,8
(fluoro)quinolones	7	7	6	6	5	7	8	7	9	8	8	6,6	5,1	3,1	2,8	3,8
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49
other	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8,1	7,8
total therapeutic sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217*	207

* *corrected data for tetracyclines*

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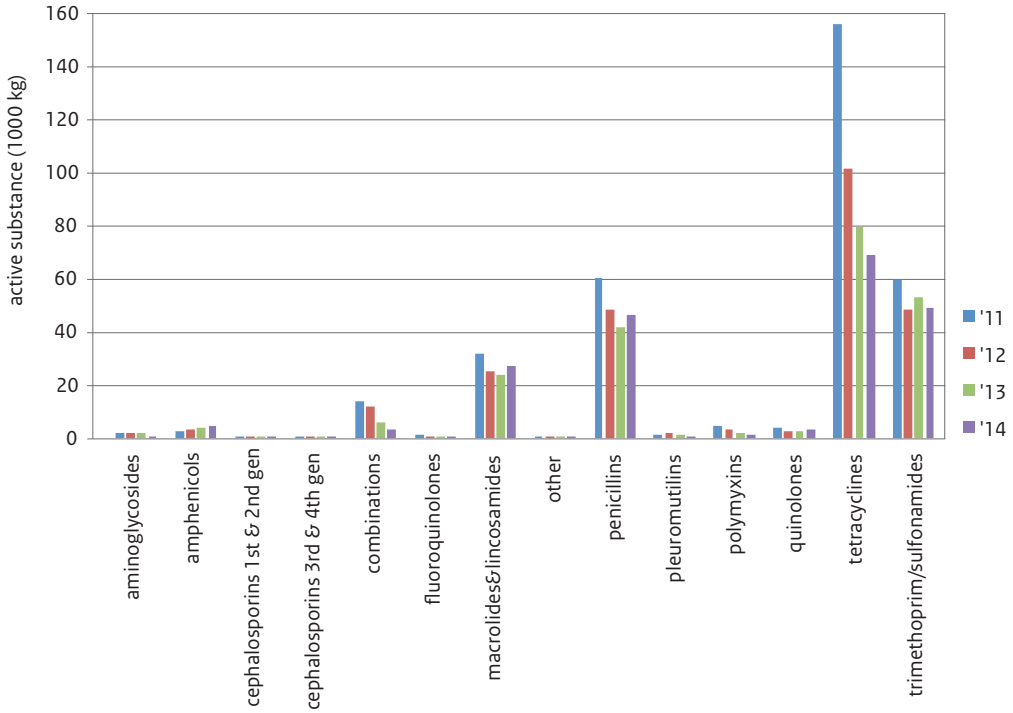
Figure ABuse02 Antimicrobial veterinary medicinal product sales by pharmacotherapeutic class from 2011-2014 in kg (thousands).



Some classes of antibiotics, tetracyclines, trimethoprim/sulphonamides, pleuromutilins, polymyxins and aminoglycosides showed a decrease in 2014, but others increased (Figure ABuse02). Increases in sales were noted for amphenicols (+18%), macrolides & lincosamides (+12%), penicillins (+10%) and quinolones (+39%). Increased sales were also noted for 3rd & 4th generation cephalosporins (+4.3%) and fluoroquinolones (+2.4%).

Tetracyclines

Tetracyclines contributed most to the 2013-2014 reduction with 10 tonnes. Doxycycline represents 41% of the total sales of tetracyclines (31% in 2013, 41% in 2012 and 34% in 2011).

Trimethoprim/sulfonamides

Trimethoprim/sulfonamides combinations are still the second contributor in mass sold, although usage decreased in 2014. Because of the high doses that are needed, with the sold mass less treatments are possible than with the sold penicillin mass.

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Penicillins
Third in mass, the penicillin sales increased with 10%. 90% of this group is represented by amoxicillin (40%), ampicillin and benzylpenicillin.

(Fluoro)quinolones
Whereas sales of fluoroquinolones halved in 2013 (0.19% of total sales), a small increase of 9 kg was noted in 2014. De sales of quinolones increased with 39% in 2014.

Cephalosporins
Cephalosporins represent 0.3% of the total sales (2014: 560 kg, 2013: 100 kg). The sales of 1st and 2nd generation cephalosporins increased due to underreporting in previous years, as mentioned earlier. The sales of 3rd and 4th generation cephalosporins increased with 1 kg. 97% of these sales is applied outside the food producing animal sectors, primarily in horses and companion animals.

Conclusion
The decrease in sales of antibiotics licenced for veterinary therapies levelled off in 2014 in the Netherlands.

2.2 Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands, trends 2007-2014

Since 2011, husbandry related consumption reports are prepared by the Netherlands Veterinary Medicines Authority (SDa) using antimicrobial delivery data from all farms in the largest food production animal sectors; pigs, veal calves, broilers and (starting 2012) cattle. In 2013 also turkeys provided delivery data. SDa reports usage on the level of the sector ($DDDA_{NAT}$), on the level of farms ($DDDA_{F}$) and on the level of veterinarians ($DDDA_{VET}$). The details on the calculation of these measures can be found in SDa publications (SDa 2015). Table ABuse03 shows the animal populations for which veterinary medicinal products consumption data are reported in 2012, 2013 and 2014 (pigs, veal calves, cattle, broilers and turkeys). In Table ABuse04 the results for the $DDDA_{NAT}$ are shown.

Reductions in use in livestock were realized for cephalosporin 3rd & 4th generation and fluoroquinolones although sales of both groups increased, probably due to usage in other, not monitored, sectors. Reductions were realized for aminoglycosides and polymyxines in most sectors (except for polymyxins in broilers and aminoglycosides in pigs), application of these pharmacotherapeutic groups is monitored closely.

In the monitored sectors, 55% of the tetracycline treatments (in DDDA) consist of doxycycline.

For reporting on long-term trends for these sectors, SDa data are combined with early data from Wageningen University (LEI WUR) expressed in defined dosages / animal year, a measure witch is

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

Sector	2012	2013	2014	
pigs	710.688	710.802	704.937	
sow/piglets	328.408	332.661	368.935	
fatttening pigs	382.280	378.141	336.003	
veal calves	156.602	159.547	158.828	
cattle	1.522.500	1.532.000	1.615.000	
diary cows	924.600	958.200	966.000	
other cattle	597.900	573.800	649.000	
broilers	43.846	44.242	47.020	
turkeys	4.961	5.046	4.763	

Figure ABuse03 Veal calves: antimicrobial veterinary medicinal product deliveries in DD/DJ (WUR-LEI, 2007-2010) and $DDDA_{NAT}$ (SDa, 2011-2014).

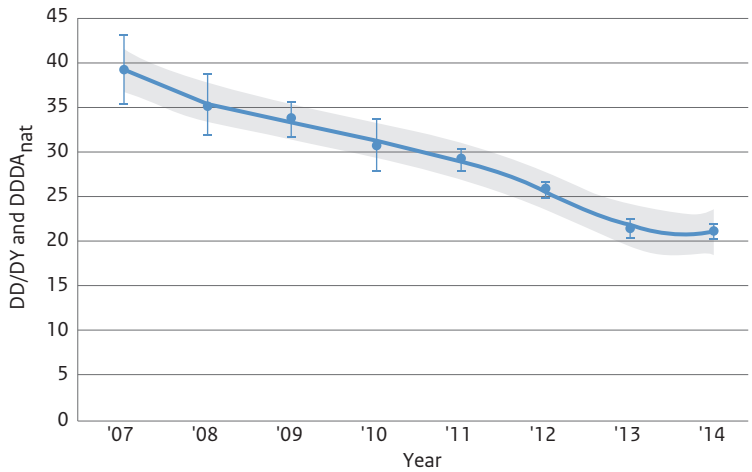

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Table ABuse04 Trends in DDDAnat in the Netherlands in livestock.

	Animalsector													
	Pigs			Veal calfs*			Cattle			Broilers			Turkeys	
Number of farms with prescriptions	6425	6588	6072	2175	2125	2061	32254	31650	31223	732	770	797	48	41
Year	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012**	2013	2014	2013	2014
Pharmacotherapeutic group														
Aminoglycosides	-	-	0.01	0.81	0.53	0.34	0.01	0.01	0.01	0.58	0.03	0.03	1.24	0.40
Amphenicols	0.06	0.09	0.17	1.23	1.23	1.52	0.05	0.07	0.08	-	-	-	0.02	-
Cefalosporins 1st & 2nd generation	-	-	-	-	-	-	0.02	0.02	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	0.42	0.09	0.01	0.85	0.66	0.30	0.52	0.37	0.08	0.00	0.00
Fluoroquinolones	0.00	0.00	0.00	0.31	0.03	0.02	0.01	0.00	0.00	0.80	0.24	0.18	1.76	1.29
Macrolides/lincosamides	1.39	1.02	1.09	3.91	3.84	3.72	0.09	0.12	0.14	1.06	0.31	0.35	3.55	2.12
Penicillins	2.91	2.17	2.05	2.80	2.11	2.15	1.22	1.45	1.27	7.46	6.34	9.96	9.34	14.89
Pleuromutilins	0.35	0.12	0.09	-	-	-	-	-	-	-	-	-	-	-
Polymyxins	0.58	0.44	0.34	0.73	0.36	0.15	0.05	0.02	0.01	0.84	0.08	0.05	0.18	0.08
Quinolones	0.03	0.03	0.05	0.27	0.30	0.49	0.00	0.00	0.01	1.97	1.65	2.22	0.23	0.02
Tetracyclines	6.79	4.58	4.34	12.61	10.87	10.66	0.48	0.48	0.42	2.40	2.52	1.77	11.19	9.58
Trimethoprim/sulfonamides	1.92	1.40	1.33	2.76	2.14	2.08	0.18	0.20	0.19	1.97	1.46	1.45	1.80	2.37
Total	14.32	9.97	9.52	25.85	21.50	21.15	3.00	3.04	2.44	17.61	13.01	15.76	29.31	30.74

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

** *Figures per pharmacotherapeutic group for 2012 based on prescriptions for approximately 60% of the farms and extrapolated with the determined treatment days ratio of 2012 / 2013*

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Figure ABuse04 Sows/piglets: antimicrobial veterinary medicinal product deliveries in DD/DJ (WUR-LEI, 2004-2010) and $DDDA_{NAT}$ (SDa, 2011-2014).

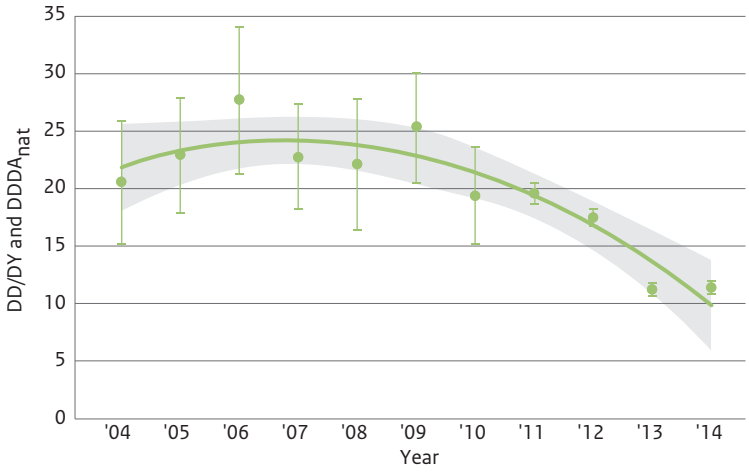

Figure ABuse05 Fattening pigs: antimicrobial veterinary medicinal product deliveries in DD/DJ (WUR-LEI, 2004-2010) and $DDDA_{NAT}$ (SDa, 2011-2014).

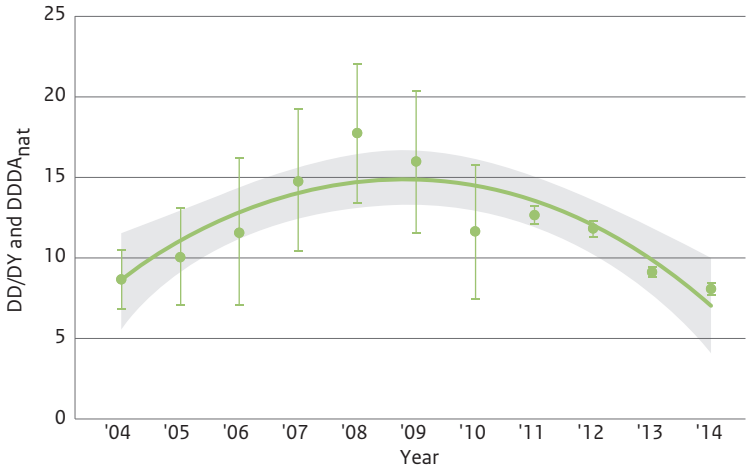

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Figure ABuse06 Broilers: antimicrobial veterinary medicinal product deliveries in DD/DJ (WUR-LEI, 2004-2010) and $DDDA_{NAT}$ (SDa, 2011-2014).

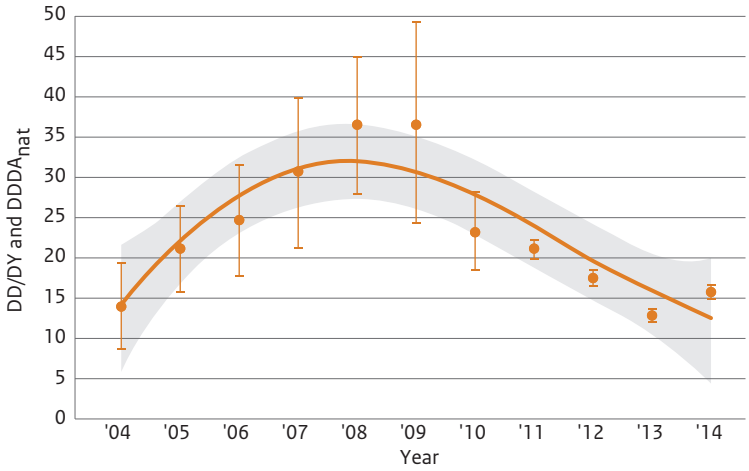
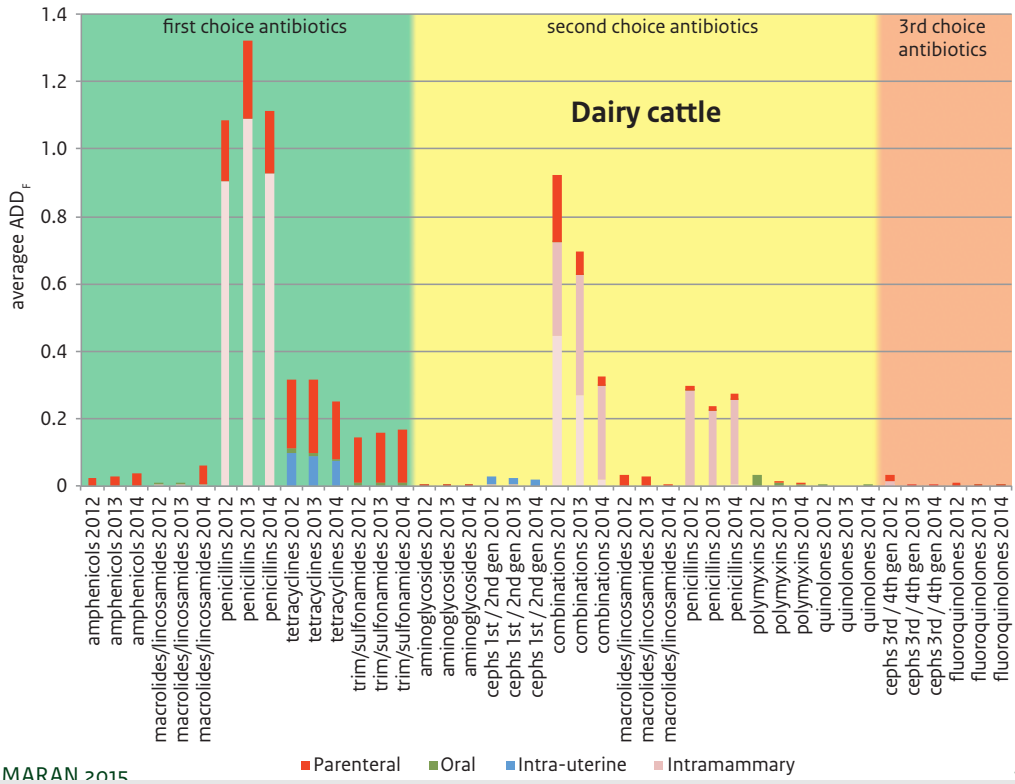


Figure ABuse07 Dairy cows: antimicrobial veterinary medicinal product deliveries in $DDDA_F$ (SDa, 2012-2014).



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comparable with the $DDDA_{NAT}$, but has been collected for a sample of farms and extrapolated to the whole sector.
Long-term sector wide usage trends for veal farming, sows/piglets farming, fattening pigs farming and broiler farming sectors as reported by LEI WUR (years 2007-2010 as DD/AY) and by SDa (years 2011-2014 as $DDDA_{NAT}$) are depicted in Figures Figure Abuse03, Figure Abuse04, Figure Abuse05 and Figure Abuse06. Point estimates (dots), 95% confidence limits (error bars), together with smoothed trend lines (penalized splines) and 95% confidence limits for the spline (shaded area) are given. For veal calves annual observations from between 2007-2010 were recalculated with average dosages of VMP's instead of maximum dosages as applied for veal calves exclusively until 2013.

Depicted in Figure Abuse07 is the average of use on the dairy cow farms, $DDDA_F$. The use is indexed per formulary choice, than by pharmacotherapeutic group per year, and categorized per route of administration.

In all sectors reduction of use is noted when comparing 2013 and 2014, except for poultry.
Of note in dairy cattle farming is the shift in antibiotics, from 3rd and 2nd choice to 1st choice VMP's, particularly in dry cow treatment between 2012 and 2013, and the reduction in dry cow treatment in 2014.

Benchmarking veterinarians

SDa introduced a benchmarking methodology for veterinarians in 2013. The details on this approach have recently been published (Bos et al., 2015). The methodology makes differences in prescription patterns of veterinarians visible and allows an analysis of causes of these differences, which is expected to lead to smaller differences in prescription practices. When prescription patterns of veterinarians are being compared, for instance by comparing the 25- and 75-percentile of the $DDDA_{VET}$, up to 7 fold differences exist between veterinarians, depending on the animal sector. For the 5- and 95-percentile these differences increase up to a factor of 33 maximally. These figures are indicative of considerable differences in prescription patterns, which cannot be explained by the effect of a few farms with higher usage figures and underlines the need for benchmarking and collegial professional discussions about antimicrobial prescription practices.

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3
Resistance data

In this chapter susceptibility test results are presented as determined in 2014 for the food-borne pathogens *Salmonella enterica*, *Campylobacter* spp. and *Escherichia coli* O157, the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*. Reduced susceptible and resistant isolates were defined using epidemiological cut-off values (www.eucast.org) for the interpretation of minimum inhibitory concentrations (MIC) values. Epidemiological cut-off values are in most cases lower than clinical breakpoints, and this can result in a proportion of non-wild type susceptible isolates being incorrectly classified as clinically resistant, depending on the MIC distribution and the antibiotic. For the purpose of this report we designate all non-wild-type susceptible isolates as "resistant", and specify this by antibiotic if necessary.

3.1 Food-borne pathogens

3.1.1 Salmonella

In this chapter resistance percentages are presented on *Salmonella* isolated 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.

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Highlights

1. In 2014 *S.* Typhimurium (N = 187) in combination with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 234), were most frequently isolated from humans suffering from salmonellosis, with S. Enteritidis (N=265) in second place.
2. In pigs, *S.* Typhimurium and its monophasic variant dominated. *S.* Derby was less prevalent compared to former years. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated.
   S. Paratyphi B var. Java was again the most predominant serovar in poultry. In 2013 and 2014 *S.* Heidelberg was frequently isolated from poultry sources. This was mainly due to contaminated poultry meat imported from Brazil, which differed from the *S.* Heidelberg isolates that resulted in human cases in 2014.
3. Highest resistance levels were observed for *S.* Heidelberg, the monophasic *S.* Typhimurium 1,4,[5],12:i:- and *S.* Paratyphi B var. Java, and to a lesser extent in *S.* Typhimurium and Infantis.
4. The dominant serovars of ciprofloxacin resistant isolates were *S.* Enteritidis (21%) predominantly derived from humans, *S.* Heidelberg (11%) , *S.* Infantis (11%), and *S.* Java (8%), mainly from poultry sources, or *S.* Typhimurium (11%) and *S.* Chester (6%) again predominantly from humans
5. In 2014, the total number of cefotaxime resistant (ESBL suspected) *Salmonella* isolates was 36/1688 (2.1%), among nine different serovars, predominantly isolated from poultry sources.
6. In 2014 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 is presented in Table S01 concerning *Salmonella* isolates recovered from humans and farm animals (swine, cattle and poultry).
Human isolates (N = 1091 in 2014) were a selection of all isolates sent to the RIVM by regional public health laboratories. All strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N = 74) and cattle (N = 45) were partially 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 = 91; layers, reproduction animals and eggs, N = 50) 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 = 244 from animal feed and food products; other animals from animal husbandry (e.g. horses, sheep, goats) and pets, samples from the environment etc.) and other poultry sources than broilers and laying hens (N=222) have been analysed as well.

Traditionally, *S.* Enteritidis or *S.* Typhimurium was most frequently isolated from human clinical infections. In 2014 *S.* Typhimurium (16%) together with the monophasic variant of Typhimurium: *S. enterica* subspecies *enterica* 1,4,5,12:i:- (20%), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (23%) in second place.

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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, but was also found (but less predominant) in cattle and poultry. *S.* Enteritidis was mainly present in poultry and more specifically layers and contaminated eggs (Table S01).
In pigs, next to *S.* Typhimurium and its monophasic variant, *S.* Derby was most predominant. In cattle, besides the *S.* Typhimurium variants, *S.* Dublin was most commonly isolated. *S.* Paratyphi B var. Java (*S.* Java) was again the most predominant serovar in poultry. In 2013 and 2014 *S.* Heidelberg was also isolated frequently in poultry. This was mainly due to contaminated poultry meat imported from Brazil, which differed from the *S.* Heidelberg isolates that resulted in human cases in 2014 (unpublished data). Depending on the serotype, reported travel contributed up to 30% of the cases of human salmonellosis in 2013/2014. Relative high contributions (between 25 and 30%) were noted for the serovars Paratyphi B var Java, Mbandaka, Typhi, Anatum, Corvallis, Minnesota and Bareilly. 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 about a factor two.

Resistance levels

The new EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in November 2013, including susceptibility testing of mandatory panels of antimicrobials. As a result for the monitoring of *Salmonella* three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice were added to the panel and three antimicrobials of less importance for treatment of human infections were deleted (florfenicol, kanamycin and streptomycin) (TableS02). 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 is demonstrated. Meropenem belongs to the carbapenems, which are last resort antimicrobials that are used to treat infections with multi-drug resistant bacteria.
Table S02 presents MIC-distributions and resistance percentages of 1688 *Salmonella*'s from different sources tested for susceptibility in 2014. Highest levels of resistance were observed for sulfamethoxazole, tetracycline, ampicillin and to a lesser extent ciprofloxacin, nalidixic acid and trimethoprim. The levels of resistance to ciprofloxacin and cefotaxime/ceftazidime have slightly decreased compared to 2013, 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 (2.8%) were found resistant to tigecycline. Using the tentative set epidemiological cut off value of 16 mg/L for azithromycin, 0.5% of the isolates (all human origin) was found resistant.

Resistance profiles varied considerably among serovars as shown in Table S03. This table presents resistance percentages for the twelve most prevalent serovars isolated in the Netherlands in 2014. Highest resistance levels were observed for *S.* Heidelberg, the monophasic *S.* Typhimurium 1,4,[5],12:i:- and *S.* Paratyphi B var. Java (referred to as *S.* Java), and to a lesser extent in *S.* Typhimurium and *S.* Infantis.

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Table S01 Most prevalent *Salmonella* serotypes isolated in 2013 and 2014 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

		Travel related	Humans		Pigs		Cattle	
		2013-2014	2013	2014	2013	2014	2013	2014
N Total			1202	1176	90	83	54	47
N tested	Tested		1103	1091	73	74	52	45
Enteritidis	650	13%	315	265		1	2	
Typhimurium	638	6%	214	187	29	28	13	25
SI 1,4,5,12:i:2-	506	2%	182	234	29	31	14	8
Infantis	161	10%	34	30	3		1	
Paratyphi B var. Java	156	29%	17	10		2		
Heidelberg	88	3%	4	46				
Dublin	75	2%	6	29			16	12
Agona	71	11%	5	9				
Derby	71	6%	12	15	18	9		
Brandenburg	56	2%	16	21	2	4		1
Senftenberg	56	8%	5	4		1		
Mbandaka	51	27%	5	10				
Livingstone	49	0%	1	4		1		
Typhi	48	30%	26	25				
Thompson	41	2%	31	10				
Kentucky	33	23%	19	9				
Newport	32	8%	14	14			2	1
Montevideo	31	13%	5	3			3	
Napoli	28	7%	17	13				
Braenderup	27	11%	7	4				
Virchow	26	14%	17	9				
Stanley	25	22%	14	10				
Rissen	24	16%	11	1				
Saintpaul	24	13%	3	18				
Anatum	23	29%	7	1	5			
Panama	23	21%	11	6	1		1	
Corvallis	21	26%	10	10				
Hadar	21	16%	8	5				
London	19	3%	6	9		1		
Indiana	15	0%	3	1				
Oranienburg	15	21%	4	5				
Bovismorbificans	14	11%	6	8			1	
Goldcoast	14	4%	5	2	1	3		
Poona	13	19%	9	5				
Muenchen	11	18%	6	2				
Javiana	10	5%	6	5				
Minnesota	10	25%						
Kottbus	7	13%	2	4				
Gallinarum	4	n.a.						
Mikawasima	4	0%	1	2				
Bareilly	2	25%	1	2				
SI 9,12:l,v:2-	1	0%	6	25		1		
Other	376	15%	131	104	2	1	1	

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Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2013 and 2014 from humans, pigs, poultry, broilers (including poultry products) and layers (including reproduction animals and eggs) and the % travel related human infections.

	Poultry		Broilers		Layers		Other	
	2013	2014	2013	2014	2013	2014	2013	2014
N Total	431	315	206	135	53	52	998	948
N tested	276	222	119	91	50	50	389	244
Enteritidis	43	45	11	10	17	25	53	27
Typhimurium	31	19	4	8	10	4	119	82
SI 1,4,5,12:i:2-	8	10	3	7	3	2	20	34
Infantis	63	48	21	28		3	42	49
Paratyphi B var. Java	100	81	54	26		2	24	19
Heidelberg	91	40	69	24			10	5
Dublin		1				1	6	11
Agona	9	7	2	5	4	1	28	27
Derby	5	3	3	2	1		30	1
Brandenburg	4	1	2				11	7
Senftenberg	3	4	2	1		2	45	89
Mbandaka	5	3	2	1	1	2	27	25
Livingstone	7	2	5	2	2		76	135
Typhi								
Thompson	1	1		1			2	4
Kentucky		1		1			7	3
Newport							3	1
Montevideo		3		2			24	9
Napoli							1	
Braenderup	9	2	3		6	2	9	3
Virchow							4	4
Stanley							2	2
Rissen	1	1			1	1	10	4
Saintpaul	1	1		1			4	2
Anatum	3	2	3			2	61	162
Panama							23	16
Corvallis	3	2						
Hadar	3	5	3	2			5	4
London		1					17	
Indiana	3	8	2	4		1	2	3
Oranienburg							11	4
Bovismorbificans							1	4
Goldcoast	1	2	1	2			3	1
Poona	1						6	2
Muenchen							4	
Javiana								
Minnesota	14		13				1	1
Kottbus	1	1		1	1		2	
Gallinarum	1	2				2	2	1
Mikawasima								2
Bareilly							1	
SI 9,12:l,v:2-	1				1		1	3
Other	19	19	3	7	6	2	301	193

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Table S02 MIC distribution (in %) and resistance percentages (R%) for all Salmonella's (N=1688) tested for antibiotic susceptibility during 2014.

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							39.6	29.9	2.5	0.1	0.1		0.2	27.6					27.8	25.7 - 30.0
Cefotaxime					96.9	1.0	0.2			2.0									2.1	1.5 - 2.8
Ceftazidime						94.9	2.8	0.4	0.4	0.5	0.9								1.9	1.3 - 2.5
Gentamicin						69.3	27.4	1.5	0.1	0.4	0.6	0.4	0.4						1.9	1.3 - 2.5
Tetracycline								63.9	7.2	0.5	0.1	0.7	2.7	25.1					28.5	26.4 - 30.7
Sulfamethoxazole										42.7	20.3	5.5	0.5		0.1			31.0	31.0	28.9 - 33.2
Trimethoprim					75.4	12.2	1.1	0.1				0.1	11.3						11.3	9.8 - 12.9
Ciprofloxacin	36.4	47.5	1.4	1.2	6.8	4.5	1.5	0.2		0.2	0.4								14.8	13.2 - 16.5
Nalidixic acid									79.9	5.4	1.9	0.7	0.1	1.1	11.0				12.9	11.3 - 14.5
Chloramphenicol										83.7	11.2	0.4	0.1	0.3	4.4				5.1	4.1 - 6.2
Azithromycin*								0.1	52.3	44.0	3.1	0.2	0.1	0.2					0.5	0.2 - 0.8
Meropenem		89.1	10.8	0.1															0.0	0.0 - 0.0
Tigecycline					42.8	48.3	6.0	2.6	0.2										2.8	2.1 - 3.7

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 the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoint are identical.

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

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Table S03 Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2014 (N tested).

	Enteritidis (302)	Typhimurium (291)	1.4,[5].12:i:- (278)	Infantis (79)	Paratyphi B var Java (63)	Heidelberg (47)	Dublin (54)	Agona (34)	Derby (29)	Brandenburg (28)	Senftenberg (33)	Mbandaka (28)
Ampicillin	4.0	48.8	77.0	12.7	44.4	44.7	0.0	8.8	10.3	9.7	3.0	0.0
Cefotaxime	0.0	1.4	0.4	2.5	7.9	42.6	0.0	2.9	3.4	3.2	0.0	0.0
Ceftazidime	0.0	0.3	0.4	2.5	7.9	42.6	0.0	2.9	3.4	0.0	0.0	0.0
Gentamicin	0.0	3.1	1.1	2.5	9.5	2.1	0.0	2.9	0.0	0.0	0.0	0.0
Tetracycline	2.3	42.3	78.8	36.7	6.3	59.6	0.0	8.8	10.3	3.2	0.0	10.7
Sulfamethoxazole	1.0	45.4	77.0	44.3	79.4	59.6	3.7	8.8	17.2	3.2	3.0	0.0
Trimethoprim	0.7	17.9	6.5	31.6	85.7	0.0	0.0	2.9	13.8	3.2	3.0	0.0
Ciprofloxacin	16.9	9.3	1.1	35.4	33.3	59.6	0.0	17.6	0.0	12.9	6.1	7.1
Nalidixic acid	16.2	7.9	0.7	35.4	33.3	59.6	0.0	11.8	0.0	12.9	6.1	0.0
Chloramphenicol	1.0	17.5	2.5	6.3	4.8	0.0	3.7	0.0	0.0	3.2	0.0	7.1
Azithromycin	1.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	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	6.9	0.4	13.9	0.0	12.8	0.0	2.9	0.0	3.2	0.0	0.0

Generally, *S.* Typhimurium and the monophasic variant have acquired resistance against a number of antimicrobials. The most common resistance pattern was resistance to amoxicillin, sulfamethoxazole and tetracycline (ASuT). High resistance levels for ciprofloxacin and nalidixic acid were commonly found in *Salmonella* strains. Highest resistance levels to the fluoroquinolones were found in *S.* Heidelberg, *S.* Infantis, *S.* Paratyphi B var Java derived from poultry and to a lesser extent *S.* Enteritidis, *S.* Agona and *S.* Brandenburg, reflecting the usage of quinolones in poultry production. Isolates suspected to be ESBL producing (cefotaxime resistant) dominated again in *S.* Heidelberg from imported poultry products.

Quinolone resistance

The class of fluoroquinolones is widely regarded as the treatment of choice for severe salmonellosis in adults. Using the epidemiological cut off value of 0.06 mg/L, 14.8% of *Salmonella* isolates (N =250/1688) demonstrated a resistant phenotype for ciprofloxacin, while 0.8% showed MICs larger than the formerly used clinical breakpoint (1 mg/L). 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). The dominant serovars of ciprofloxacin resistant isolates were *S.* Enteritidis (21%) predominantly derived from humans, *S.* Heidelberg (11%) , *S.* Infantis (11%), and *S.* Java (8%), mainly from poultry sources, or *S.* Typhimurium (11%) and *S.* Chester (6%) again mainly from humans.

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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 2014, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 36/1688 (2.1%), among nine different serovars. Eight isolates were derived from humans (four *S.* Typhimurium, two *S.* Infantis, one *S.* Brandenburg, and one *S.* Derby), almost all other isolates (n=24) were derived from poultry sources (seventeen *S.* Heidelberg, five *S.* Java, one *S.* Abony, one monophasic *S. enterica subspecies enterica* 1,4,[5],12:i:-). Again, like in 2013, *S.* Heidelberg derived from poultry products imported from Brazil were most predominant. Cefotaxime resistant *S.* Heidelberg comprised 43% of total *S.* Heidelberg isolated and cefotaxime resistant *S.* Java comprised 8% of total *S.* Java isolated.

S. Typhimurium

As shown in Table S01, *S.* Typhimurium represented 15.9% (187/1176) of all human *Salmonella* isolates as characterized by the RIVM in 2014. This is slightly less than in 2013 (17.8%, (214/1202)). In animals *S.* Typhimurium is a common serotype. If the monophasic SI 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 and it is also frequently isolated from poultry sources.
Resistance in *S.* Typhimurium was very high for ampicillin, tetracycline and sulfonamides (Table S04). Resistance to the clinical important drug cefotaxime was only seen in isolates from humans at a low level (2.1%), which suggests a non-domestic source. Resistance to the also important antimicrobial class fluoroquinolones had low to moderate levels (0% in pigs -22.2% in poultry). Resistance to chloramphenicol and trimethoprim was common. In *S.* Typhimurium derived from humans, cattle and

Table S04 Resistance percentages of S. Typhimurium (N tested) isolated from different sources in 2014.

	S. Typhimurium (291)				
	Humans (193)	Cattle (23)	Pigs (27)	Poultry (18)	Food products (30)
Ampicillin	48.7	56.5	59.3	44.4	36.7
Cefotaxime	2.1	0.0	0.0	0.0	0.0
Ceftazidime	0.5	0.0	0.0	0.0	0.0
Gentamicin	4.7	0.0	0.0	0.0	0.0
Tetracycline	45.6	39.1	48.1	27.8	26.7
Sulfamethoxazole	48.2	69.6	48.1	27.8	16.7
Trimethoprim	18.7	34.8	14.8	5.6	10.0
Ciprofloxacin	11.4	4.3	0.0	22.2	0.0
Nalididixic acid	9.3	4.3	0.0	22.2	0.0
Chloramphenicol	18.7	13.0	22.2	22.2	6.7
Azithromycin	1.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	8.8	4.3	7.4	0.0	0.0

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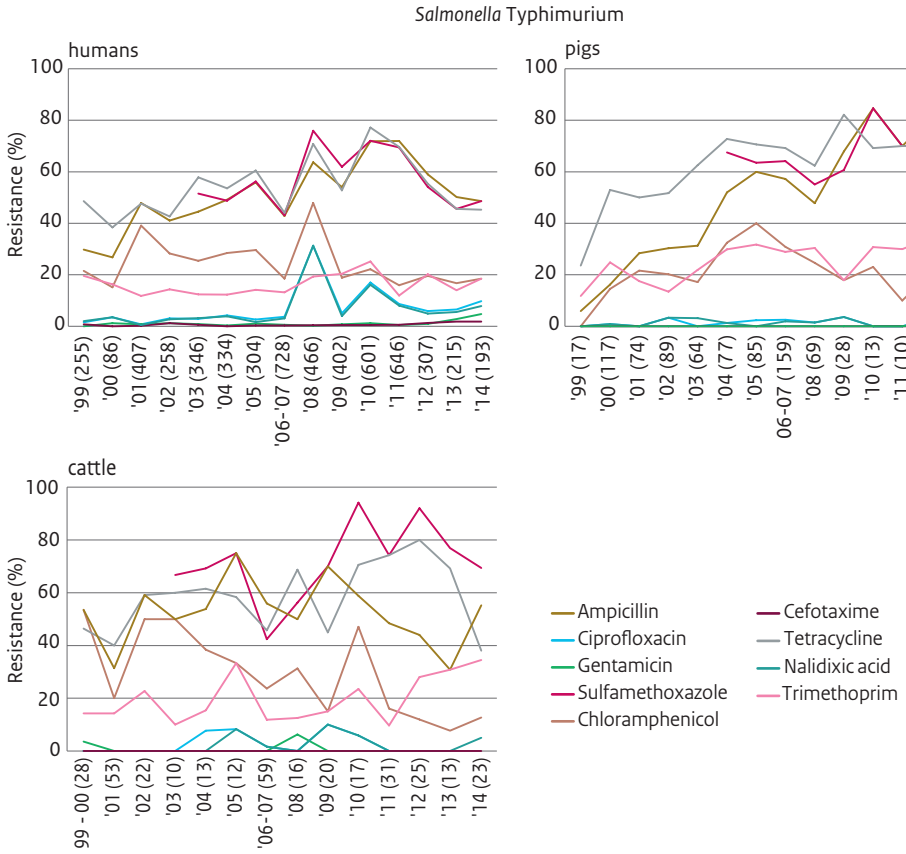
Figure S01 Trends in resistance (%) of *S. Typhimurium* isolated from humans and food-animals in 1999 - 2014.



pigs low level resistance to tigecycline (4.3-8.8%) was found. Azithromycin resistance occurred at a very low level (1%) in S. Typhimurium and only in isolates derived from humans.

With regard to trends, resistance levels in S. Typhimurium isolates from human samples have increased over the years until 2010 after which resistance showed a constant tendency to decrease until 2013. In 2014 resistance levels for almost all antimicrobials tested stabilized or tended to increase again (Figure S01). With regard to animal strains, resistance levels vary considerably over the years and interpretation should be done with caution because of the relatively small number of the isolates per year.

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

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Table S05 Resistance percentages of *S. Enteritidis* (N tested) isolated from different sources in 2014.

	S. Enteritidis (302)		
	Humans (253)	Laying hens (25)	Other sources* (24)
Ampicillin	4.3	4.0	0.0
Cefotaxime	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Tetracycline	2.8	0.0	0.0
Sulfamethoxazole	1.2	0.0	0.0
Trimethoprim	0.8	0.0	0.0
Ciprofloxacin	19.0	0.0	12.5
Nalididixic acid	18.2	0.0	12.5
Chloramphenicol	1.2	0.0	0.0
Azithromycin	1.2	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*

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. Interesting is the moderate resistance of strains from human infections compared to the lack of resistance in Dutch layers, which indicates that other sources of infection exist. These 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 was very low, except resistance to the quinolones as shown in Table S05. The trends in resistance levels over the years are summarized in Figure S02. It should be noted that the variation in quinolone resistance levels over the years is also reflected by the relative proportion of certain MLVA types. Apart from this, similar to the situation for *S.* Typhimurium, resistance levels vary considerably over the years because of the relatively small number of animal isolates per year and interpretation should be done with great caution. In humans, there was an apparent increase in quinolone resistance levels since 2012. In 2014, quinolone resistance decreased only slightly.

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

As in previous years, in 2014 *S.* Java was the most predominant serovar isolated in broiler production. (Table S01). From poultry, 42 *S.* Java strains were included for susceptibility testing (Figure S03). All harboured the phenotype typical for the clone, which is characterized by high level resistance to trimethoprim. This occurs frequently in combination with acquired resistance against the quinolones and third generation cephalosporins (cefotaxime and ceftazidime). A large proportion of *S.* Java isolates from poultry expressed resistance to ciprofloxacin and nalidixic acid (both 42.9%); Resistance to

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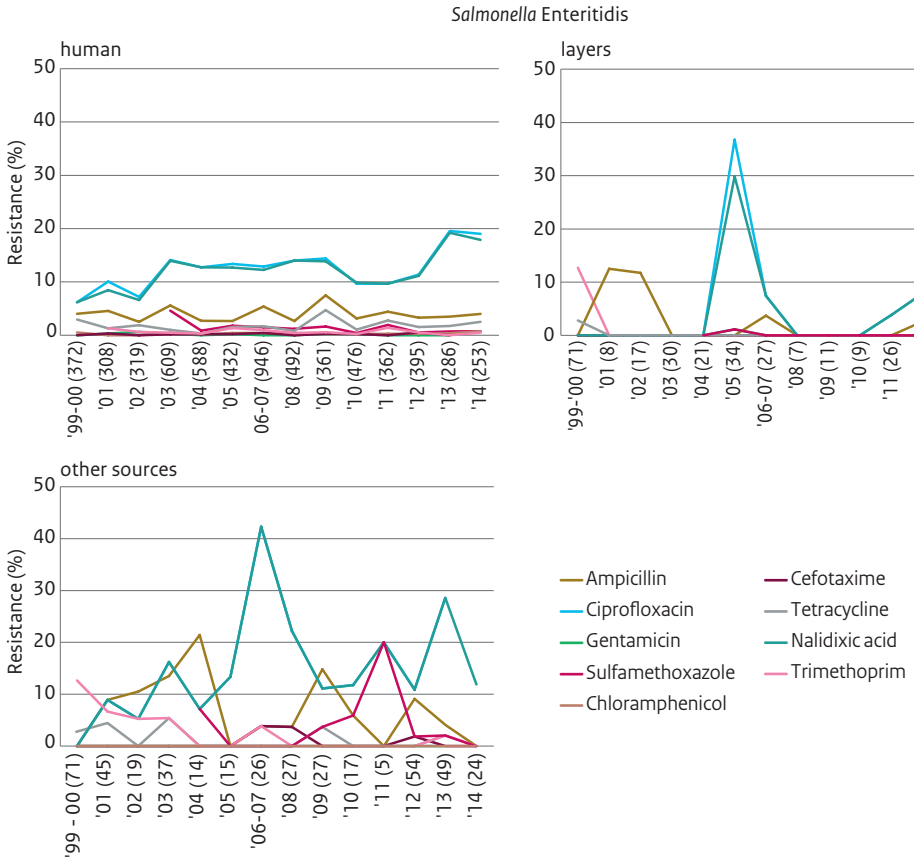
Figure S02 Trends in resistance (%) of S. Enteritidis isolated from humans, layers and other sources from 1999 - 2014.



cefotaxime/ceftazidime (ESBL-producers) was detected in 11.9% of the isolates from poultry, which is substantially higher than last year (1.8%) and comparable to 2012 (11.4%). This phenomenon is not fully understood and could partially be due to sampling bias. Remarkable increases of resistance were also found for sulfamethoxazole, ampicillin, and gentamicin, whereas resistance levels to the quinolones decreased (Figure S03).

A number of *S.* Java strains were isolated from human infections in 2014 (n=10). All strains tested, except for one, were trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related. The single human *S.* Java strain resistant to trimethoprim was susceptible to third generation cephalosporins and quinolones.

Salmonella in raw meats from poultry, animal feed and other sources at retail

Resistance data in meat are presented for poultry meat only, because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Table S06, Figure S03). In 2014 *S.* Java

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Figure S03 Trends in resistance (%) of *S. Paratyphi B var. Java* isolated from poultry sources from 1999 - 2014 and humans (Separate data on the rigt indicate all human S. java isolates from 1999 - 2014).

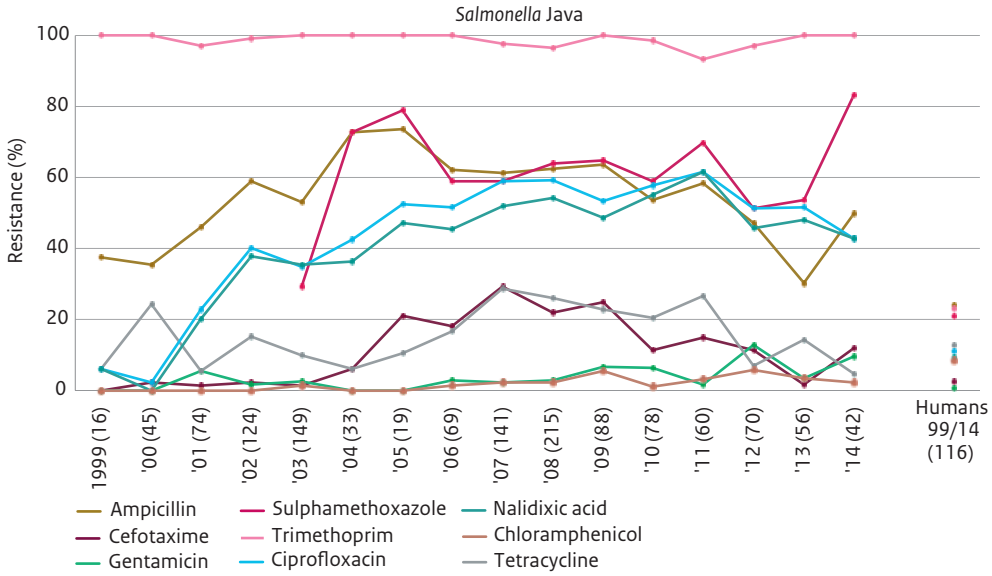

Table S06 Resistance (%) of *Salmonella* enterica isolated from raw meats from poultry and other meat sources, herbs and spices and animal feed in the Netherlands in 2014.

	poultry meat S. Java	poultry meat other serovars	other raw meat all serovars	herbs/spices all serovars	Animal feed all serovars
	N = 31	N = 61	N = 35	N = 22	N = 70
Ampicillin	45.2	21.3	37.1	0.0	1.4
Cefotaxime	3.2	13.1	8.6	0.0	0.0
Ceftazidime	3.2	13.1	8.6	0.0	0.0
Gentamicin	9.7	3.3	0.0	0.0	0.0
Tetracycline	6.5	57.4	40.0	9.1	1.4
Sulfamethoxazole	83.9	52.5	37.1	9.1	1.4
Trimethoprim	100.0	16.4	14.3	9.1	0.0
Ciprofloxacin	45.2	59.0	22.9	18.2	0.0
Nalididixic acid	45.2	57.4	22.9	0.0	0.0
Chloramphenicol	3.2	0.0	2.9	0.0	0.0
Azithromycin	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	18.0	2.9	0.0	0.0

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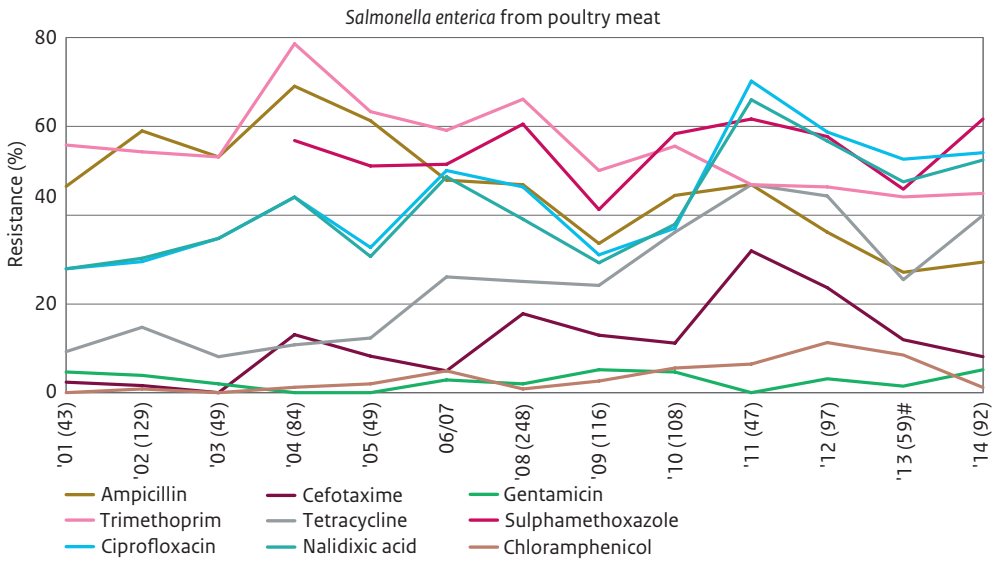
was the dominant serovar found in raw meat products (19.2%), followed by S. Infantis (14.3%) and S. Heidelberg (9.9%), mainly isolated from poultry sources.
Overall resistance levels in poultry meat are higher than in meat from other sources. Noteworthy in poultry meat isolates other than S. Java is the high level of resistance against quinolones (57.4-59.0%) and the relatively high level of resistance to tigecycline (18%). Resistance to ciprofloxacin was present in 18.2% (n=4) of herbs/spices isolates, interestingly no resistance to nalidixic acid was found in those isolates. This might be explained by the presence of plasmid mediated quinolone resistance (PMQR) genes, exhibiting resistance to ciprofloxacin, but not nalidixic acid. Eleven different *Salmonella* serotypes were found among 21 samples from herbs and spices. Among those were five of the twelve most prevalent serotypes described earlier in Table S03: S. Enteritidis (n=1), S. Typhimurium (n=2), S. Mbandaka (n=3), S. Senftenberg (n=3) and S. Agona (n=3).

Figure S04 shows the overall resistance levels of *Salmonella* from poultry products over the years. It should be noted that this not necessarily reflects the situation in humans to resistant salmonellae. For instance S. Java, with a substantial contribution to the resistance levels, is hardly infective for humans.

Figure S04 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meat in the Netherlands from 2001-2014.



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

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3.1.2 Campylobacter

This chapter describes the resistance in *Campylobacter jejuni* and *C. coli* isolated from food animals, meat and from humans suffering from diarrhoea. Samples from food animals, as well as meat samples have been collected. Data on human isolates were derived from sixteen regional public health laboratories. In previous years also MIC data on isolates from veal calves, dairy cows, pigs and turkeys were included. 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 also included in the surveillance. Also *C. coli* isolated from pork were included.

In Table C01 the MIC-distributions and resistance percentages are summarized for all *Campylobacter jejuni* and *C. coli* strains isolated at CVI from broilers in 2014. Table C02 shows the more detailed resistance profiles of *C. jejuni* and *C. coli* according to the different sources (meat as well as from faecal samples from different animal species). Figure C01 and C02 present trends over the last decade in resistance of *C. jejuni* and *C. coli* from broilers and broiler meat products.
National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and 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. In the last five years resistance rates seem to have stabilized in *C. jejuni* from broilers and poultry meat.
3. 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*.
4. Macrolide resistance was not detected in *C. coli* from pork.
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, first choice antibiotic in human medicine for campylobacteriosis, is still 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 new EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in November 2013, including susceptibility testing of mandatory panels of antimicrobials. As a result for the monitoring of *Campylobacter spp,* six out of twelve antimicrobials were no longer included in the survey (ampicillin, chloramphenicol, clarithromycin, tulathromycin, sulfamethoxazole and neomycin). The remaining six antimicrobials (ciprofloxacin and nalidixic acid (quinolones), erythromycin (macrolides), tetracycline (tetracyclines), gentamicin and streptomycin (aminoglycosides)) all represent antimicrobial classes important in human

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Table C01 MIC distribution (in %) for all *Campylobacter jejuni* (N = 98) and *C. coli* (N = 39) isolated from faecal samples of broilers and pigs in 2014.

C. jejuni	MIC (%) distribution mg/L												R%	95% CI
(N = 98)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	29.6	5.1	1.0				26.5	24.5	13.3				64.3	54.6 - 73.9
Nalidixic acid					11.2	18.4	8.2	1.0			61.2		61.2	51.3 - 71.0
Erythromycin				87.8	12.2								0.0	0.0 - 3.7
Gentamicin	75.5	24.5											0.0	0.0 - 3.7
Streptomycin		11.2	65.3	23.5									0.0	0.0 - 3.7
Tetracycline			37.8	12.2	1.0	2.0		1.0	4.1	2.0	39.8		50.0	39.8 - 60.1

C. coli	MIC (%) distribution mg/L												R%	95% CI
(N = 39)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	35.9	12.8				10.3	20.5	15.4	5.1				51.3	35.2 - 67.2
Nalidixic acid					2.6	38.5	7.7				51.3		51.3	35.2 - 67.2
Erythromycin				79.5	12.8	5.1						2.6	2.6	0.0 - 7.6
Gentamicin	2.6	82.1	15.4										0.0	0.0 - 9.0
Streptomycin			15.4	66.7	5.1		2.6	5.1	5.1				12.8	2.1 - 23.5
Tetracycline			38.5	2.6							59.0		59.0	4.2 - 74.7

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 raw meat from pigs (only *C. coli*) and poultry and from faecal samples of broilers and laying hens in 2014.

	C. jenuni			*C. coli*			
	Poultry meat	Broilers	Laying hens	Pigs	Poultry meat	Broilers	Laying hens
N	145	98	61	46	84	39	90
Ciprofloxacin	63.4	64.3	34.4	13.0	76.2	51.3	53.3
Nalidixic acid	63.4	61.2	27.9	15.2	76.2	51.3	53.3
Erythromycin	0.7	0.0	0.0	0.0	21.4	2.6	1.1
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptomycin	1.4	0.0	0.0	69.6	4.8	12.8	3.3
Tetracycline	37.2	50.0	18.0	84.8	72.6	59.0	50.0

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medicine for treatment of campylobacteriosis. In the last five years resistance in *C. jejuni* from broilers and poultry meat seems to have stabilized. In *C. coli*, more fluctuation was observed over the years than in *C. jejuni* due to the relative low number of isolates included in the survey. In 2014 the highest resistance levels of *C. jejuni* in poultry were detected for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table CO1). Low resistance levels were observed for streptomycin and erythromycin and no resistance for gentamicin. In laying hens, resistance levels for the quinolones and tetracycline were substantially lower compared to broilers (Table Co2). This most probably reflects the lower use of antimicrobials on laying hen farms. In *C. coli* from poultry meat levels of resistance were similar to *C. jejuni* with the exception of erythromycin resistance which is more commonly observed in *C. coli*. Like in *C. jejuni*, no resistance was detected for gentamicin in *C. coli*. In *C. coli* from pork, erythromycin resistance was not observed which might reflect the decreasing use of macrolides (tylosine, tilmicosin and tulathromycin) in pigs husbandry.

Quinolones

The increasing trend in the percentage of isolates resistant to the quinolones, both in strains from animal origin (Figure Co1 and Co2) as in those 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. In *C. jejuni* from poultry meat the resistance level was identically high with 63.4%. High levels of quinolone resistance were also observed in *C. coli* from broilers (51.3%) and poultry meat (76.2%). Also in laying hens ciprofloxacin resistance rates were relatively high in both *C. jejuni* (34.4%) and *C. coli* (53.3%). In pigs quinolones are not used very frequently. As a result resistance levels in isolates from pigs are relatively low (13.0%). In human *C. jejuni* in 2014 the resistance level for ciprofloxacin was higher than in 2013 (60.7%) versus 57.6%. These figures indicate that ciprofloxacin resistance in Campylobacters is still rising, both in poultry (products) and human patients.

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. The level of resistance for macrolides reported in animals and humans is low for *C. jejuni*, on average 3.3% of strains from animal origin in 2014 and 2.2% of human isolates from 2012-2014 (n=8083) 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*).

As in former years, erythromycin resistance is scarce in *C. jejuni* with no resistance in broilers and 0.7% in poultry products. In contrast, erythromycin resistance is more frequently present in *C. coli* from broilers (2.6%) and poultry meat (21.4%). The large difference in macrolide resistance of *C. coli* from animals and meat products maybe a result of the inclusion of foreign poultry products in the survey. Macrolide resistance was not detected among *C. coli* isolates from pork which might reflect the decreasing use of macrolides in pigs.

Broiler chickens, laying hens and poultry meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from animals as well as from meat samples. In 2014, *Campylobacter* isolated from faecal samples of broilers and laying

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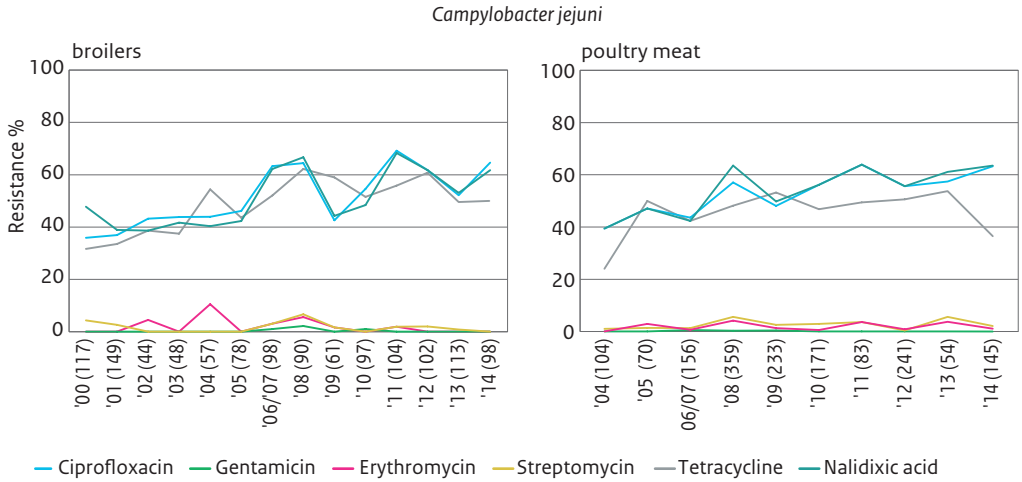
Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and poultry meat in the Netherlands.



hens were included. In laying hens the antibiotic use is on average considerably less than in conventionally raised animals.
As shown in Table C02, 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 are more comparable. In *C. jejuni* isolates from poultry meat the overall resistance rates were similar to isolates from broilers. Differences between meat and animals seemed larger in *C. coli*. More specifically, macrolide resistance in *C. coli* was clearly higher in meat than in animals. 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 from animals and meat products maybe a result of the samples of foreign poultry products included in the survey.

Pigs

As a result of prioritization and changes in legislation, from 2014 onwards *C. coli* from pigs will no longer be part of the surveillance. However, a total of 46 *C. coli* isolates from pork were included in the survey and the results will be briefly discussed. In *C. coli* from pork, highest resistance levels were observed for tetracycline (84.8%), followed by streptomycin (69.6%). Resistance to nalidixic acid and ciprofloxacin was relatively low (15.2% and 13.0%, respectively) compared to levels in Dutch broilers (> 75%), reflecting the low use of quinolones in swine. Trends in resistance of *C. coli* from pork are difficult to determine, because of the low number of isolates tested each year.

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 (discontinuous) increasing tendency of ciprofloxacin resistance in human patients. For the first time since 2008, a lower resistance rate was observed for tetracycline in 2014. Resistance to erythromycin seems to stabilize at a low level.

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

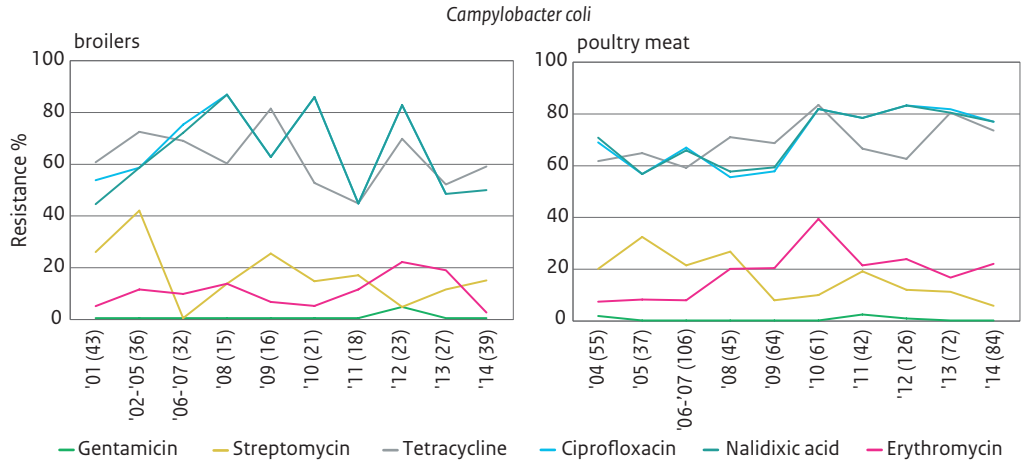

Table C03 Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans from 2002 - 2014 from all 16 Public Health Services (PHLS) covering >50% of the Dutch population.

	2003-2006							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	9118	35.5	552	38	745	55	73	53
Tetracycline	6625	19	487	22	513	28	65	15
Erythromycin	7544	1.4	520	3.3	625	1.8	69	2.9

	2012-2014							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	9059	58	663	64	431	69.6	56	70
Tetracycline	4699	39	369	53	109	51	19	63
Erythromycin	7737	2.1	524	15	346	4	46	28

	Campylobacter spp. (R%)						
	2014	2013	2012	2011	2010	2009	2003/6
Fluoroquinolone	60.7	57.6	59.4	57	53.3	51.4	37.7
Tetracycline	33.3	38.5	35.4	25.5	22.1	20.3	20.5
Erythromycin	3.3	3.2	3	3.7	2.7	2.6	1.7

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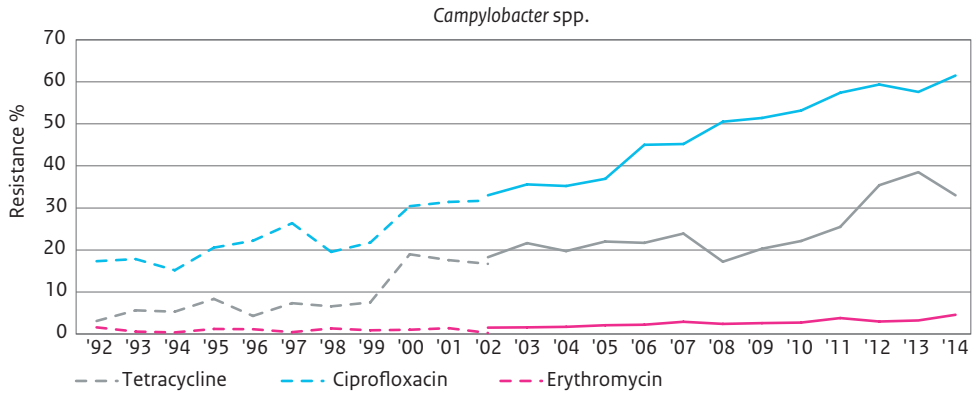
Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2002 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002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



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

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

Highlights

1. Over the last decade, STEC isolates show a tendency of increasing resistance to ampicillin, tetracycline, sulfamethoxazole and trimethoprim resulting in approximately 10% resistance for all four antibiotics in 2014.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) decreased from 4.2% in 2013 to 2.4% in 2014.
3. As in the former four years, no ESBL-producing isolates were detected.

In 2014, 125 Shiga-toxin producing *E. coli* O157 (STEC) isolates were tested for susceptibility. Since 2012, isolates were only obtained from human patients and not reported any more for cattle. MIC results are presented in Table STEC01 and the trends over time in Figure STEC 01.

Trends in resistance

In the last decade, resistance rates of STEC isolates show a tendency to increase for a number of antimicrobials as shown in Figure STEC 01. Traditionally, resistance levels in *E. coli* O157 have been very low. Clear increases have been observed over the years for ampicillin, tetracycline, sulfamethoxazole and trimethoprim with resistance percentages ranges from 9.6 – 12.0%. After the first occurrence of quinolone resistant isolates in 2013, resistance for ciprofloxacin and nalidixic acid decreased from 4.2% resistance in 2013 to 2.4% in 2014. As in former four years, no ESBL-producing isolates were detected.

Figure STEC01 Trends in resistance (%) of *E. coli* O157 (STEC) isolated in the Netherlands from humans from 1999-2014.

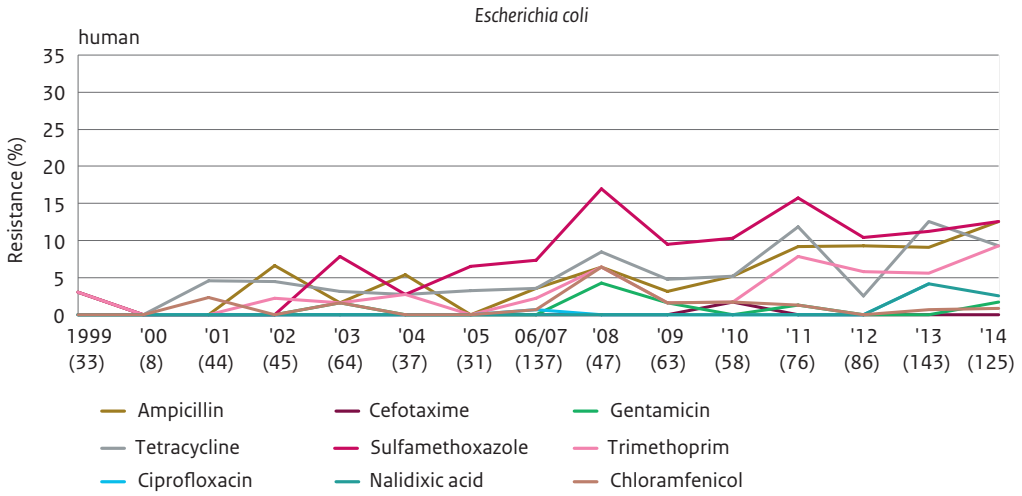

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Table STEC01 MIC distribution (in %) and resistance percentages (R%) for all E. coli O157 (N=125) isolated from humans the Netherlands in 2014.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 125	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								7.2	79.2	1.6		0.8		11.2					12.0	6.1 - 17.8
Cefotaxime					100														0.0	0.0 - 2.9
Ceftazidime						100													0.0	0.0 - 2.9
Gentamicin						32.0	63.2	3.2			0.8	0.8							1.6	0.0 - 3.8
Tetracycline								59.2	31.2			0.8		8.8					9.6	4.3 - 14.8
Sulfamethoxazole										88.0								12.0	12.0	6.1 - 17.8
Trimethoprim					83.2	7.2							9.6						9.6	4.3 - 14.8
Ciprofloxacin	68.8	28.8			0.8	0.8			0.8										2.4	0.0 - 5.1
Nalidixic acid									96.8	0.8					2.4				2.4	0.0 - 5.1
Chloramphenicol										93.6	5.6				0.8				0.8	0.0 - 2.3
Azithromycin*								37.6	52.8	8.0	0.8			0.8					0.8	0.0 - 2.3
Colistin							97.6	1.6											0.8	0.0 - 2.3
Meropenem		99.2	0.8																0.0	0.0 - 2.9
Tigecycline					96.0	4.0													0.0	0.0 - 2.9

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)*

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3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal micro-organisms of the gastro-intestinal tract in food-producing animals. 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. In addition, monitoring programs in veal calves at farms stopped, from 2012 and onwards samples of veal calves were taken at slaughterhouses. Resistance levels were reported separately for white veal calves and rosé veal calves, for the first year in 2012. Furthermore, in 2014 besides broilers, also layer hens were included in the surveillance.

It should be noted, that these sampling strategies are inherently insensitive to detect resistance as only one randomly selected isolate is tested for susceptibility from a single sample taken from one animal per epidemiological unit (herd or flock). 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 1% of the *E. coli* bacteria are resistant. Because each animal harbours about 10^6 cfu/g faeces *E. coli* in its gut, 1% would be approximately 10^4 cfu/g faeces. 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.

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3.2.1 *Escherichia coli*

Highlights

1. In most animal species resistance levels of indicator *E. coli* from faecal samples stabilized in 2014. This may reflect the use patterns of antibiotics in the different livestock species.
2. In isolates from broiler meat, beef and pork, resistance showed a tendency to decrease. In veal the trends are variable due to low numbers annually examined.
3. Resistance to third-generation cephalosporins was low in most animal species, most likely the result of the stringent limitations in usage of cephalosporins in food producing animals.
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, tetracyclines, sulfonamides and trimethoprim was 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. In *E. coli* from laying hens levels of resistance were substantially lower than those from broilers for almost all antibiotics tested.

In this chapter information is presented on resistance in *E. coli* from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-negative bacteria present in the gastro-intestinal tract of food-producing animals.
The new EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in November 2013, including susceptibility testing of mandatory panels of antimicrobials. As a result, in 2014 for *E. coli* three antibiotics (streptomycin, kanamycin and florfenicol) were excluded from the national monitoring and three new antibiotics were included: meropenem, azithromycin and tigecycline. Carbapenems (including meropenem), azithromycin and tigecycline are used in human medicine for treatment of infections with highly resistant Gram-negative bacteria.

Resistance levels

Resistance levels of a total of 1519 *E. coli* isolates obtained from broilers, laying hens, 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 2014 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.
In addition, resistance levels of 1313 *E. coli* isolates collected from raw meat products are presented in Table Eco03. Trends in resistance of *E. coli* isolated from poultry meat products, beef, pork, veal and lamb in the Netherlands from 2002 to 2014 are presented in Figure Eco03.

Table Eco02 shows that for most drugs or drug classes there are notable variations in resistance levels between the different animal species. Highest levels are recorded for broilers, white veal calves and slaughter pigs, lower levels for rosé veal calves and laying hens and lowest levels for dairy cattle.

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Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1519) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2014.

E. coli	MIC (%) distribution mg/L																	R%	95% CI
N = 1519	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	22.2	44.2	4.5	0.1	0.1	0.1	27.5					27.8	25.5 - 30.1
Cefotaxime					98.8	0.3	0.1	0.1	0.1	0.7									1.2	0.6 - 1.7
Ceftazidime						98.8	0.5	0.2		0.2	0.3								1.2	0.6 - 1.7
Gentamicin						22.1	63.1	11.5	1.5	0.1	0.7	0.7	0.4						3.4	2.4 - 4.3
Tetracycline								42.8	22.3	0.8	0.3	0.5	6.2	27.1					34.1	31.6 - 36.5
Sulfamethoxazole										69.6	0.1		0.1	0.1	0.2			30.0	30.3	27.9 - 32.6
Trimethoprim					58.1	16.7	1.2					0.2	23.8						24.0	21.8 - 26.2
Ciprofloxacin	76.6	9.7	0.6	1.1	6.3	3.0	1.3		0.1	0.7	0.5								13.0	11.3 - 14.7
Nalidixic acid									85.1	1.9	0.5		1.6	3.8	7.1				12.5	10.8 - 14.2
Chloramphenicol										81.2	9.5	1.1	1.6	1.6	4.9				9.2	7.7 - 10.7
Azithromycin*								2.5	43.9	48.5	4.3	0.5	0.3	0.1					0.9	0.3 - 1.3
Colistin							89.9	10.1											0.0	0.0 - 0.0
Meropenem		99.7	0.3																0.0	0.0 - 0.0
Tigecycline					86.0	13.5	0.5												0.0	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)*

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Table Eco02 Resistance (in %) of *E. coli* isolated from faecal samples of broilers, laying hens, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2014.

Faecal samples	Broilers	Laying hens	Pigs	Dairy cows	Veal calves	
	N=377	N=190	N=392	N=268	White, N=149	Rosé, N=143
Ampicillin	62.1	13.7	24.0	1.5	35.6	8.4
Cefotaxime	2.9	0.5	0.5	0.4	2.0	0.0
Ceftazidime	3.2	0.5	1.0	0.0	0.7	0.0
Gentamicin	6.4	1.1	3.6	0.4	4.7	2.8
Tetracycline	42.4	14.2	49.2	3.0	70.5	17.5
Sulfamethoxazole	52.5	5.8	41.3	2.6	43.0	12.6
Trimethoprim	44.6	5.8	30.9	0.0	34.2	9.8
Ciprofloxacin	46.4	2.1	0.0	0.0	12.1	0.7
Nalidixic acid	44.6	2.1	0.3	0.0	10.7	0.7
Chloramphenicol	13.5	0.0	12.0	1.1	20.1	6.3
Azithromycin	2.1	0.0	0.0	0.0	2.0	1.4
Colistin	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
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0

In general, highest resistance is seen for ampicillin, tetracycline, trimethoprim and sulfamethoxazole. These include the drug classes that are most frequently used in veterinary medicine.

Quinolones

Resistance to quinolones was most commonly found in *E. coli* from broiler chickens; 45 - 46% of all isolates showed resistance to nalidixic acid and ciprofloxacin. Although resistance rates are still high, these figures indicate a decrease of resistance to quinolones compared to 2012 and 2013 (50% and 54%, respectively) possibly as a result of the recent reduction in usage of quinolones in broiler chickens. In 2014 high level resistance (MIC >1 mg/L) to ciprofloxacin in broiler chickens was detected in 5.0% of the isolates, which is similar to former years. The percentage of *E. coli* with resistance to ciprofloxacin was 2.1% in laying hens, 12.1% in white veal calves compared to 0.7% in rosé veal calves, and 0% in pigs and dairy cattle. This likely reflects the use of quinolones in various animal husbandry systems.
In meat samples the highest resistance levels were detected in poultry and turkey meat products which are comparable to the situation in slaughter animals. In meat samples from poultry, turkey and veal higher resistance levels were observed for ciprofloxacin compared to nalidixic acid. This is possibly due to the increase of *E. coli* with PMQR genes exhibiting resistance to ciprofloxacin, but not to nalidixic acid. This difference was not observed in slaughter animals, which might be explained by the inclusion of foreign meat in the survey.

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Cefotaxime resistance

Resistance to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL producing *E. coli*, was detected in most animal host species included in this survey, except for rosé veal calves. Resistance levels for cefotaxime ranged from 0.4% in *E. coli* from dairy cattle to 2.9% in broiler chickens. The data show a similar low rate of cefotaxime resistance in broilers compared to 2013 (2.7%) (Figure Eco01). Among *E. coli* isolated from meat, resistance against third generation cephalosporins in poultry meat decreased from 22.5% in 2011 to 8.0% in 2012. In 2013 the values remained stable at 10.7%, but in 2014 it sharply decreased to 1.9% (Figure Eco 03). This 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. However, targeted sampling to detect cefotaxime resistant *E. coli* in fresh poultry meat samples using selective media resulted in 67% prevalence (see appendix 1) demonstrating the high prevalence of ESC on poultry meat samples. Despite the high prevalence, 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 chickens and laying hens

In commensal *E. coli* isolated from caecal samples from broiler chickens resistance to all antimicrobials tested was commonly present as summarized in Table Eco 02. Remarkably, for some antibiotics tested an increase of resistance was observed compared to 2013. This included ampicillin (62.1%), tetracycline (42.4%), sulfamethoxazole (52.5%) and trimethoprim (44.6%). Although quinolone resistance still showed a tendency to decrease, the levels of resistance to nalidixic acid (44.6%) and ciprofloxacin (46.4%) were still quite high. Slightly lower resistance was observed for gentamicin and chloramphenicol.

In laying hens resistance levels of *E. coli* were substantially lower compared to broilers for all antibiotics tested most likely reflecting the difference in antimicrobial usage between the two farm types.

Slaughter pigs

In 2014, high levels of resistance in *E. coli* isolates from swine were recorded for tetracycline (49.2%), sulfamethoxazole (41.3%), trimethoprim (30.9%) and ampicillin (24.0%). However, these levels of resistance were lower for all four antibiotics compared to 2013 showing an ongoing decrease in resistance (Figure Eco 01).

Resistance to the 3rd generation cephalosporins was found at low levels in 2014, indicating that ESBLs are still present in low concentrations.

Veal calves

Since 2012, we report resistance data on two veal calf husbandry types separately: white veal and rosé veal calves. 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 the 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 Eco 02).

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Figure Eco01 Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2014.

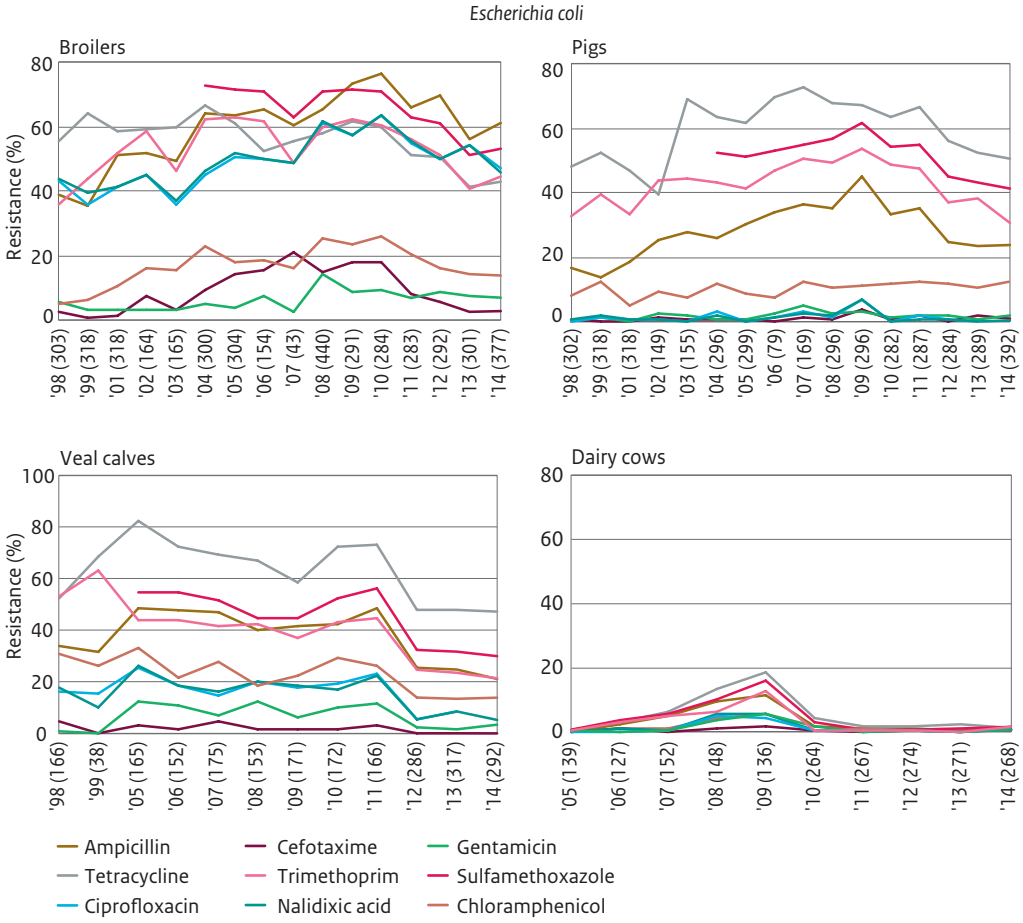

Figure Eco 01 illustrates the trends in resistance in *E. coli* isolated from both types of veal calves combined. Resistance levels have been relatively stable over time, with a clear decrease in 2012, which was also the year in which the sample strategy changed (see the description at the beginning of chapter 3.2), which might have influenced the results from 2012 and onwards (sampling at slaughterhouse) compared to the results before 2012 (sampling at farm). In 2013 and 2014 the resistance levels stabilised for most antibiotics tested. Similar to 2013, a low resistance rate was recorded for 3rd generation cephalosporins (2.0%) in white veal calves. In rosé animals this type of resistance was not detected. Furthermore, resistance to ciprofloxacin was higher in white veal calves (12.1%) than in rosé veal calves (0.7%).

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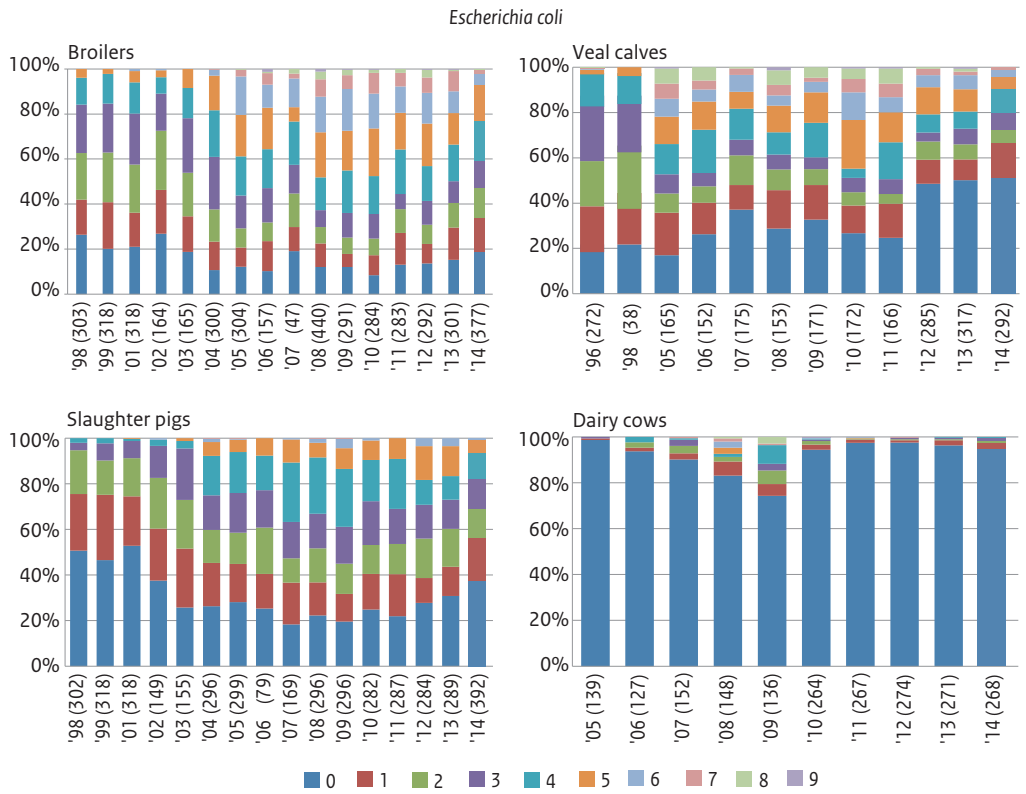
Figure Eco02 Resistance (%) to 0 - 9 antimicrobial classes among *E. coli* strains from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2014.



Dairy cattle

Resistance in indicator *E. coli* isolated from dairy cattle is very low compared to resistance levels observed in pigs, broilers and veal calves. Compared to 2013, slightly higher resistance rates were observed for some antibiotics, but all 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. Mainly because (the frequently detected) resistance to streptomycin was no longer included, the determined level of multidrug resistance was expected to decrease in 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.

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High levels of multidrug resistance (resistant to three or more classes of antibiotics) were still present among *E. coli* originating from broilers (48.0%), pigs (30.9%) and veal calves (27.4%). In dairy cattle multidrug resistance was rare in *E. coli* with 1.5% of the isolates showing resistance to three or four classes of antimicrobials in 2014. Despite the shift in the test panels the data indicate a decreasing trend in the level of multidrug resistance in broilers and pigs. In veal calves, the level of multidrug resistance seems to have stabilized in the last 3 years.

Finally, the overall increasing tendency of the number of totally susceptible *E. coli* isolates in all animal species 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.

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

Table Eco03 shows resistance percentages of *E. coli* strains 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), and the trends in resistance are presented in Figure Eco03. Although the results are more variable than in isolates from faeces, probably due to the annual inclusion of foreign meat products, the resistance rates in poultry, pork and beef show a tendency to decrease over the last 5 years. Cefotaxime resistance in *E. coli* isolates from poultry products has rapidly decreased from 10.7% in 2013 to 1.9% in 2014, while isolates from pork and beef are incidentally resistant to 3rd generation cephalosporins. This decrease is in line with what was observed in isolates from faeces. Although still 67% of fresh poultry meat products are positive for ESC resistant isolates (see appendix 1), it indicates that the concentration of ESC-resistant *E. coli* on meat has decreased.

In 2014, resistance rates of *E. coli* isolated from poultry meat have markedly decreased compared to 2013 which partly could reflect the recent decrease in antibiotic usage in poultry in the Netherlands (Table Eco02). However, an unknown proportion of the meat samples originates from foreign meat products. Compared to the other types of meat resistance rates of *E. coli* from beef are traditionally among the lowest and remain 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 tested. This uncertainty is demonstrated by the variability in resistance rates over the years as shown in Figure Eco03.

Table Eco03 Resistance (in %) of *E. coli* isolated from raw meat products at retail in the Netherlands in 2014.

Meat products	Poultry	Pork	Veal	Beef	Lamb	Turkey
	N = 531	N = 331	N = 19	N = 370	N = 18	N = 44
Ampicillin	40.7	12.7	57.9	7.8	11.1	65.9
Cefotaxime	1.9	0.9	0.0	1.9	0.0	2.3
Ceftazidime	3.0	0.9	0.0	2.7	5.6	6.8
Gentamicin	5.3	2.4	5.3	1.4	0.0	4.5
Tetracycline	38.0	19.3	68.4	9.2	11.1	52.3
Sulfamethoxazole	41.6	20.2	52.6	22.4	22.2	34.1
Trimethoprim	29.6	14.2	42.1	7.0	5.6	25.0
Ciprofloxacin	31.3	3.0	10.5	2.7	0.0	36.4
Nalidixic acid	27.3	2.7	5.3	1.4	0.0	22.7
Chloramphenicol	6.6	5.7	15.8	2.7	0.0	15.9
Azithromycin	0.0	0.9	0.0	1.6	0.0	4.5
Colistin	1.5	0.0	0.0	0.3	0.0	4.5
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.6	0.3	5.3	0.3	0.0	2.3

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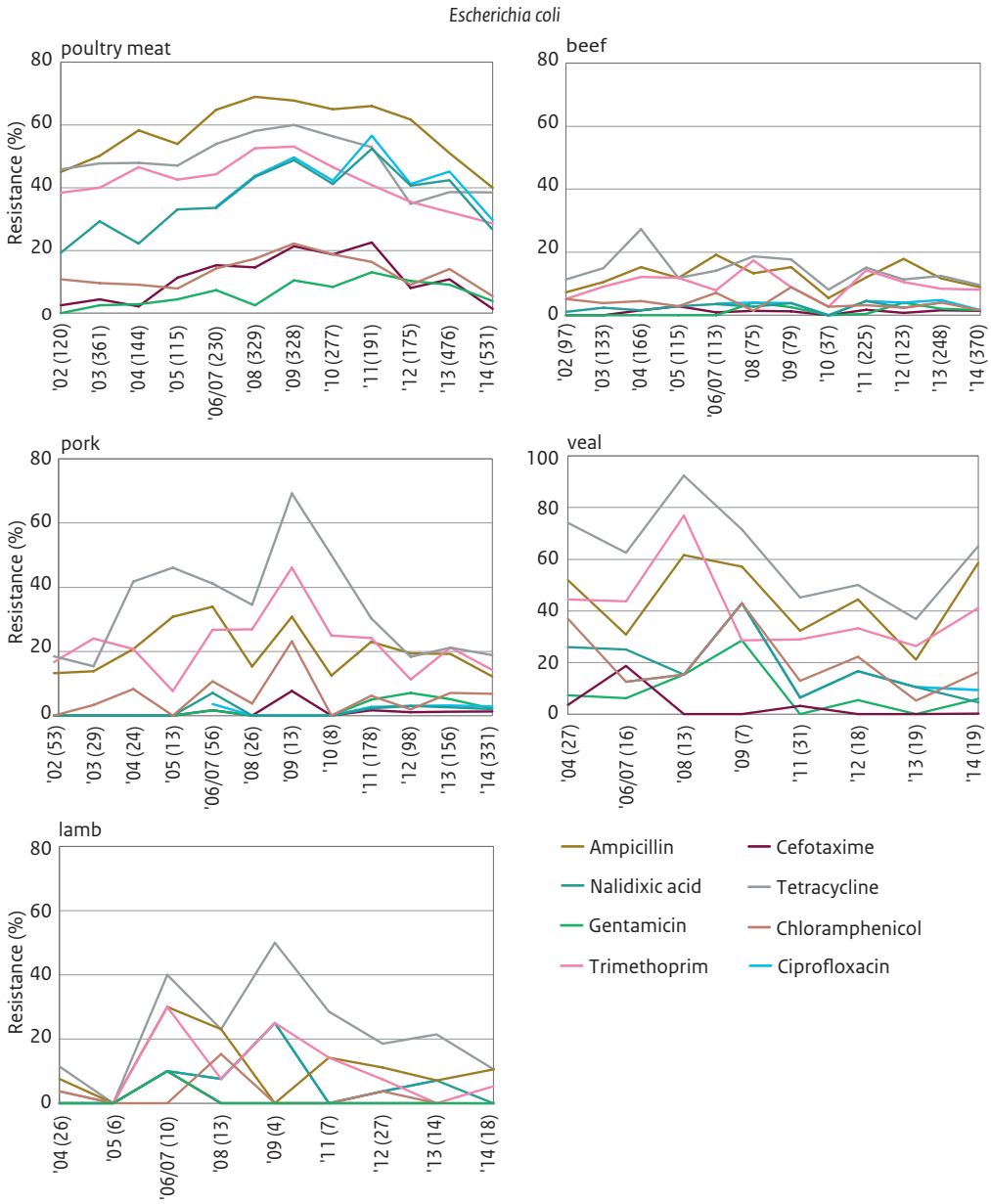
Figure Eco03 Trends in resistance (%) of *E. coli* isolated from raw poultry meat products, pork, veal, beef and lamb in the Netherlands from 2002 - 2014.



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3.2.3 *Enterococcus faecalis* and *E. faecium*

This chapter presents information on resistance in *Enterococcus* species from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-positive bacteria. In 2014 *Enterococcus faecalis* and *E. faecium* isolates were isolated from faecal samples of pigs only. From 2013 onwards, as a result of less priority for including enterococci in the surveillance, poultry, pigs and cattle and meat thereof will be sampled every three years. Supplementary to isolates from live animals, susceptibility profiles of *E. faecalis* and *E. faecium* isolated from raw pork products are presented as well.

The new EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in November 2013, including 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 were included: teicoplanin, daptomycin and tigecycline. All three antimicrobials are used for treatment of human infections with resistant enterococci. For early detection of possible spread of resistance for these new agents in bacteria from food-producing animals it is important to implement these new antimicrobials into the monitoring system.

Highlights

1. In 2014, for the first year, only isolates from pigs 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 every three years instead of annually.
2. In slaughter pigs, highest resistance levels were observed for tetracycline (71.1% in *E. faecalis* and 81.2% in *E. faecium*), erythromycin (39.5% in *E. faecalis* and 19.4% in *E. faecium*). In *E. faecium*, additional high levels of resistance were observed for quinu/dalfopristin (86.7%), and to a lesser extent to ampicillin (18.2%).
3. Isolation rates of *E. faecalis* and *E. faecium* differ between faeces and meat. 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.
4. Vancomycin resistant enterococci were not detected in pigs in 2014.

Resistance levels

In 2014 MIC values have been determined for 38 *E. faecalis* and 165 *E. faecium* strains isolated from faecal samples of pigs as well as for 847 *E. faecalis* and 162 *E. faecium* isolates from pork samples. Table Ento1 presents MIC-distributions and Table Ento2 the resistance percentages specified for the isolates from slaughter pigs. Trends over the years are depicted in Figure Ento1.
Data for 2014 on *E. faecalis* and *E. faecium* from poultry meats are presented in Table Ento3. Trends over the years for enterococci from poultry meat sources are presented in Figure Ento2.

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Table Ent01 MIC distributions (in %) for *E. faecalis* (N=38) and *E. faecium* (N=165) isolated from pigs in the Netherlands in 2014.

E. faecalis	MIC (%) distribution mg/L																	R%	95% CI
N = 38	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						7.9	78.9	10.5	2.6										0.0	0.0 - 9.3
Chloramphenicol									18.4	60.5	2.6		10.5	7.9					18.4	5.8 - 30.9
Ciprofloxacin						7.9	78.9	13.2											0.0	0.0 - 9.3
Daptomycin						2.6	23.7	73.7											0.0	0.0 - 9.3
Erythromycin							21.1	23.7	15.8	2.6					36.8				39.5	23.6 - 55.3
Gentamicin										13.2	73.7	10.5						2.6	2.6	0.0 - 7.8
Linozelid							15.8	84.2											0.0	0.0 - 9.3
Quino/dalfopristin*						2.6			5.3	34.2	57.9								n.a.	n.a.
Teicoplanin						100													0.0	0.0 - 9.3
Tetracycline							26.3		2.6	2.6		5.3	18.4	44.7					71.1	56.3 - 85.7
Tigecycline				65.8	34.2														0.0	0.0 - 9.3
Vancomycin							52.6	44.7	2.6										0.0	0.0 - 9.3

E. faecium	MIC (%) distribution mg/L																	R%	95% CI
N = 165	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin						2.4	13.3	14.5	51.5	18.2									18.2	12.1 - 24.1
Chloramphenicol									5.5	90.3	3.0	1.2							0.0	0.0 - 2.2
Ciprofloxacin					3.0	28.5	27.9	27.9	11.5	1.2									1.2	0.0 - 2.9
Daptomycin					6.7	24.2	30.9	28.5	9.7										0.0	0.0 - 2.2
Erythromycin							12.7	57.0	10.9	3.6	1.2				14.5				19.4	13.2 - 25.5
Gentamicin										81.2	15.2	3.6							0.0	0.0 - 2.2
Linozelid							1.2	87.3	11.5										0.0	0.0 - 2.2
Quino/dalfopristin						7.9	5.5	7.9	72.7	6.1									86.7	81.3 - 91.9
Teicoplanin						99.4		0.6											0.0	0.0 - 2.2
Tetracycline							18.8						62.4	18.8					81.2	75.1 - 87.2
Tigecycline				93.9	6.1														0.0	0.0 - 2.2
Vancomycin							97.6	2.4											0.0	0.0 - 2.2

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*

n.a. not applicable

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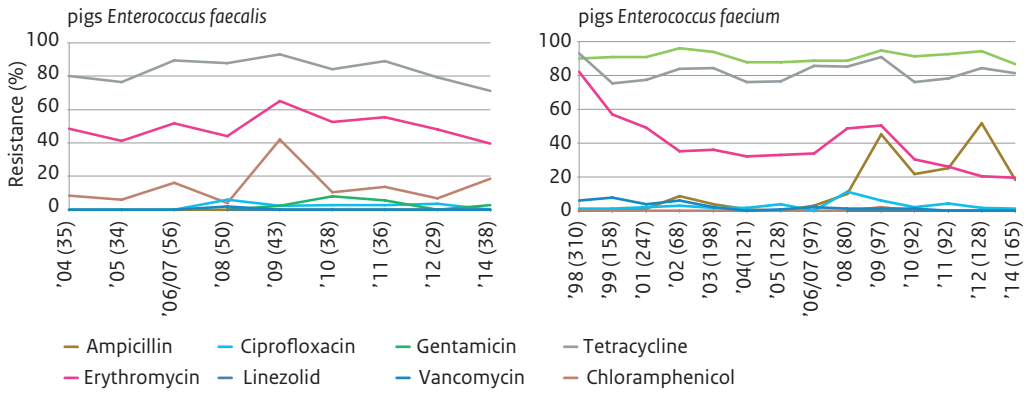
Table Ent02 Resistance percentages (%) of *Enterococcus faecalis* and *E. faecium* isolated from slaughter pigs in the Netherlands in 2014.

	Slaughter pigs	
	***E. faecalis* (N = 38)**	***E. faecium* (N = 165)**
Ampicillin	0.0	18.2
Chloramphenicol	18.4	0.0
Ciprofloxacin	0.0	1.2
Daptomycin	0.0	0.0
Erythromycin	39.5	19.4
Gentamicin	2.6	0.0
Linozelid	0.0	0.0
Quinu/dalfopristin*	-	86.7
Teicoplanin	0.0	0.0
Tetracycline	71.1	81.2
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 pigs in the Netherlands from 1998 - 2014.



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Pigs

Highest resistance levels were observed for tetracycline (71.1% in *E. faecalis* and 81.2% in *E. faecium*), erythromycin (39.5% in *E. faecalis* and 19.4% in *E. faecium*) (Table Ento2). In *E. faecium*, additional high levels of resistance were observed for quinu/dalfopristin (86.7%), and to a lesser extent to ampicillin (18.2%).

Over the years, resistance to the tested antimicrobials remained relatively stable in *E. faecalis* showing a decreasing tendency for resistance to tetracycline and erythromycin in the last five years. In *E. faecium*, a discontinuous decrease in ampicillin resistance was observed during the total test period. Vancomycin resistance was not detected in *E. faecium* since 2011 (Figure Ento1).

Raw meat products of pigs

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

For some antimicrobials, differences were observed in resistance between enterococci obtained from faecal samples and meat samples. In pork, resistance rates of *E. faecalis* were lower for chloramphenicol, erythromycin and tetracycline compared to isolates from faeces. These data indicate lower resistance rates of enterococci in meat. However, in *E. faecium* resistance rates in meat were more variable with lower resistance for ampicillin and higher resistance for erythromycin compared to faeces.
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-in-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 unexpected high numbers of strains with resistance were observed for both antibiotics. Since this is in conflict with the data from faeces, these data are not reported until they are confirmed.

Variable resistance levels were observed between *E. faecalis* and *E. faecium* isolated from pork (Table Ento3). For erythromycin, a large difference in resistance levels was observed among *E. faecalis* and *E. faecium* with 2.2% and 41.4%, respectively. However, tetracycline resistance was similar among both species (18%). Vancomycin and teicoplanin resistance was not observed and resistance to linezolid was detected in a small number of *E. faecalis* (n = 1) and *E. faecium* (n = 4) isolates.
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 have stabilized at a relatively low level. The resistance percentages in *E. faecium* demonstrate large fluctuations over the years (Figure Ento2). This fluctuation is most likely due to the variation in sample size over the years.

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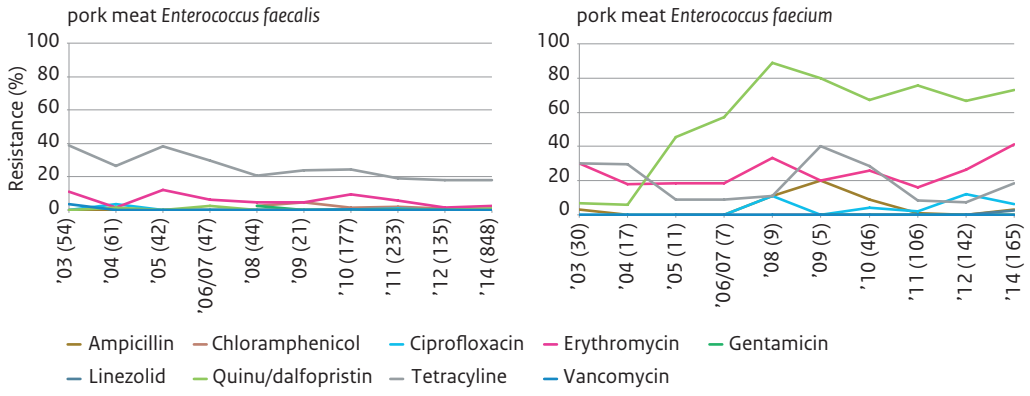
Table Ent03 Resistance % of *Enterococcus faecalis* and *E. faecium* strains isolated from raw meat products from pigs in the Netherlands in 2014.

	Pork meat	
	E. faecalis (N = 847)	*E. faecium* (N = 162)
Ampicillin	0.1	3.1
Chloramphenicol	0.9	0.0
Ciprofloxacin	0.0	6.2
Daptomycin	-	-
Erythromycin	2.2	41.4
Gentamicin	0.0	0.0
Linozelid	0.1	2.5
Quinu/dalfopristin*	-	72.8
Teicoplanin	0.0	0.0
Tetracycline	18.2	18.5
Tygecycline	-	-
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 pigs in the Netherlands from 2003 - 2014.



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4 Appendix I

Results of the screening for ESBL, AmpC and carbapenemase-producing Enterobacteriaceae in food producing animals in the Netherlands in 2014

Highlights

1. The decrease in cefotaxime resistant *E. coli* from 2008 – 2013, has levelled off in 2014.
2. The prevalence of livestock being positive for ESBL/AmpC producing *E. coli* in the faeces was 67% in broilers, 34% in laying hens, 18% in slaughter pigs, 23% in white veal calves, 14% in rosé veal calves and 9% in dairy cows.
3. Highest ESBL/AmpC-prevalence was observed in poultry meat (67%), which was lower than found in former years (83% in 2013 and 73% in 2012).
4. ESBL/AmpC prevalence in processed meat products was higher compared to raw meat. Cross-contamination during processing of the meat might explain these differences.
5. The dominant human ESBL-gene ($bla_{CTX\text{-}M\text{-}15}$) was more frequently found in animals or their products. This is an unwanted development that warrants extra attention in the surveillance in food-animal sources
6. The prevalence of ESBL-producing *Salmonella* was in 2014 2.1%, almost half the amount of 2013 (4%). In isolates from human sources a variety of ESBL-genes were found: $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}65}$. These isolates were all highly multidrug resistant, which could affect the success of a therapy in infected humans. No resistance was detected in *Salmonella* against the last resort antibiotic class of the carbapenems (meropenem).
7. In 2014 in 1601 faecal samples from broilers, veal calves, slaughter pigs and dairy cows no carbapenemase-producing *Enterobacteriaceae* were detected.

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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[1]. 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* isolates selected from non-selective media derived from broilers, slaughter pigs (1998 – 2014), veal calves and dairy cows (2005 – 2014). In broilers after 2001 and more in particular after 2003 an apparent increase was observed up to levels that varied from 15 – 20%. The prevalence in broilers declined to 2.7% in 2013, and levelled off to 2.9% in 2014. The decline until 2013 is most likely the result of decreased usage of antibiotics in broilers until 2013 and the fact that since spring 2010 no ceftiofur was used (off label) at Dutch hatcheries. In 2014, the decrease in usage stopped in broilers, which may have resulted in the observed levelling off.
From a total of 1519 randomly selected *E. coli* isolates that were tested in 2014, sixteen displayed resistance (MIC>0.25 mg/L) to cefotaxime (see also 3.2.1). Eleven were isolated from poultry (ten from broilers and one from a laying hen), three from veal calves (white) and two from slaughter pigs (TableESBL01). In dairy cows no ESBL-suspected *E. coli* isolates were found in 2013/2014. Cefotaxime resistant isolates were screened for beta-lactamase gene families using the Check-Points CT101 miniaturised micro-array or PCR. Subsequently the genes were identified by dedicated PCR and sequence analysis. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutants in the chromosomal *ampC*-genes. The results of this molecular typing are displayed in Table ESBL01. In the poultry isolates two plasmid mediated ESBL genes were present: $bla_{CTX\text{-}M\text{-}1}$ (n=8) and $bla_{SHV\text{-}12}$ (n=4). 2014 is the first year in which $bla_{CMY\text{-}2}$ and $bla_{TEM\text{-}52c}$ were not found in cefotaxime resistant isolates from broilers derived from the monitoring program. One of the two pig isolates contained $bla_{TEM\text{-}52c}$. In the other pig isolate no plasmid-mediated ESBL/AmpC-gene was found, but a mutation in the chromosomal *ampC* gene was detected. Only one of the three isolates with cefotaxime resistance from veal calves contained an ESBL gene ($bla_{CTX\text{-}M\text{-}1}$), in the others no plasmid-mediated ESBL/AmpC genes were found. The MIC of those latter two isolates was only slightly reduced (MIC 0.5 mg/L). As found in other years, $bla_{CTX\text{-}M\text{-}1}$ is still the gene which is mostly detected in isolates from food-producing animals and for the first year no $bla_{CMY\text{-}2}$ was found.
It can be concluded that in 2014 by random isolation, only fourteen plasmid mediated ESBLs were found in 1519 isolates. This is a major improvement compared to 2008 before the antibiotic use in Dutch livestock was reduced where 67 ESBL/AmpC-producing isolates where found in 1062 isolates.

Active surveillance of ESBLs in 2014

In former years (2011-2013) active surveillance on ESBL-producers was done by analysing faecal samples from 10 animals per batch of animals (for pigs and veal calves) or from individual dairy cows. There was

[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.

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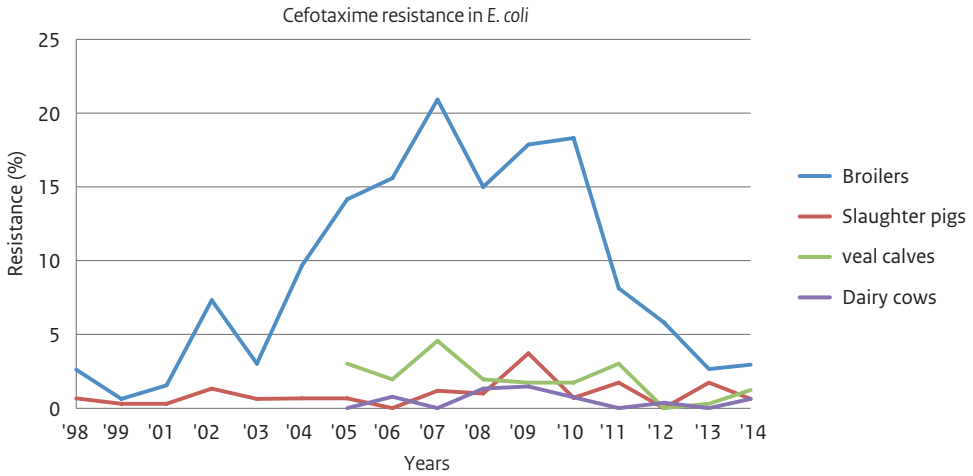
Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows.



no active surveillance in broilers as all batches were expected to be positive. In 2014 active surveillance for ESBL producers was implemented in the monitoring program on antimicrobial resistance. The same faecal samples taken at slaughterhouses (from slaughter pigs, veal calves and broilers) and at farms (dairy cows) used from the monitoring of antimicrobial resistance in food-producing animals were also used for detection of ESBL/AmpC-producing *E.coli* by selective methods. This resulted in the screening for ESBL/AmpC-producing *E. coli* in 1601 faecal samples. Screening was done by overnight incubation of the faecal sample in Tryptic Soy Broth with 1 mg/L cefotaxime followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime. Moreover, in 2014, 2909 meat samples were analysed for ESBL/AmpC-producing *E. coli*. Meat samples by pre-enrichment in Luria Bertani broth with 1 mg/L cefotaxime, 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 the typical morphology of *Enterobacteriaceae* were selected for identification of the bacterial species and if confirmed for *E. coli*, confirmation of the ESBL/AmpC-genes present was done. One positive *E. coli* isolate per sample was screened for beta-lactamase gene families as described above.

Results of active surveillance of ESBL/AmpC-producing *E. coli* in faeces

The prevalence of ESBL/AmpC producing *E. coli* in faeces is shown in Table ESBL02. Suspected ESBL isolates comprised all *E. coli* growing on MacConkey with 1 mg/L cefotaxime. Those include isolates in which no ESBL/AmpC-gene was found and in which a promoter mutant of the chromosomal *ampC*-gene was found (of which relatively high numbers were present in pig, calf and cow isolates). Confirmed ESBL isolates comprised all isolates in which an ESBL or AmpC gene was detected, most likely located on a plasmid, which can be horizontally transferred. Each sample represents one slaughter batch of animals from one farm. Of the 1601 samples analysed for ESBL-producing *E. coli*, 27.5% were positive, mainly due to the high prevalence in broilers. Prevalence differed from 6% in dairy cows (comparable to former years) to 66% in broilers. Traditionally the levels in white veal calves are higher than found in rosé veal

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Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2014.

	ESBLs isolated from						ESBL-genes detected											
Year	Poultry	Veal calves	Slaughter pigs	Dairy cows	Turkey	Total ESBL suspected (n)	CTX-M-1-group#	CTX-M-2	CTX-M-9	TEM-52c	TEM-20	SHV-12*	SHV-2	CMY-2	chromosomal ampC	no gene found	Total *E.coli* (n)	% ESBL of total *E. coli*
2007	9	6	2			17	3	1		3				1	2	7	539	3.2
2008	66	4	3	2		75	38	5	1	9			2	12	3	5	1026	7.3
2009	53	2	11	2		68	34	7		2	1	8	1	12	3		894	7.6
2010	52	3	2	2		59	21	6		5	1	9	4	5	3	5	1002	5.9
2011	23	5	5		6	39	9			8		9	2	3	3	5	1096	3.6
2012	26	2		1		29	8			4		8		5		4	1328	2.2
2013	13	1	4			18	7			4		3		3	1		1371	1.3
2014	11	3	2			16	8			1		4			1	2	1519	1.1
Total	253	26	29	7	6	321	128	19	1	36	2	41	9	41	16	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.*

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Table ESBL02 Prevalence of *E. coli* isolates reduced susceptable for cefotaxime derived from selective culturing of faecal samples from broilers, laying hens, pigs, veal calves and dairy cows taken at slaughter in 2014.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	400	267	264	66.0
Laying hens	200	68	65	32.5
Pigs	400	73	49	12.3
Veal calves white	151	35	27	17.9
Veal calves rosé	150	21	17	11.3
Dairy cows	300	26	18	6.0
Total	1601	490	440	27.5

calves (respectively 17.9% and 11.3%). It is not known whether these numbers are fully representative for the farms in the Netherlands. For broilers it would indicate that compared to 2009, when an overall prevalence of 85% (based on 25-42 animals per farm (n=26)) was found, the prevalence has decreased substantially (Dierikx et al, 2013). For laying hens, pigs and veal calves the data cannot be compared with previous years. In dairy cows the prevalence slowly decreases from 14% in 2011 to 6% in 2014.

Table ESBL03 shows the ESBL/AmpC genes detected in the faeces of these animal species. Compared to former years (MARAN 2011-2013), more variation in different ESBL types was found. This might be related to a change in surveillance method. Between 2011 and 2013 about 100 slaughter batches of pigs and veal calves were sampled by taking 10 animals per batch, which resulted in samples from about 100 different farms per animal species. The method used in 2014 resulted in a collection of samples derived from a minimum of 150 to 400 different farms per animal species (Table ESBL02), which might have led to more variation in the detected types. Like in former years, $bla_{CTX\text{-}M\text{-}1}$ was the dominant ESBL-variant in all animal species examined. In broilers, compared to cefotaxime resistant isolates derived from the passive surveillance (see Table ESBL01) a very high variation in ESBL-types was found. Also types not earlier described in isolates derived from faecal samples of broilers in the Netherlands were found, like the more classical human associated ESBL-types $bla_{CTX\text{-}M\text{-}9}$, $_{\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$, which is an unwanted development. Next to $bla_{CTX\text{-}M\text{-}1}$, also $bla_{SHV\text{-}12}$ and $bla_{CMY\text{-}2}$ were abundantly found in broilers. In laying hens, interestingly, not $bla_{CTX\text{-}M\text{-}1}$ but $bla_{CMY\text{-}2}$ was the predominant beta-lactamase gene. Chromosomal *ampC* types seem to play a larger role in conferring cefotaxime resistance than in 2013, but predominantly in pigs.

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

Table ESBL04 shows the prevalence of ESBL suspected and confirmed isolates in meat. The first category is based on phenotypical characterisation of isolates resistant to cefotaxime. This included species like *Serratia, Citrobacter, Enterobacter, Acinetobacter* and *Hafnia* that are intrinsically resistant due to resistance genes on the chromosome. The vast majority of the species isolated that were not *E. coli* were negative for plasmid-mediated ESBLs/AmpCs. Highest prevalence of ESBL/AmpC confirmed

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Table ESBL03 Beta-lactamases identified in *E. coli* from broilers, laying hens, veal calves, pigs and dairy cows in 2014. Data derived from the active surveillance of ESBL-producing *E. coli* at slaughter.

		Broilers	Laying hens	Veal calves White	Veal calves Rose	Slaughter pigs	Dairy cows	Total
CTX-M-1 group								
	CTX-M-1	115	22	16	7	29	8	197
	CTX-M-3				1		1	2
	CTX-M-15	4	3	5	4			16
	CTX-M-32			1				1
CTX-M-2 group								
	CTX-M-2	2			1			3
CTX-M-9 group								
	CTX-M-9	1						1
	CTX-M-14	1	1	2		4	3	11
	CTX-M-27				1			1
	CTX-M-65						1	1
TEM								
	TEM-20					1		1
	TEM-52	10				1		11
	TEM-52c	8	1	1		11		21
	TEM-52cVar	4	3				1	8
SHV								
	SHV-12	41		1		1		43
CMY								
	CMY-2	76	35	1	2	2	4	120
Combinations								
	CTX-M-15&CMY-2				1			1
	CTX-M-1&TEM-52c	1						1
	TEM-52c&SHV-12	1						1
Chromosomal ampC								
	ampC-type-3	1	3	5	4	19	7	39
	ampC-type-5	1						1
	ampC-type-11					3		3
	ampC-type-18	1					1	2
	ampC-type-45	1						1
Unknown								
				3		2		5
Total		268	68	35	21	73	26	491

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Table ESBL04 ESBL-suspected and confirmed isolates from raw meat products in the Netherlands in 2014.

Animal source		N screened	N suspected ESBL	% suspected ESBL	% ESBL confirmed positive in 2014
Cattle					
	fresh meat	403	36	8,9	2,2
	meat product	514	59	11,5	7,8
Calf					
	fresh meat	16	2	12,5	3,1
	meat product	13	4	30,8	21,0
Pig					
	fresh meat	757	85	11,2	2,7
	meat product	549	32	5,8	4,0
Lamb					
	fresh meat	31	0	0,0	0,0
	meat product	17	0	0,0	0,0
Chicken					
	fresh meat	526	376	71,5	67,0
	import	39	38	97,4	84,4
Turkey					
	fresh meat	35	19	54,3	50,9
	import	9	7	77,8	58,3
Total		2909	658		

isolates was observed in poultry meat (67%), which was lower than found in former years (83% in 2013 and 73% in 2012) and lower than published prevalence data in poultry meat (84 – 100%) (Cohen-Stuart et al. 2012). Fifty one percent of turkey meat was found positive (in 2013 this was 35%) while in beef and pork the prevalence of confirmed ESBLs (respectively 2.2 -3.1 % in beef and 2.7 % in pork) was comparable to 2013 (5% for beef and 2% for pork respectively). Prevalence in processed meat products tended to be higher compared to raw meat. Cross-contamination during processing of the meat might explain the differences found.

Table ESBL05 shows the different ESBL/AmpC types detected in meat. All genotypes found in beef were also found in faecal samples of veal calves or dairy cows. This strongly suggests that faecal contamination during slaughter or processing of the meat was the source of these genes. In chicken meat all genotypes were also found in broilers, accept for $bla_{\text{CTX-M-8}}$, which was not found in faecal samples from broilers. This genotype (together with $bla_{\text{CTX-M-2}}$) is known to be present in broilers from South-America and this suggests that these meat samples were imported from South America. Other frequently found genes in isolates from meat were $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{TEM-52}}$, all typically associated with the food animals the meat originates from. $bla_{\text{CTX-M-15}}$ was found eleven times (7.4%) in meat from all animal sources, which is slightly higher than in 2013 (4.9%).

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Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2014.

ESBL gene		Chicken	Beef	Pork	Turkey	Total
CTX-M-1 group						
	CTX-M-1	23	17	10	5	55
	CTX-M-15	1	5	1	4	11
	CTX-M-32		1			1
CTX-M-2 group						
	CTX-M-2	23		1	2	26
CTX-M-8/25 group						
	CTX-M-8	2				2
CTX-M-9 group						
	CTX-M-14	1	1			2
	CTX-M-65			1		1
TEM						
	TEM-52c	2				2
	TEM-52cVar	3		1		4
SHV						
	SHV-12	12	1	1	2	16
CMY						
	CMY-2	19	1		2	22
Chromosomal ampC						
	ampC-type-11	3				3
	ampC-type-18	3			1	4
Total		92	26	15	16	149

ESBL/AmpC-producing Salmonella

Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is also done in *Salmonella enterica*. Annually a selection of ± 2000 salmonella's sent to RIVM for sero- or MLVA-typing were tested for susceptibility to cefotaxime and ceftazidime. In 2014, the cefotaxime resistant *Salmonella* isolates were mainly from human and poultry sources. The prevalence of ESBL-producing *Salmonella* was in 2014 2.1%, almost half the amount of last year (4% in 2013). In 2013 the higher prevalence was attributed to an extra import project in which poultry meat from South America was oversampled. These samples were often positive for ESBL-producing S. Heidelberg isolates. In 2014 this was still going on, but only a subset of those samples was included in the surveillance. Next to S. Heidelberg, a wide variation of eight other serovars was identified to carry ESBLs. In these isolates the genes were identified as described above for *E. coli*.

Table ESBL06 shows that S. Heidelberg is still most prevalent, carrying predominantly bla_{CMY-2}, which is frequently reported in North and South-America. In isolates from human sources a variety of ESBL-genes were found: $bla_{CTX-M-1}$, $bla_{CTX-M-8,}$ $bla_{CTX-M-9}$ and $bla_{CTX-M-65}$. Table ESBL07 shows that all cefotaxime resistant *Salmonella* isolates were all highly multidrug resistant, which could affect the

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Table ESBL06 Beta-lactamases in *Salmonella* isolated in 2014.

Serovar	Humans	Poultry	Other	CTX-M-1 group	CTX-M-1 & CMY-2	CTX-M-8 group	CTX-M-9 group		TEM	CMY	gene not found	Total
				CTX-M-1		CTX-M-8	CTX-M-9	CTX-M-65	TEM-52	CMY-2		
1.4.5.12:i:-		1								1		1
Abony		1								1		1
Agona			1			1						1
Brandenburg	1										1	1
Derby	1			1								1
Heidelberg		17	3		1					19		20
Infantis	2							2				2
Paratyphi B var Java		5		4					1			5
Typhimurium	4					1	1				2	4
Total	**8**	**24**	**4**	**5**	**1**	**2**	**1**	**2**	**1**	**21**	**3**	**36**

Table ESBL07 Resistance and multidrug resistance percentages of ESBL-producing *Salmonella* in the Netherlands in 2014.

Antimicrobials	R%
Ampicillin	100
Cefotaxime	100
Ceftazidime	89
Gentamicin	8
Tetracycline	75
Sulfamethoxazole	86
Trimethoprim	22
Ciprofloxacin	78
Nalididixic acid	75
Chloramphenicol	14
Azithromycin	3
Meropenem	0
Tigecycline	19

Multi drug resistance	N = 36
0	0%
1	0%
2	8%
3	22%
4	42%
5	19%
6	0%
7	8%
8	0%
9	0%
10	0%

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Table ESBL08 ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime derived from human and chicken sources during 2007-2014.

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
Total	80	43	8	14	44	4	10	101	3	307		

 # contains $bla_{CTX-M-1}$ (n=59, in all years), $bla_{CTX-M-55}$ (n=6, 2008-2010,2012), $bla_{CTX-M-15}$ (n=6, 2011-2013), $bla_{CTX-M-3}$ (n=3 , 2010, 2012) and one combination with bla_{CMY-2} (2014).

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=7, 2008-2009, 2012-2014), $bla_{CTX-M-14}$ (n=4, 2009-2012) and $bla_{CTX-M-65}$ (n=3, 2013-2014).*

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

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success of a therapy in infected humans. No resistance was detected against the last resort antibiotic class of the carbapenems (meropenem).

In Table ESBL08 the ESBL-types found in *Salmonella* since 2007 are summarized. Every year genes belonging to bla_{CMY-2}, bla_{TEM-52} and the $bla_{CTX-M-1}$-group, were found in several *Salmonella* isolates derived from different sources. This is the first year no $bla_{CTX-M-2}$ genes were found. The relatively high prevalence of bla_{CMY-2} positive isolates in 2014 can (as was also the case in 2013) be attributed to the extra sampling of imported meat from South America.

It can be concluded that the occurrence of ESBL/AmpC-producing *E. coli* and *Salmonella* is widespread in Dutch food-producing animals and in raw meat products mainly of poultry origin. No further decrease in cefotaxime resistance was found in the monitoring of antimicrobial resistance in animals. Also the active surveillance in faecal samples of food-producing animals did not show an apparent decline in prevalence.
The potential attribution to infections in humans warrants strict measures to control antibiotic usage and possibilities of transmission of these organisms in animal production chains. The dominant human ESBL-gene ($bla_{CTX-M-15}$) was more frequently found in animals or their products. This is an unwanted development that warrants extra attention in the surveillance in food-animal sources. $Bla_{CTX-M-1}$ was still the predominant ESBL gene identified in all animal species (except laying hens) and sources tested.

4.2 Carbapenemases

Carbapenemases including metallo-beta-lactamases are beta-lactamases with an extended spectrum that can also hydrolyse the carbapenems. These antibiotics are considered 'last-resort' antibiotics in human medicine and therefore usage is restricted to humans only. However, recently carbapenemase producing *E. coli* and *Salmonella* were found in samples derived from pigs, broilers and dogs in Germany (Fisher et al., 2012, 2013, Stolle et al., 2013). The Netherlands has intensive contact with Germany in terms of trade of live animals, which is a risk for introduction in the Netherlands. Therefore since 2012 extra screening was conducted with the aim to detect carbapenemase-producing *Enterobacteriaceae* in food-producing animals in the Netherlands. The results from 2012 and 2013 are described in Nethmap/MARAN 2013.
As in 2013, in 2014 a sensitive method was applied to screen for carbapenemase producers. This is important in an environment with a very low anticipated prevalence of carbapenem resistance. This method included a commercial RT-PCR (Check-Points, CarbaCheck MDR RT), which can detect the most important carbapenemase gene families (KPC, NDM, VIM, IMP and OXA-48) in samples. All faecal samples sent to the Central Veterinary Institute (CVI) by the Dutch Food and Consumer Protection Authority (NVWA) for antimicrobial resistance surveillance in broilers, slaughter pigs, veal calves and dairy cows (N =1601) were screened with this method. The samples were grown overnight in Tryptic Soy Broth with 50 mg/L vancomycin. After incubation the culture was centrifuged and the pellet stored at -20°C. The RT-PCR was performed according to the manufacturer's description on the isolated pellet DNA. If the RT-PCR gave suspicious or positive results, a three step analysis was performed to confirm the results as stated below:

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1. The DNA-lysate was used to run the CT102 micro array (Check-Points). This array detects the carbapenemase gene families NDM, KPC, VIM, IMP and OXA-48.
2. If the micro array was positive, the result was further confirmed by dedicated PCR and sequencing.
3. Moreover, for samples suspected to be positive the original faecal sample and the broth culture were inoculated on commercial selective plates (ChromID CARBA and ChromID OXA (Biomerieux).

In 2014, this sensitive screening method resulted in one positive signal in the RT-PCR (pig faecal sample). For the first time a bacterial isolate was cultured from a PCR-positive sample and identified as a *Shewanella* spp. with a chromosomally located $bla_{OXA-199}$ gene. This gene is very closely related to bla_{OXA-48} (> 99% homology) and had also been found in three faecal samples in 2013 (Nethmap/MARAN 2013). Finding this gene on the chromosome of a *Shewanella* spp. that are known to occur in the environment was considered the result of the high sensitivity of the method used and not a concern for public health.

Screening for carbapenemase producing isolates in faecal samples of food-producing animals (N > 1500) will continue in 2015. In addition, screening will also take place at clinical samples in pet animals at the veterinary faculty in Utrecht, and in imported ornamental fish. Active screening in food products will be conducted in 2015 in imported fish and shrimps for South-East Asia based on the incidental finding of a carbapenemase producing *Pseudomonas fluorescens* in a squid imported from South Korea into Canada (Rubin et al, 2014).

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5
Appendix II

Materials and methods

Detailed information on microbiological methods used is available on the website www.maran.wur.nl .

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