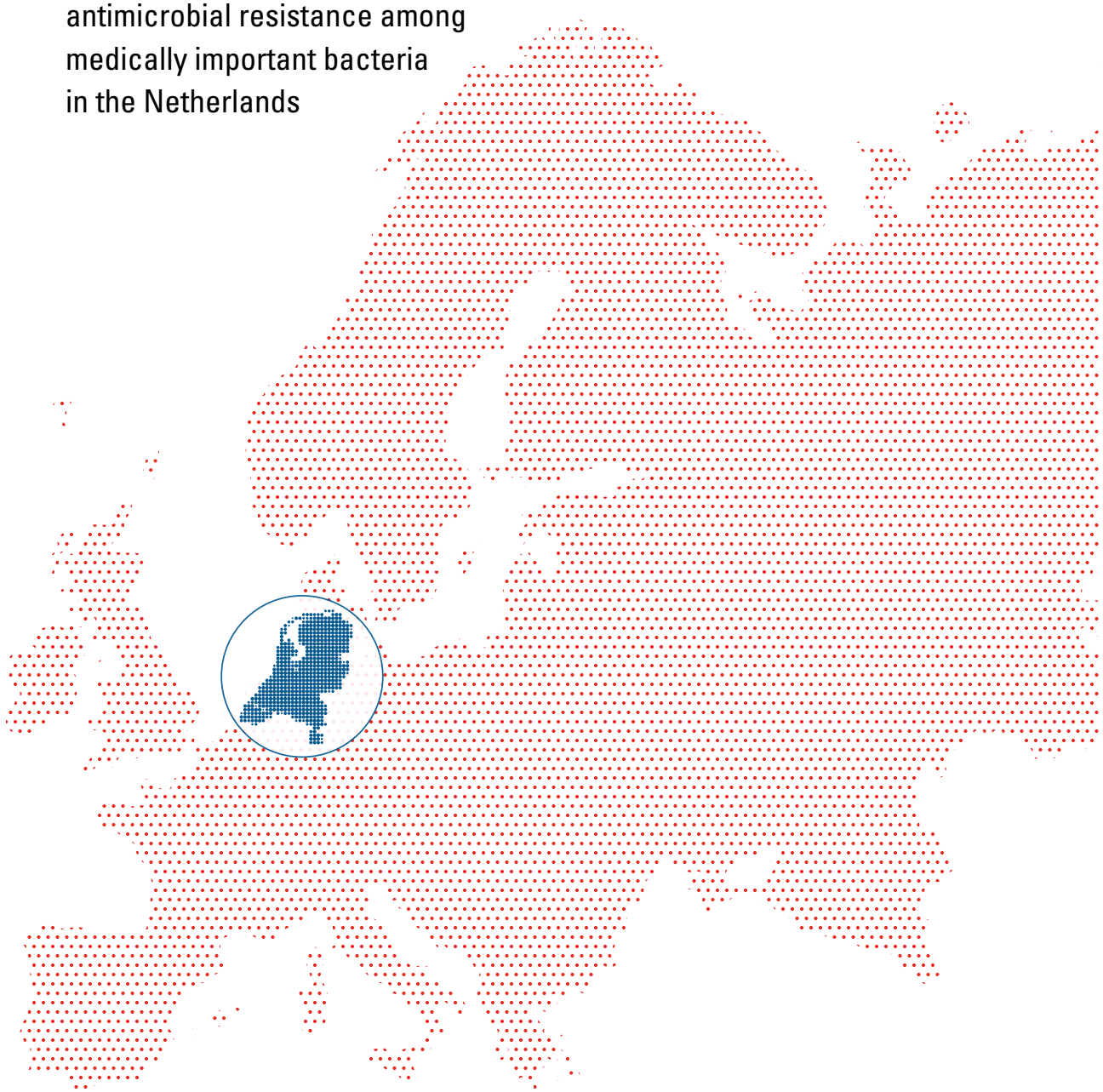
NethMap 2014

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

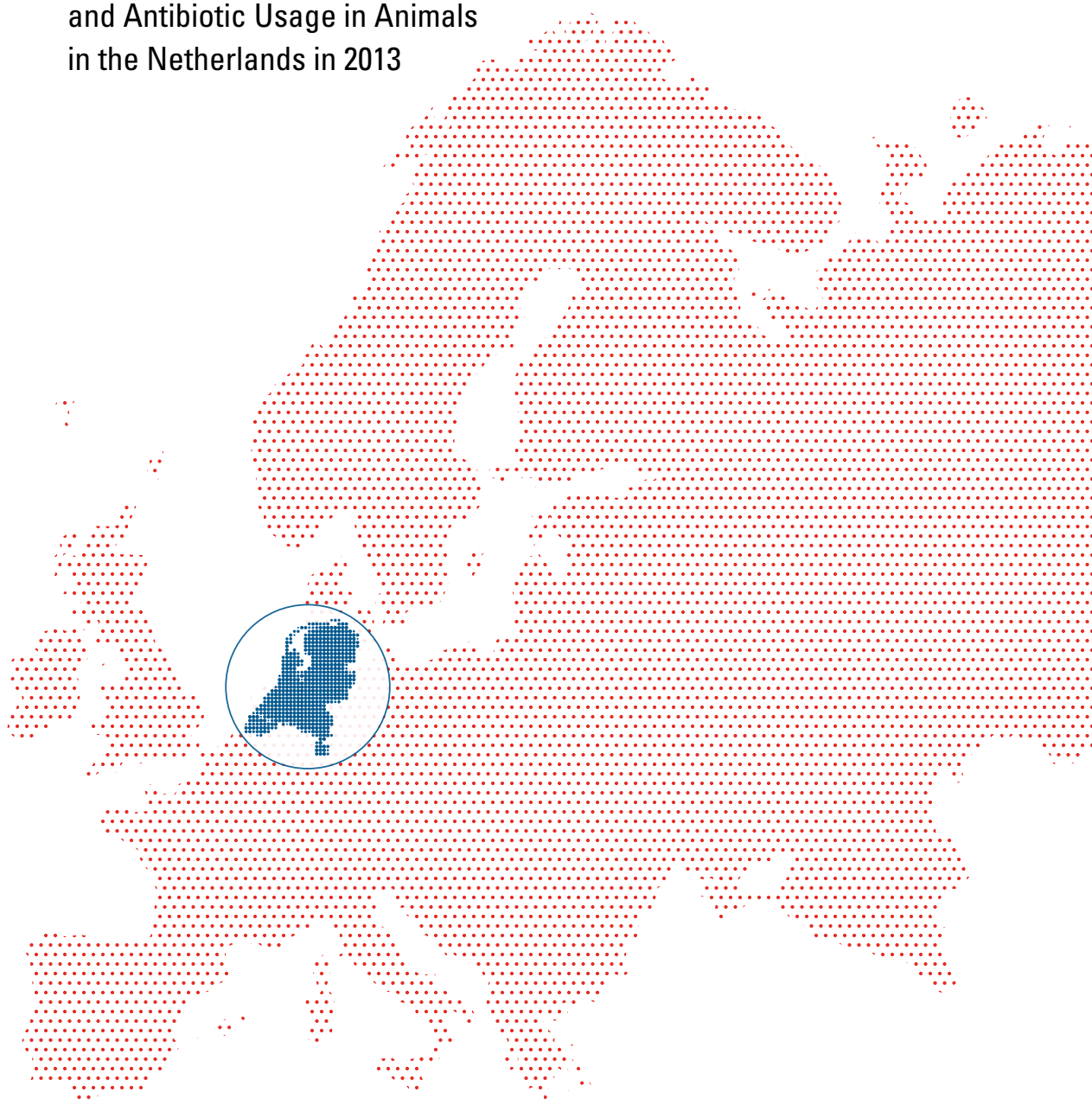


National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

SWAB

MARAN 2014

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013



National Institute for Public Health and the Environment
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NethMap 2014

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in The Netherlands
in 2013

June 2014

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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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Contents

Colophon 2
Acknowledgements 3

Contents 5

1 Introduction 7

2 Extensive summary 9

3 Use of Antimicrobials 17
3.1 Primary care 17
3.2 Hospital care 20
3.3 Care in nursing homes 31

4 Surveillance of resistance 35
4.1 Methods of surveillance 35
4.2 Primary care 38
4.2.1 ISIS-AR 38
4.2.2 APRES-study 42
4.3 Hospital departments 43
4.3.1 Outpatient departments 43
4.3.2 Unselected hospital departments 48
4.3.3 Intensive care units 55
4.3.4 Blood isolates in unselected hospital departments and intensive care units 61
4.3.5 Urology services 66
4.3.6. Respiratory pathogens 71
4.4 BRMO 73
4.4.1 Carbapenemase producing *Enterobacteriaceae* (CPE) 73
4.4.2 Vancomycin Resistant Enterococci in Dutch hospitals 76
4.4.3 Methicillin resistant *Staphylococcus aureus* (MRSA) 77
4.5. Resistance in specific pathogens 79
4.5.1. *Neisseria meningitidis* 79
4.5.2. *Neisseria gonorrhoeae* 81
4.5.3. *Mycobacterium tuberculosis* 84
4.5.4. Resistance to influenza antiviral drugs 86

Download from SWAB.nl | 2026-11-12 14:20

4.5.5. Resistance among anaerobic pathogens 89
4.5.6. *Clostridium difficile* 92
4.5.7. Azole resistance in *Aspergillus fumigatus* 94

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1 Introduction

This is NethMap 2014, the SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in The Netherlands in 2013 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).

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 goal 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 guidelines for antibiotic prophylaxis and therapy and an integrated nationwide surveillance system for antibiotic use and resistance.

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 2014 extends and updates the information of the annual reports since 2003. Many things have changed since the first edition – e.g. internet has become a standard for access to information in general and ISIS-web has been developed for individualized reporting of resistance. It was therefore felt that Nethmap required a facelift – more comprehensive and easier to handle, highlighting significant

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developments with respect to emergence of resistance. The reader is encouraged to visit www.isis-web.nl for tailored overviews of resistance development.

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.

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 microorganisms 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.
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 SWAB and thereby contribute to the general benefit and health of the people.

The editors:
Dr Ir SC de Greeff
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2 Extensive summary

In the Netherlands, several surveillance programs have been developed 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 2014.

2.1 Most important trends in antimicrobial use

In GPs

- After years of slow increase and stabilizing over the last 2 years, antibiotic use declined from 11.34 DDD/1000 inhabitants per day in 2012 to 10.81 DDD/1000 inhabitants per day in 2013.
- The use of azithromycin stabilized more or less after an increase over the last 10 years, whereas the use of clarithromycin declined further to 0.44 DID in 2013. Total use of macrolides decreased by 9% in 2013.
- The rapidly increasing use of nitrofurantoin observed over the last few years seems to have stopped in 2013.
- Overall use of quinolones decreased by 5% compared to 2012.

In nursing homes

- Specific antibiotic consumption data in nursing homes are provided for the second time. The mean use in 25 nursing homes was 74 DDD/1000 residents/day but varied widely between 33 and 177 DDD/1000 residents/day.
- The most frequently used antibiotic is amoxicillin with clavulanic acid (24 %), followed by nitrofurantoin (17%) and fluoroquinolones (15%).

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Table 2.1 Overview of Current surveillance programs in the Netherlands.

Surveillance program[1]	Origin of isolates	availability	Sources 2012	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in major pathogens					
SERIN	GP	1996-	20 GP practices from NIVEL	Central testing	Microdilution
ISIS-AR	GP, Hospital, Nursing homes	2008-	32 laboratories	Decentral testing	Various methods used in routine susceptibility testing
Specific surveillance program aimed at resistance surveillance in specific pathogens					
CPE	community, GP, nursing home, hospital	2010-	Nationwide	Central testing	Phenotypic and genotypic (PCR) confirmation of carbapenemases
VRE	Hospital	2011-	Nationwide	Central testing	PCR confirmation of VAN genes en genotyping
MRSA	community, GP, nursing home, hospital	1989-	Nationwide	Central testing	PCR confirmation of MecAgene, Spa typing, MLVA
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

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Table 2.1. Continued Overview of Current surveillance programs in the Netherlands.

Surveillance program[1]	Origin of isolates	availability	Sources 2012	Central or decentral susceptibility testing	Method of susceptibility testing
azole resistance in *Aspergillus fumigatus*	Hospital	2011-	8 University hospitals	Central testing	EUCAST methodology

* SERIN = Surveillance of Extramural Resistance in The Netherlands; ISIS-AR = Infectious Disease Surveillance Information System on Antibiotic Resistance; GP = general practitioner; CPE = Carbapenemase producing Enterobacteriaceae; VRE = vancomycin-resistant *Enterococcus faecium*; STI = sexually transmitted infections ; MGIT = Mycobacteria Growth Indicator Tube; EUCAST = European Committee on Antimicrobial Susceptibility Testing; NIVEL = Netherlands institute for health services research; NIC = National influenza center; WHO-CC = WHO Collaborating Centre

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In hospitals

- Compared to 2011, the in-patient use of antibiotics in 2012 stabilized at a level of 71.3 DDD/100 patient-days.
- Although overall use has declined there is general trend of more broadspectrum antibiotic use, in particular carbapenems. University hospitals account for most of the meropenem use with 2.7 DDD/100 patient-days compared to 1.00 and 0.8 DDD/100 patient-days in large teaching and general hospitals respectively This should be a continuing point of attention in the coming years.
- Antibiotic use per 100 admissions showed a further decline to 295.7 DDD/100 admissions from 306.4 in 2011.
- After a peak in total use of 1.061 DDD/1000 inhabitants/day in 2010, this value decreased in 2012 to 0.963.
- The point prevalence study in 25 hospitals by the PREZIES network showed that 32% of all admitted patients (N = 7542) received antibiotics, the same figure as last year. Antibiotics most often prescribed were amoxicillin with clavulanic acid (24%), ciprofloxacin (11%) and cefuroxim (7%).

2.2 Most important trends in antimicrobial resistance

In GPs

- With a few exceptions – notably nitrofurantoin and fosfomycin – resistance did not increase significantly compared to 2012.
- 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.

In hospitals

- Compared to 2012, overall resistance rates for many antimicrobials were similar or slightly lower. The major exception was nitrofurantoin, which is slightly increasing.
- Strains harbouring carbapenemases were isolated occasionally. However, for strains sent to the RIVM with meropenem MICs > 1 mg/L carbapenemases could not be found in a significant number of cases indicating other mechanisms of resistance.
- The prevalence of MRSA remains low.
- Resistance to vancomycin remained rare in enterococci (<0.5%)
- Resistance to penicillin (0.4%) in pneumococci was still rare in the Netherlands.
- Alterations in the *pen* gene were found in 10% of *N. meningitidis*, explaining the continuing MIC creep towards less susceptibility.

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2.3 Antibiotic use and resistance in veterinary sector

Total sales of antibiotics licensed for therapeutic usage in the Netherlands decreased by 63% since 2007, to 209 tons in 2013. The reduction in sales from the National authority defined index year, 2009, is 58%. This means that the reduction target defined by the authorities for 2013 (50% reduction) is abundantly reached. Relatively largest reductions were realized for cephalosporin 3rd and 4th generation (-76%) en fluoroquinolones (-50%), which is in accordance with Dutch antimicrobial formularies and stimulated by new legislation limiting the use of these (third choice) antimicrobial drugs to bacterial culture proven infections.

One sector was added to the monitoring program (turkeys), resulting in a further narrowing down of discrepancies between sales data and consumption data, although differences are still recognizable due to unmonitored sectors like companion animals and horses. In all major livestock producing sectors a steady decrease in use of antimicrobials is observed since 2009.

- Since 2011 resistance to the fluoroquinolones in *C. jejuni* isolates from broiler feces show a tendency to decrease from 69.2% in 2011 to 52.2% in 2013. In organic raised broilers resistance levels are even lower (42.6%). Although these lower levels are not found in poultry meat, probably due to inclusion of meat from non-domestic origin. Resistance to ciprofloxacin in *Campylobacter spp* in humans is still very high (57.6%). Resistance to the macrolides is still low in all sources sampled (humans, broilers, poultry meat and pigs).
- In 2013, resistance levels for almost all antibiotics testes, further decreased in commensal *E. coli*, used as an indicator organism for the Gram-negative intestinal flora. For all *E. coli* from food-producing animals 26.2% were resistant to amoxicillin (37% in 2012) and 1.5 % to ciprofloxacin (4.9% in 2012) based on EUCAST MIC-breakpoints.
- Prevalence of ESBL-producing *E. coli* from broilers using non-selective methods has decreased in 2013 (to 2.7%) compared to former years (18.3% in 2011 and 8% in 2012). However, active surveillance of broiler meat, using selective media to detect ESBL/AmpC producers still resulted in high prevalence (83% of 728 samples) of ESBL/AmpC producers. Also in other food-producing animals and meat thereof ESBL/AmpC producing *E. coli* and to a lesser extent in *Salmonella* are frequently encountered. The dominant enzymes detected in *E. coli* and *Salmonella* from all sources is CTX-M-1 and CMY-2. The dominant human ESBL variant CTX-M-15 was only found incidentally in broiler meat, beef and feces from veal calves.
- Targeted screening for carbapenemase-producing strains in all feces samples (>1000) from broilers, veal calves, slaughter pigs and dairy cows did not result in isolates with plasmid-mediated carbapenemase genes.

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2.4 Implications for therapy

Although the resistance rates in The Netherlands have increased over the last decade, the resistance rates in 2013 did not increase further for most antibiotics. Yet, there is a continuing concern. For some micro-organisms where resistance rates are apparently similar over the last years, an MIC creep is observed below the clinical breakpoint, indicating that most of the iceberg is not seen. 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 important to tailor therapy to the individual patient. If broad spectrum therapy was initially chosen, antibiograms should be used to narrow down antimicrobial therapy to prevent even further emergence of resistance. In the summary below, some of the most important implications for therapy are provided, based on the general trends of resistance. As implications differ by category of patient and indication of use, the summary is organized as such. It should be borne in mind that the majority of conclusions below are based on agents used as intravenous therapy, except for agents that are available as oral drugs only or have a specific indication such as UTI. Non-susceptible rates can be higher than resistance rates in some cases.

In GPs

Urinary tract infections

- Approximately 80% of Gram-negatives cultured were *E. coli, K. pneumoniae* and *P. mirabilis.* High levels of resistance to amoxicillin, trimethoprim and co-trimoxazole (all $\geq$ 20%) make these agents less suitable for empirical treatment in UTI both in children and adults.
- The best suitable treatment options for uncomplicated UTI are nitrofurantoin (3% resistance in *E. coli* , though increased from 2% in 2012) and fosfomycin (1% resistance in *E. coli*, but >10% in *K. pneumoniae* and *P. mirabilis*). However, care must be taken with nitrofurantoin in the elderly, because of potential toxicity.
- Resistance to co-amoxiclav was > 10% in *E. coli* indicating that care should be taken with empirical treatment without further diagnostic work-up. Multi-drug resistance, defined as resistance to all oral treatment agents for complicated UTI was 3% reduces the oral treatment possibilities of complicated UTI among GP patients.
- 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; and culture, antibiograms and tailored therapy are necessary.
- Resistance rates are comparable to, or slightly higher than in GP patients, thus the treatment strategies will be largely similar

Unselected hospital patient departments

- High levels of resistance to amoxicillin, co-amoxiclav, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections. The ciprofloxacin

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resistance rate of 15% in *E.coli* has further increased and is especially worrisome.
- Piperacillin/tazobactam, cefotaxime/ceftriaxone, ceftazidime and aminoglycoside resistance rates are all between 5 and 10% and in the range that is generally considered to be acceptable for patients not severely ill.
- Combination therapy of a beta-lactam with an aminoglycoside are still the best suitable options for empirical treatment in serious infections.

Intensive care patients
- High levels of resistance to amoxicillin, co-amoxiclav, cefuroxime, co-trimoxazole and ciprofloxacin, make these agents less suitable for empirical treatment in serious infections. The ciprofloxacin resistance rate of 13% in *E.coli* is especially worrisome, but compares well with the values in unselected hospital patients.
- There are significant differences in resistant rates between hospitals as well as over time. This clearly indicates that empiric therapy should be based on the local epidemiology of resistance.
- Piperacillin/tazobactam, cefotaxime/ceftriaxone, ceftazidime and aminoglycoside resistance rates are all between 5 and 10%. This is in a range that warrants combination therapy or at least close monitoring for the severely ill. However, resistance to combinations of a beta-lactam and an aminoglycoside is 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 major European and global public health problem and is, for a large part, driven by (mis) use of antibiotics. As a consequence, patients who are infected with resistant bacteria, that are often resistant to multiple antibiotics (multi-drug resistance), have limited options for treatment. Over the last years there has been a significant increasing trend of combined resistance/multidrug resistance, defined as resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides, in *E. coli* (ECDC) in many European countries.

In the Netherlands, there is a general increase in resistance for almost all compound-pathogen combinations and multi-drug resistance in *E. coli* in all patient groups including GPs over the last years, although the data in 2013 indicate levelling off of this trend. This reflects a general trend, suggesting an increase in ESBL-producing Enterobacteriaceae in community onset and health care associated infections. The increasing trend of combined resistance means that, for patients who are infected with these multidrug-resistant bacteria, only few therapeutic options remain available, such as the carbapenems. Due to the frequent use of antibiotics and the vulnerable population especially patients in hospitals and long term care facilities are at risk for infections with these multi-drug resistant bacteria. Likewise, hospitals and nursing homes may spread these microorganisms within or between settings, and sometimes to the general population. In addition, introductions of resistant bacteria from abroad, from livestock, from the environment and from the general population play a role in the spread of resistance. To control the increase and spread of antibiotic resistance, trends in resistance and antibiotic use should

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be carefully monitored to allow intervention if necessary. This requires intensive collaboration between professionals in the private and public domain.
In 2013 the Minister of Health announced several actions to further improve the surveillance and monitoring of antibiotic resistance in human health care, which are implemented from 2014 onwards. The Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) will be extended to cover all medical microbiological laboratories in the Netherlands. Furthermore, a nationwide system will be developed to monitor resistance at a molecular level to better understand the spread of resistant pathogens and to enable timely actions to control the spread. Finally, a surveillance network in nursing homes is set up to obtain insight in the prevalence and spread of resistant micro-organisms as well as the use of antibiotics in these settings. For successful embedding this requires a change in local policies by performing more diagnostics. Besides control of the spread of resistant bacteria among patients in and between nursing homes, this helps to develop antibiotic therapy guidelines.

Conclusions

The data presented in NethMap 2013 once more demonstrate that the overall rise in resistance requires 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. Diagnostic cultures and in particular susceptibility testing are becoming increasingly important to guide antimicrobial treatment choices. Furthermore, to control the increase and spread of antibiotic resistance, intensive collaboration between professionals in the private and public domain in both human and veterinary health care is necessary.

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3
Use of Antimicrobials

Introduction

In this chapter the use of antimicrobials over the past ten years is reported. First the extramural antibiotic use from 2004 until 2013 is presented; this includes total use as well as the use of individual and groups of antibiotics. Second, antibiotic use in hospital care from 2003 until 2012 is depicted by several measures: DDD/100 patient-days, DDD/100 admissions, as well as in DDD/1000 inhabitant-days (DID). Furthermore, 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.

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 extrapolate their data to 100%. Data are presented as DDD per 1000 inhabitants per day (DID).

Results

Compared to 2012, antibiotic use in 2013 declined from 11.34 to 10.81 DID. Until 2012, there was a steady increase in antibiotic use from 9.87 in 2004 to 11.34 DID in 2012. (Table 3.1).

From 2012 to 2013, use of amoxicillin with clavulanic acid showed a clear decrease of more than 8% to 1.67 DID, whereas the use of amoxicillin slightly increased to 1.99 DID. Only penicillins with extended spectrum show an increase in 2013 compared to 2012 (up to 1.99DID). (Fig.3.1)
Slight decreases were furthermore seen for tetracyclines, macrolides and fluoroquinolones. The rapidly increasing use of nitrofurantoin seems to have stopped in 2013.

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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), 2004-2013 (Source: SFK).

ATC Group*	Therapeutic group	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	2.24	2.41	2.37	2.57	2.66	2.67	2.67	2.60	2.49	2.33
J01CA	Penicillins with extended spectrum	1.71	1.86	1.87	1.91	1.91	1.89	1.81	1.91	1.94	1.99
J01CE	Beta-lactamase sensitive penicillins	0.43	0.44	0.50	0.46	0.42	0.39	0.37	0.35	0.33	0.31
J01CF	Beta-lactamase resistant penicillins	0.28	0.29	0.31	0.32	0.36	0.38	0.38	0.39	0.41	0.41
J01CR	Penicillins + beta-lactamase-inhibitors	1.39	1.50	1.59	1.66	1.71	1.74	1.80	1.82	1.82	1.67
J01D	Cephalosporins	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04
J01EA	Trimethoprim and derivatives	0.26	0.25	0.23	0.22	0.21	0.21	0.20	0.20	0.19	0.17
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	0.39	0.38	0.37	0.36	0.36	0.35	0.35	0.34	0.33	0.29
J01FA	Macrolides	1.32	1.42	1.39	1.39	1.36	1.33	1.31	1.34	1.34	1.22
J01FF	Lincosamides	0.07	0.08	0.09	0.10	0.11	0.12	0.14	0.15	0.16	0.17
J01GB	Aminoglycosides	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03
J01MA	Fluoroquinolones	0.83	0.84	0.87	0.91	0.89	0.86	0.85	0.82	0.80	0.76
J01MB	Other quinolones	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01
J01XB	Polymyxins	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofuran derivatives	0.81	0.90	1.00	1.07	1.13	1.17	1.23	1.31	1.38	1.37
J01XX05	Methenamine	0.02	0.02	0.03	0.03	0.02	0.03	0.04	0.03	0.04	0.03
J01	**Antibiotics for systemic use (total)**	**9.87**	**10.51**	**10.73**	**11.10**	**11.24**	**11.21**	**11.23**	**11.37**	**11.34**	**10.81**

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

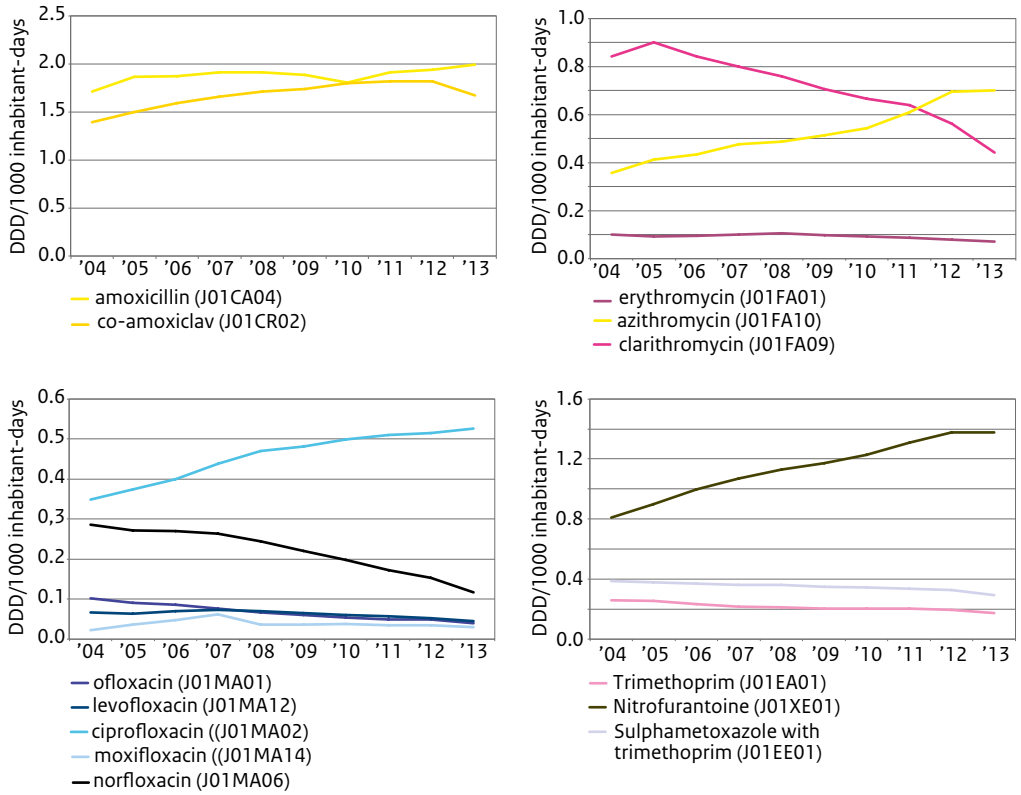
With respect to the macrolides, the use of azithromycin stabilized more or less after an increase over the last 10 years, whereas the use of clarithromycin declined further to 0.44 DID in 2013. Total use of macrolides decreased by 9% in 2013.

Ciprofloxacin use still showed a small increase in use compared to 2012, whereas all other fluoroquinolones showed a decline. Overall use of quinolones decreased by 5% compared to 2012.

Use of tetracyclines (mainly doxycycline) decreased by 6.4% to 2.33 DID in 2013.

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Figure 3.1 a-d Use of antibiotics for systemic use in primary health care, 2004-2013 (Source:SFK).



Discussion

Overall antibiotic use in primary care declined by 4.5% to a total of 10.8 DID in 2013. All groups of antibiotics showed a decline except for penicillins with extended spectrum. Most remarkable changes were seen for macrolides, amoxicillin with clavulanic acid, tetracyclines and fluoroquinolones. Probably, the very mild winter season of 2013/14 could explain decrease in overall and second choice antibiotic use in primary care. In the winter months of 2013/14, less seasonal variation was observed, as compared to previous winter seasons (http://www2.sfk.nl/producten/swab/landelijk last access on 24 March 2014).

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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. We received data from 72 out of 91 hospitals. For each hospital, the annual number of bed-days and admissions were registered. Data were entered in the ABC-calculator (www.escmid.org) to convert them 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.

Extrapolated data calculated as DDD/1000 inhabitants per day, 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. First, an algorithm combining linear interpolation, first value carried backward and last value carried forward was used, followed by up-scaling of the dataset to the total number of university hospitals, large teaching hospitals or general hospitals in the Netherlands. Finally, hospital antibiotic consumption was expressed as DDDs per 1000 inhabitants per day. Statistical analyses were performed using R 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria). Data on annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS).

Dutch hospitals collected again 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 2011, the in-patient use of antibiotics in 2012 stabilized at a level of 71.3 DDD/100 patient-days (Table 3.2). From 2003 to 2009, there was a steady increase in the overall use from 51.9 to about 71 DDD/100 patient-days. From then on, antibiotic use per 100 patient-days remained about stable. Antibiotic use per 100 admissions showed a further decline to 295.7 DDD/100 admissions.

Broken down by hospital category, university hospitals use the least antibiotics (on average 67.6 DDD/100 patient-days), whereas large teaching hospitals used the most (73.7 DDD/100 patient-days). General hospitals used 71.1 DDD/100 patient-days on average.
Figure 3.2 shows the distribution of use per antibiotic class, for the different types of hospitals in 2012. Notable is the large difference in the relative use of combinations of penicillins (mainly amoxicillin with clavulanic acid) between university hospitals (15.8%), large teaching hospitals (19.6%) and general hospitals (25.2%). Most carbapenems and glycopeptides were used in university hospitals, while relatively more tetracyclines and nitrofuran derivates were used in general hospitals. Large teaching hospitals were the highest users of aminoglycosides and cephalosporins.

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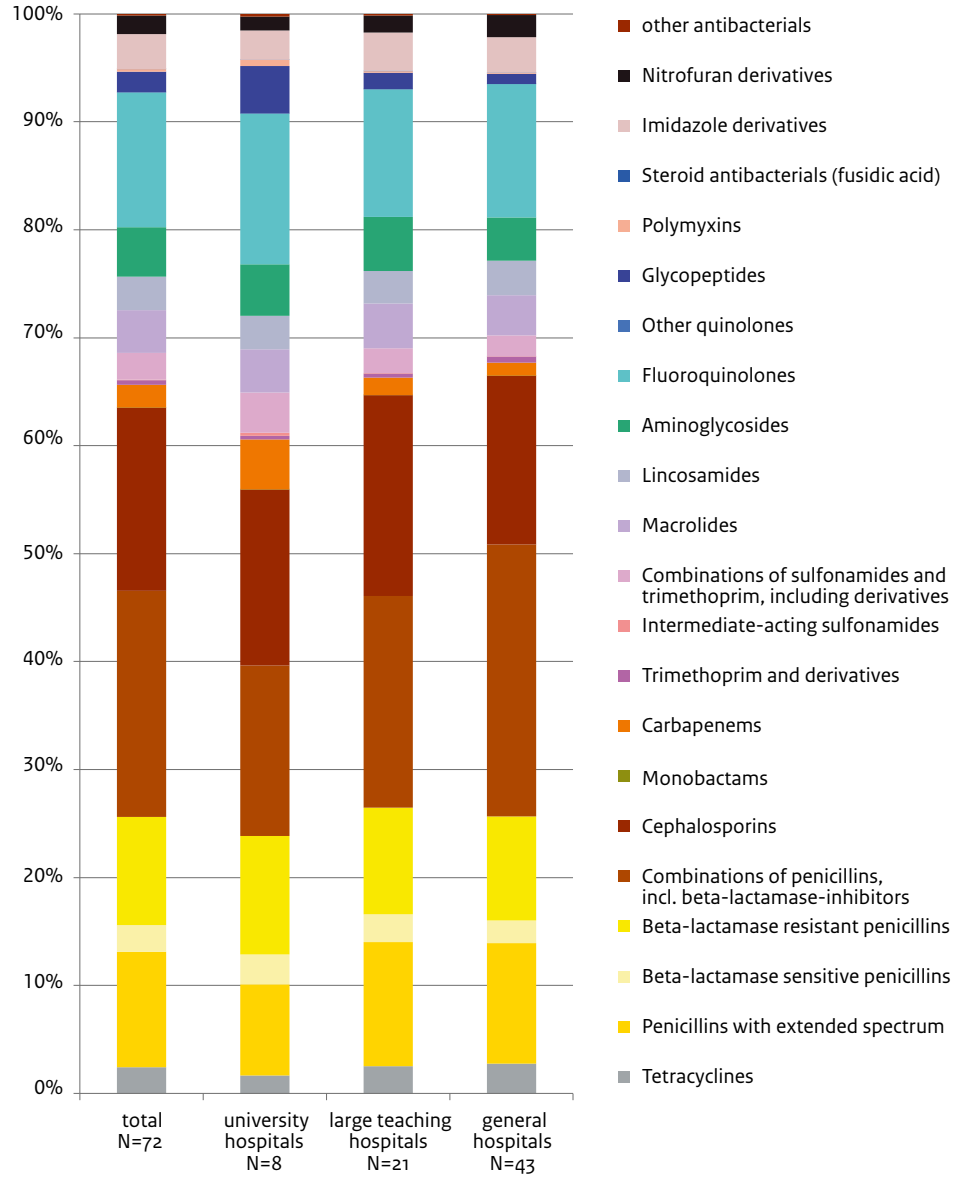
Table 3.2. Ten years use of antibiotics for systemic use (J01) in hospitals, 2003-2012 (Source: SWAB).

ATC group*	Therapeutic group	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01AA	Tetracyclines	1.4	1.5	1.6	1.6	1.4	1.7	1.6	1.7	1.8	1.7
J01CA	Penicillins with extended spectrum	6.0	6.0	6.7	7.6	7.3	6.5	7.6	7.3	7.3	7.6
J01CE	Beta-lactamase sensitive penicillins	1.2	1.4	1.4	1.4	1.2	1.3	1.6	1.5	1.5	1.7
J01CF	Beta-lactamase resistant penicillins	5.4	5.7	5.8	5.9	5.7	6.4	6.6	6.8	6.7	7.1
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	12.1	12.8	13.9	15.1	14.5	16.2	16.5	16.0	15.8	15.0
J01DB -DE	Cephalosporins	6.5	7.0	7.4	8.4	8.4	8.8	10.1	10.2	11.1	12.1
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	0.5	0.6	0.6	0.8	1.0	1.1	1.2	1.4	1.5
J01EA	Trimethoprim and derivatives	0.5	0.4	0.6	0.8	0.5	0.4	0.4	0.5	0.4	0.3
J01EC	Intermediate-acting sulfonamides	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.3	2.1	2.3	2.1	2.3	2.4	2.0	2.0	1.9	1.8
J01FA	Macrolides	2.4	2.3	2.8	2.5	2.8	2.7	2.6	2.7	2.9	2.8
J01FF	Lincosamides	1.6	1.8	1.9	2.0	2.1	2.1	2.4	2.3	2.3	2.2
J01GB	Aminoglycosides	2.5	2.2	2.6	2.5	2.6	3.9	4.2	4.1	3.9	3.3
J01MA	Fluoroquinolones	6.4	6.5	7.3	8.0	7.6	8.8	9.3	9.0	9.2	8.9
J01MB	Other quinolones	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0
J01XA	Glycopeptides	0.5	0.6	0.8	0.7	1.0	1.1	1.3	1.3	1.3	1.4
J01XB	Polymyxins	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.2
J01XC	Steroid antibacterials (fusidic acid)	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
J01XD	Imidazole derivatives	1.6	1.7	1.5	1.7	1.8	1.7	1.8	1.9	2.2	2.3
J01XE	Nitrofuran derivatives	0.7	0.9	1.0	1.0	1.1	1.2	1.1	1.2	1.2	1.2
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	0.0	0.1	0.1	0.1	0.1	0.1
J01	**Antibiotics for systemic use (total)**	**51.9**	**53.7**	**58.5**	**62.2**	**61.6**	**66.8**	**70.9**	**70.2**	**71.3**	**71.3**
	expressed in DDD/100 admissions										
J01	**Antibiotics for systemic use (total)**	**333.3**	**306.8**	**316.9**	**335.9**	**337.5**	**344.7**	**321.3**	**315.9**	**306.4**	**295.7**

* From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

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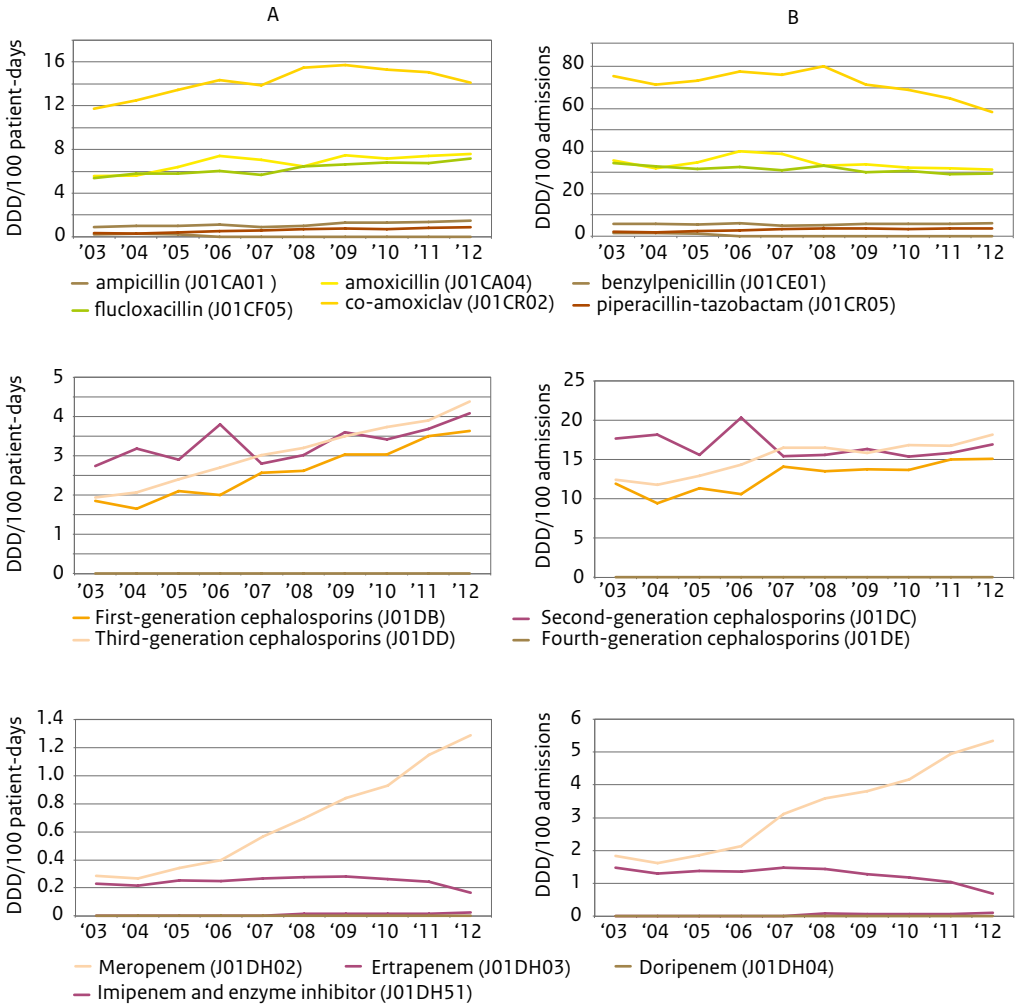
Figure 3.2 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2012 (Source:SWAB)



With respect to subgroups of antibiotics (Fig.3.3 and 3.4), amoxicillin with clavulanic acid showed a marked decrease of 6.6%, from 15.1 in 2011 to 14.1 DDD/100 patient-days in 2012. The use of other penicillins remained stable.

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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), 2003-2012 (Source:SWAB).

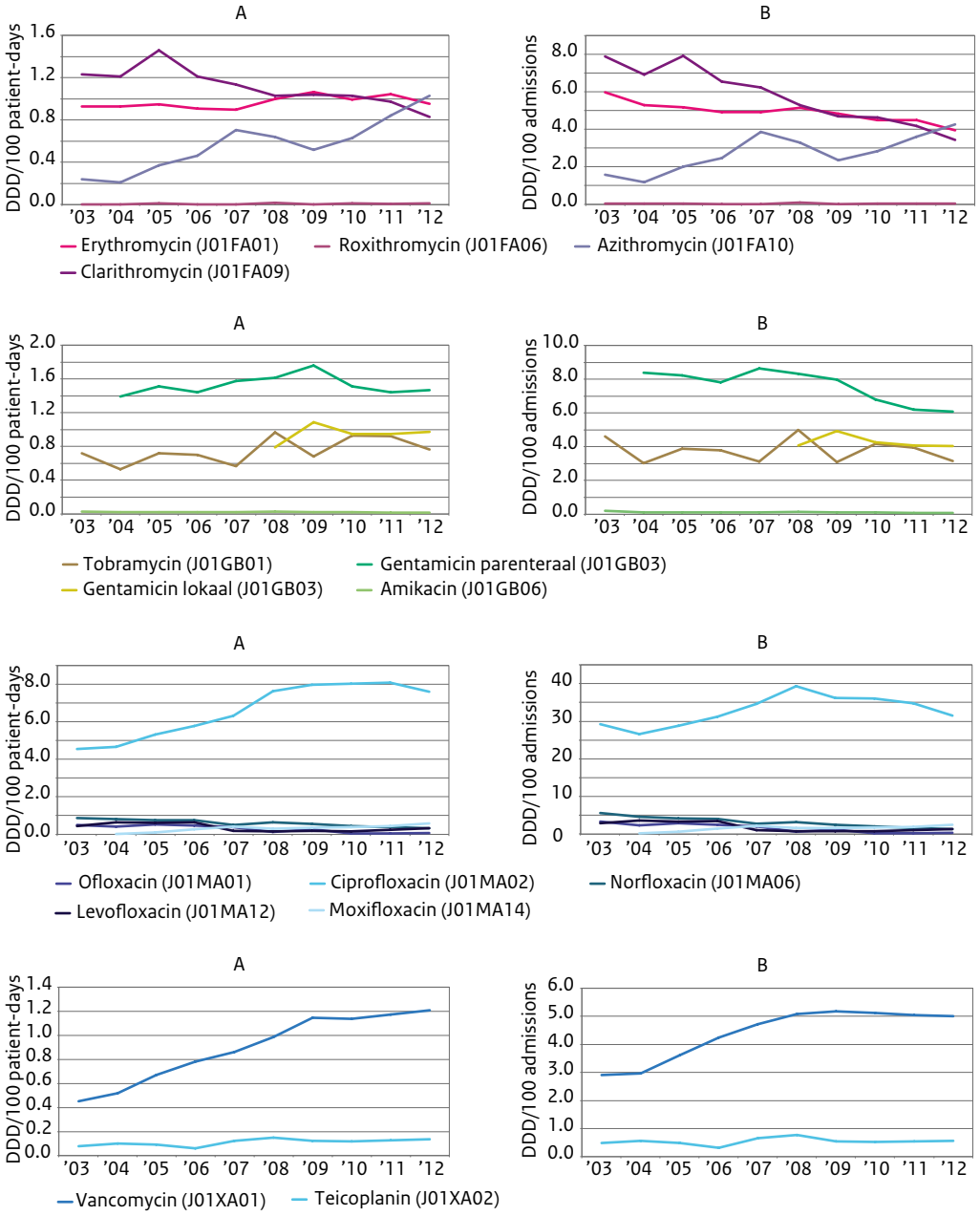


Cephalosporins showed an increase of 4.3% for first-generation, 10.9% for second-generation and 12.1% for third-generation cephalosporins when calculated in DDD/100 patient days. An increase was also seen when calculated in DDD/100 admissions.

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

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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), 2003-2012 (Source: SWAB).



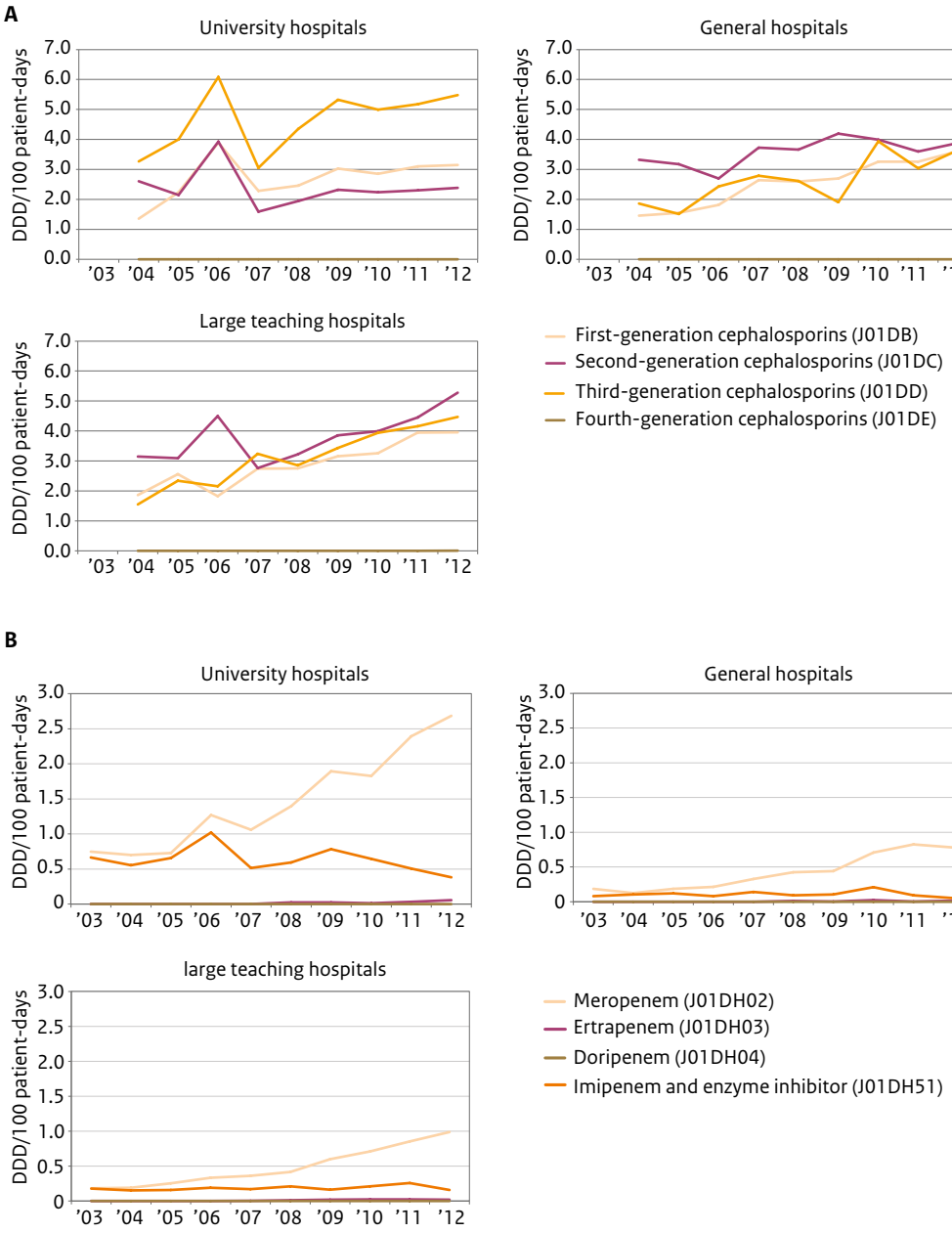
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Table 3.3 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1000 inhabitant-days), 2003-2012 (Source: SWAB).

ATC Group*	Therapeutic group	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01AA	Tetracyclines	0.022	0.025	0.027	0.027	0.025	0.023	0.025	0.027	0.026	0.024
J01CA	Penicillins with extended spectrum	0.087	0.093	0.106	0.113	0.110	0.101	0.111	0.110	0.103	0.100
J01CE	Beta-lactamase sensitive penicillins	0.016	0.019	0.021	0.022	0.020	0.019	0.023	0.023	0.020	0.023
J01CF	Beta-lactamase resistant penicillins	0.068	0.080	0.089	0.091	0.087	0.086	0.093	0.097	0.089	0.093
J01CR	Penicillins + beta-lactamase-inhibitors	0.189	0.212	0.231	0.239	0.233	0.229	0.241	0.256	0.223	0.211
J01DB-DE	cefalosporins	0.088	0.103	0.121	0.127	0.124	0.118	0.137	0.147	0.145	0.158
J01DF	monobactams	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J01DH	Carbapenems	0.004	0.006	0.008	0.009	0.010	0.011	0.014	0.015	0.018	0.019
J01EA	Trimethoprim and derivatives	0.008	0.008	0.009	0.009	0.009	0.007	0.007	0.009	0.006	0.005
J01EC	Intermediate-acting sulphonamides	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.001
J01EE	Sulphonamides + trimethoprim	0.030	0.032	0.035	0.034	0.033	0.029	0.030	0.030	0.026	0.024
J01FA	Macrolides	0.032	0.036	0.042	0.040	0.040	0.037	0.039	0.041	0.037	0.038
J01FF	Lincosamides	0.024	0.027	0.030	0.031	0.031	0.029	0.033	0.035	0.032	0.031
J01GB	Aminoglycosides	0.029	0.031	0.038	0.039	0.041	0.048	0.055	0.058	0.054	0.044
J01MA	Fluoroquinolones	0.092	0.104	0.115	0.121	0.124	0.139	0.129	0.138	0.127	0.124
J01MB	Other quinolones	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000
J01XB	Polymyxins	0.001	0.002	0.005	0.005	0.006	0.008	0.009	0.006	0.003	0.002
J01XE	Nitrofuran derivatives	0.012	0.014	0.017	0.016	0.018	0.016	0.017	0.018	0.015	0.018
J01XX05	Methenamine	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.001
J01XX08	Linezolid	0.000	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001
	other antibiotics	0.028	0.032	0.035	0.038	0.039	0.038	0.043	0.048	0.045	0.047
J01	**Antibiotics for systemic use (total)**	**0.734**	**0.827**	**0.931**	**0.965**	**0.952**	**0.941**	**1.008**	**1.061**	**0.971**	**0.963**

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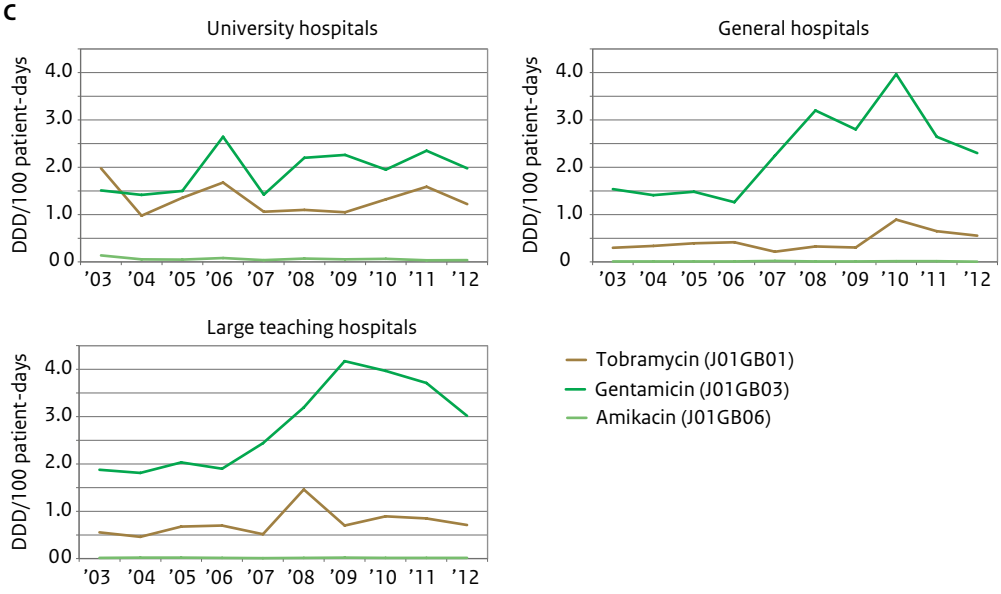
Figure 3.5 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C) and glycopeptides (D) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2003-2012 (Source: SWAB)



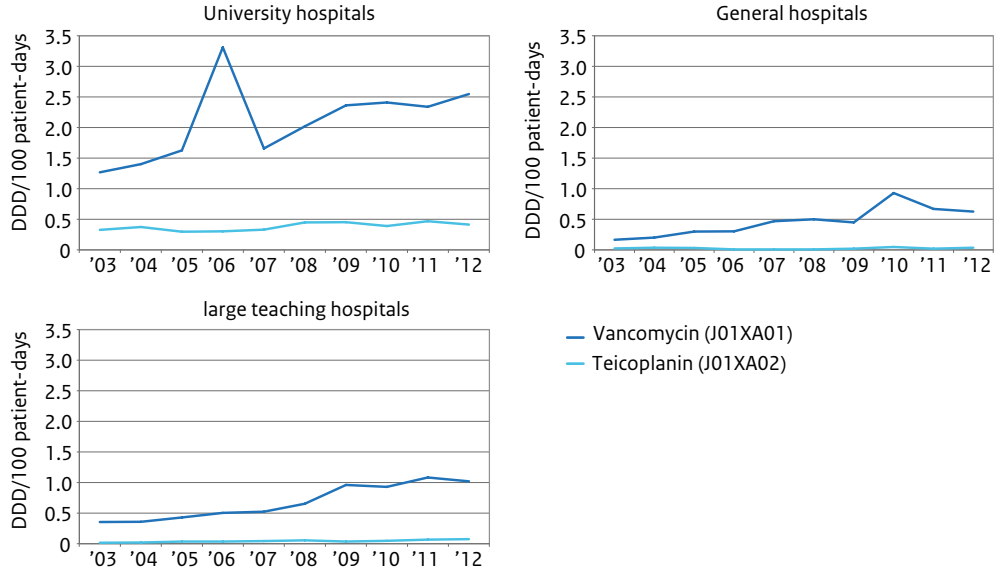
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Figure 3.5 continued

C



D



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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), 2007-2012 (Source: SWAB).

ATC group *	Therapeutic group	2007	2008	2009	2010	2011	2012
J02AA01	Antibiotics (amphotericin B)	4.44	1.12	1.35	1.65	1.77	2.43
J02AB02	Imidazole derivatives (ketoconazole)	0.12	0.11	0.08	0.15	0.09	0.10
J02AC	Triazole derivatives	5.18	6.36	6.72	6.31	5.83	6.25
J02AX	Other antimycotics for systemic use	0.19	0.40	0.61	0.56	0.57	0.55
J02	**Antimycotics for systemic use (total)**	**9.93**	**7.98**	**8.77**	**8.66**	**8.26**	**9.33**
J04AA	Aminosalicylic acid and derivatives	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
J04AC	Hydrazides (mainly isoniazide)	0.39	0.29	0.40	0.34	0.30	0.40
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.00	0.01	0.00
J04AK	Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol)	0.38	0.31	0.34	0.37	0.26	0.31
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.00	0.01
J04BA	Drug for treatment of leprosy (dapson)	0.53	0.39	0.33	0.45	0.49	0.62
J04	**Antimycobacterials for systemic use (total)**	**2.74**	**2.33**	**2.35**	**2.58**	**2.62**	**2.57**
J05AB	Nucleosides excl. Reverse transcriptase inhibitors (J05AB)	1.72	2.00	2.22	2.02	2.18	2.24
J05AD	Phosphonic acid derivatives (J05AD)	0.06	0.11	0.13	0.10	0.10	0.15
J05AE	Protease inhibitors (J05AE)	0.70	0.92	0.75	0.78	0.55	0.81
J05AF	Nucleoside reverse transcriptase inhibitors (J05AF)	0.83	0.74	0.64	0.67	0.63	0.69
J05AG	Non-nucleoside reverse transcriptase inhibitors (J05AG)	0.20	0.25	0.23	0.22	0.14	0.18
J05AH	Neuraminidase inhibitors (J05AH)	0.02	0.05	n.a.#	0.21	0.42	0.19
J05AR	Antivirals for the treatment of HIV, combinations (J05AR)	0.33	0.52	0.55	0.76	0.69	0.91
J05AX	Other antivirals (J05AX)	0.00	0.06	0.06	0.15	0.17	0.24
J05	**Antivirals for systemic use (total)**	**3.86**	**4.65**	**4.59**	**4.91**	**4.89**	**5.41**

* from the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system
# Total use not to be assesed because of alternative distribution during the pandemic

As for the past 10 years, meropenem use showed a continued increase in use up to 1.3 DDD/100 patient-days in 2012. University hospitals account for most of the meropenem use with 2.7 DDD/100 patient-days compared to 1.00 and 0.8 DDD/100 patient-days in large teaching and general hospitals respectively (figure 3.5).
Overall macrolide use in hospitals remained stable over the past 10 years, whereby azithromycin showed a clear increase in 2012 again, and both clarithromycin and erythromycin showed a decline compared to 2011, after a more or less stable use in the previous years.

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Use of gentamicin remained stable. In the case of gentamicin, large teaching and general hospitals show a higher use than university hospitals (figure 3.5).
Fluoroquinolone use slightly decreased by 3.3% compared to 2011, whereas glycopeptides showed an only very small increase to 1.4 DDD/100 patient-days. Most of it is used in university hospitals with 2.6 DDD/100 patient-days, compared to 1.0 and 0.6 DDD/100 patient-days in large teaching and general hospitals respectively (figure 3.5).
Over 75% of the antimycotics (J02), antimycobacterials (J04) and antivirals (J05) for systemic use were used in university hospitals. General and large teaching hospitals only used these substances occasionally. In table 3.4 use of J02, J04 and J05 in university hospitals is presented from 2007 until 2012, expressed in DDD/100 patient-days. The use of antimycotics increased in 2012 compared to 2011, while the use of antimycobacterials remains stable. Also the use of antivirals was increasing to 5.41 DDD/100 patient-days in 2012.

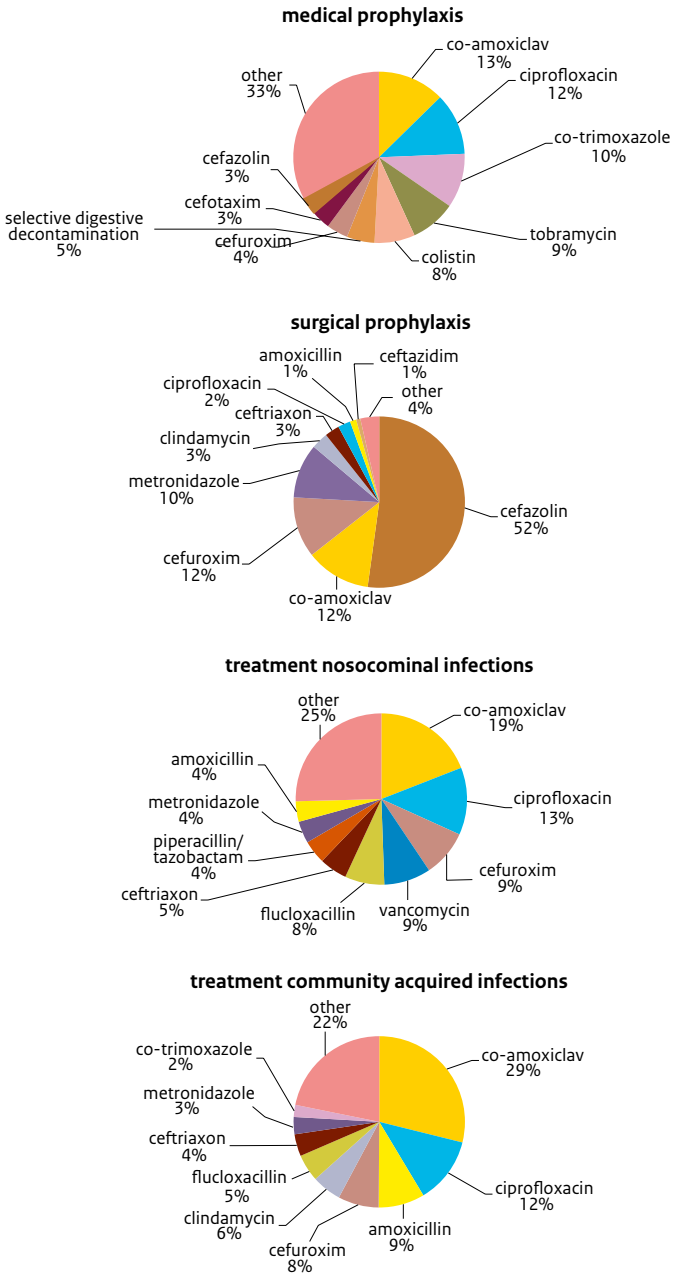
From PREZIES, in 2013 we received data from twenty five hospitals participating in the point prevalence study, including 7542 patients of which 2427 received antibiotics, with a total of 3071 prescriptions (1756 for community acquired infections, 362 for nosocomial infections, 419 for medical prophylaxis, 253 for surgical prophylaxis and 281 for other or unknown indications.) (Fig. 3.6). Antibiotics most often prescribed were amoxicillin with clavulanic acid (24%), ciprofloxacin (11%) and cefuroxim (7%). The respective distribution for community acquired and nosocomial infections are shown in Figure 3.5. Amoxicillin with clavulanic acid was most often used in both types of infection. Also surgical and medical prophylaxis are depicted. Cefazolin was used in 52% cases of surgical prophylaxis. The use for medical prophylaxis was more diverse, amoxicillin with clavulanic acid was most often used (13%), followed by ciprofloxacin resp. trimethoprim/sulfamethoxazole.

Discussion

The same pattern of overall use of systemic antibiotics in Dutch hospitals is seen in 2012 as in previous years: a stable use when calculated as DDD/100 patient-days, whereas it decreases when expressed in DDD/100 admissions. The number of hospital admissions increases, while length of stay decreases. This means that, on average, individual patients were exposed to the same amount of antibiotics, but because more patients were admitted to the hospital, total use of antibiotics in Dutch hospitals increased. A consequence of a reduction in the duration of hospital stay is that more patients with antibiotic treatment can be admitted per bed during a specific period. This results in an intensification of antibiotic treatment per patient-day and per hospital bed, which may cause increased selection pressure towards resistance. For an in-depth discussion of these developments, see Kwint et al (2).
Despite a stable total use of systemic antibiotics over the last 4 years, there are marked shifts of use between different subgroups of antibiotics. The steady increase of use of 3rd-generation-cephalosporins and meropenem is of particular interest, even though, on a European level, the use is still low.
For the first time, we see a decrease in the use of fluoroquinolones. After steady increases every year to a total of 9.2 DDD/100 patient-days in 2011, total use declines to 8.9 DDD/100 patient-days 2012. Fluoroquinolone use is higher in university hospitals compared to the other two groups of hospitals.

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Figure 3.6 Distribution of the use of antibiotics for systemic use (J01) ; results of the point-prevalence studies 2013 (Source: PREZIES)



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3.3 Care in nursing homes

Methods

All hospital pharmacists participating in the surveillance of antibiotic use in hospitals were again asked to provide the antibiotic consumption data from nursing homes their pharmacy is serving. Data from 25 nursing homes were received. The size of these homes varied from 19 to 889 residents per home, with a mean of 248 residents. In total, the antibiotic use of 5943 residents was included. For each nursing home the amount of DDD/1000 residents/day was calculated, and their weighed mean was calculated.

Results

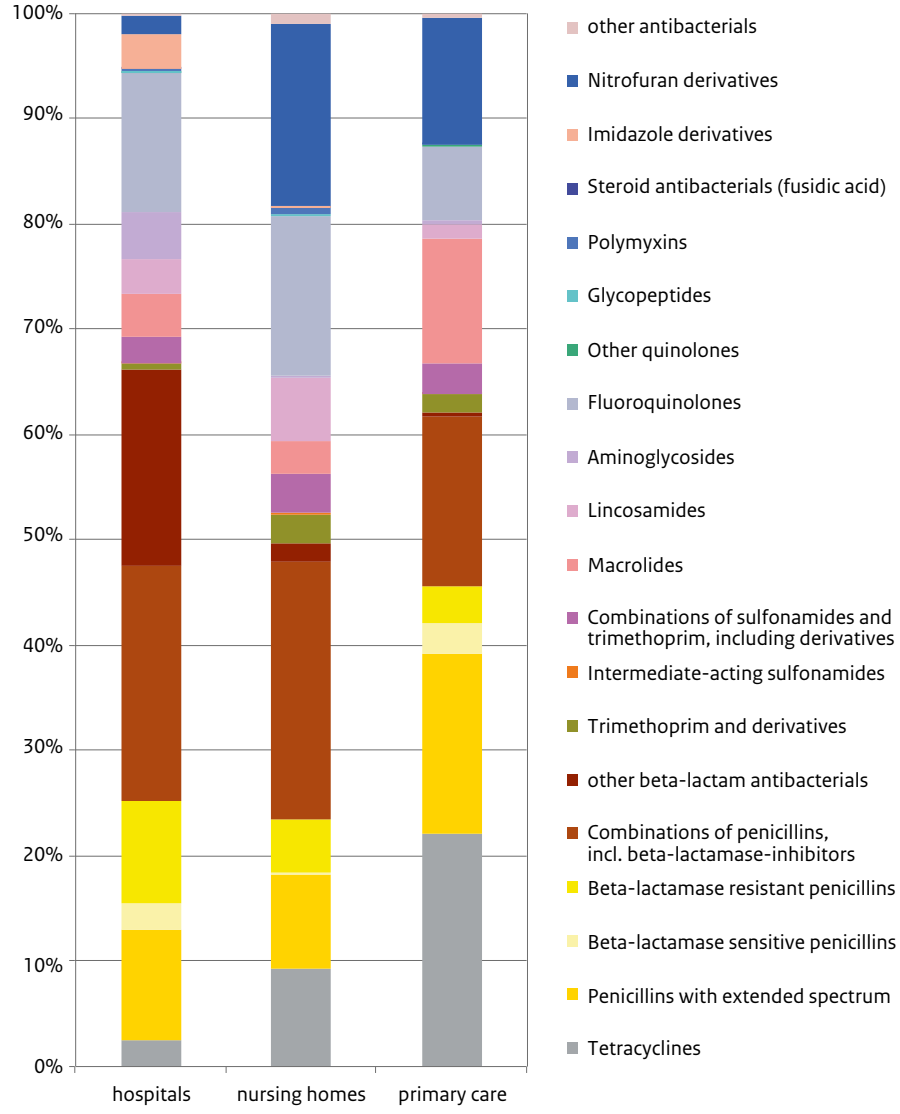
The use of antibiotics varied hugely for the different nursing homes with a minimum of 33 and a maximum of 177 DDD/1000 residents/day. The mean use was 74 DDD/1000 residents/day. Combinations of penicillins (mainly amoxicillin with clavulanic acid), with 18.1 DDD/1000 residents/day, nitrofurantoin derivates (12.8 DDD/1000 residents/day) and fluorquinolones (11.2 DDD/1000 residents/day) were most frequently used (Table 3.5).

Table 3.5 Distribution of the use of antibiotics (J01) in nursing homes, expressed as DDD/1000 residents/day in 2011 and 2012 (Source: SWAB).

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

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Figure 3.7 Comparison of the distribution of antibiotic usage (J01) in primary care, hospital care and care in nursing homes in 2012.



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Discussion

For the second year, the use of antibiotics in nursing homes is reported in NethMap. Unfortunately, data from only 25 nursing homes could be retrieved, about half of the amount of 2011. Nevertheless, more or less the same pattern of usage is seen. The most frequently used antibiotic is amoxicillin with clavulanic acid (24 %), followed by nitrofurantoin (17%) and fluoroquinolones (15%).

Notable is the relatively lower use of tetracyclines (9%). The high use of nitrofurantoin is not surprising, because there are a lot of urinary tract infections among elderly patients. With respect to broad spectrum antibiotics, the high use of fluoroquinolones is especially worrisome.

The broad range of use suggests that there is considerable variation in antimicrobial use in nursing homes across the Netherlands. However, details about differences in characteristics of residents and care provided (rehabilitation, palliative care) are still lacking. Nursing homes provide a significant service and more information should be available in order to optimize antimicrobial use and limit the development of antimicrobial resistance.

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

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4 Surveillance of resistance

4.1 Methods of surveillance

In the Netherlands, the surveillance of resistance in GPs, nursing homes and hospitals, is based on ISIS-AR (Infectious Disease Surveillance Information System on Antibiotic Resistance). Below, a brief overview of the methods are decribed; more details can be found at www.swab.org.

Since 2008, routinely available antimicrobial susceptibility data of all isolates from Dutch medical laboratories, including underlying 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 2013, ISIS-AR received data from 32 laboratories of which 26 laboratories continuously reported to ISIS-AR from 2009 to 2013. To avoid bias in time trends due to incomplete data we used for all analyses in the current report data from these 26 continuously reporting laboratories only. Three of these laboratories were serving university hospitals, 22 laboratories were serving non-university hospitals and general practitioners and one laboratory was only serving general practitioners. We calculated resistance levels and linear time trends over the five most recent years (2009 to 2013) 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), unselected hospitals departments, ICU departments, 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), unselected hospital departments (chapter 4.3.2), and ICU departments (chapter 4.3.3), the selected isolates originated from blood, liquor, wound, lower respiratory tract and urinary isolates combined. Additionally, we conducted a separate analysis for blood isolates in

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non-ICU hospital departments and ICU departments combined (chapter 4.3.4). Finally, for the analysis on respiratory pathogens (*Haemophilus influenzae*, *Streptococcus pneumoniae, Moraxella catarrhalis*) we selected isolates from blood, liquor, higher respiratory tract, and lower respiratory tract isolates combined (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 for screening and inventory purposes. Furthermore, to avoid bias due to selective testing, for each pathogen-compound combination we included only data from laboratories in which at least 50% of isolates was tested for that specific compound. Finally, for representativeness of the results, the resistance level and time trend of each pathogen-compound combination is only shown if at least 50% of laboratories could be included.

Calculation of resistance levels

The percentage of resistant isolates ("R") was calculated. To avoid bias because of 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 2013 breakpoints. For most included pathogens (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Staphylococcus aureus,* and coagulase-negative staphylococci (CNS) including *Staphylococcus epidermidis*) at least 80% 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 15 laboratories that used EUCAST recommendations in 2013, were included for calculating the percentage of resistant isolates.

In some tables, data are presented for a combination of compounds against which comparable resistance mechanisms exist, namely amoxicillin/ampicillin, ceftriaxone/cefotaxime, imipenem/meropenem, and doxycylin/tetracyclin. For these combinations, we calculated the resistance percentage against at least one of both compounds. Additionally, we calculated resistance to specific combinations of compounds that are frequently used for empiric therapy (gentamicin + amoxicillin/ampicillin, gentamicin + co-amoxiclav, gentamicin + cefuroxime, gentamicin + ceftriaxon/cefotaxime, gentamicin + ceftazidime, gentamicin + piperacillin-tazobactam, tobramycin + ciprofloxacin, and tobramycin + ceftazidim). For these combinations, resistance was defined as resistance to both compounds.
To calculate the percentage of highly resistant micro-organisms (HRMO) we used the definitions of the Working Group on Infection Prevention (WIP, http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_WIP). Enterobacteriaceae except *Enterobacter cloacae* were considered a HRMO if they were 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. *E. cloacae* was considered a HRMO if resistant to both fluoroquinolones and aminoglycosides. *P. aeruginosa* was considered a HRMO if resistant to ≥3 compounds per category/compound 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 the GP and urology outpatient departments, multidrug resistance in Enterobacteriaceae was calculated, defined as resistance to all of the following oral compounds: co-trimoxazole, co-amoxiclav and ciprofloxacin.

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Calculation of time trends

In addition to resistance levels in 2013, we calculated time trends over the five most recent years (2009 to 2013) for each pathogen-compound combination, 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 significant. Significantly increasing trends are shown in the tables as a red coloured font, whereas decreasing trends are shown as a green coloured font. In addition, to facilitate the interpretation of time trends for pathogen-compound combinations with low resistance levels, the trends for the pathogen-compound combinations are shown in the figures when the percentage resistant isolates is between 0.5% and 30% in at least three years.

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4.2 Primary care

Surveillance data on resistance in patients attending a general practice (GP) is available from (1) the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) database and the APRES project (Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance).

4.2.1 ISIS-AR

For the resistance data on GP patients in ISIS-AR, only urinary isolates were included. GPs usually send urine samples for culture and susceptibility testing in case of complicated UTI or when there is no response to antimicrobial therapy. Urinary 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 presenting at the GP. Therefore, these patients are further referred to as 'selected GP patients'.

Table 4.2.1 shows the distribution of pathogens isolated from urine samples in selected GP patients and table 4.2.2 and figure 4.2.1 show the resistance levels for selected GP patients. Results are presented for patients aged ≤12 years and patients aged >12 years separately.

Table 4.2.1 Distribution of isolated pathogens N (%) in clinical specimens from general practitioners, presented per age category, ISIS-AR 2013

Pathogen	Age ≤12 N (%)	Age >12 N (%)
E. coli	7726 (68)	62043 (58)
K. pneumoniae	188 (2)	6528 (6)
P. mirabilis	562 (5)	6351 (6)
P. aeruginosa	196 (2)	2427 (2)
Other Enterobacteriaceae*	525 (5)	8131 (8)
Other non-fermenters**	199 (2)	1996 (2)
Enterococcus spp.	1324 (12)	10269 (10)
Other gram-positives	663 (6)	10114 (9)

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

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

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Table 4.2.2 Resistance levels (%) of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* among clinical isolates from general practitioners, presented per age category, ISIS-AR 2013

	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	63	4	72	3	73	3	78
Antibiotic								
amoxicillin/ ampicillin	37	41	-	-	18	21	-	-
co-amoxiclav	12	15	9	7	7	6	-	-
cefuroxime	3	7	4	12	1	1	-	-
cefotaxime/ ceftriaxone	2	3	1	3	1	1	-	-
ceftazidime	1	1	0	2	1	0	0	3
gentamicin	2	4	2	2	4	4	0	3
tobramycin	-	-	-	-	-	-	0	1
trimethoprim	21	27	14	23	25	35	-	-
co-trimoxazole	20	25	11	12	21	28	-	-
norfloxacin	7	15	4	22	6	12	-	-
ciprofloxacin	3	10	2	4	3	8	0	6
nitrofurantoin	0	3	-	-	-	-	-	-
fosfomycin	1	1	15	29	14	14	-	-
Multi-drug resistance								
HRMO*	2	5	1	3	2	3	-	-
multidrug-resistance**	1	3	1	1	0	1	-	-

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

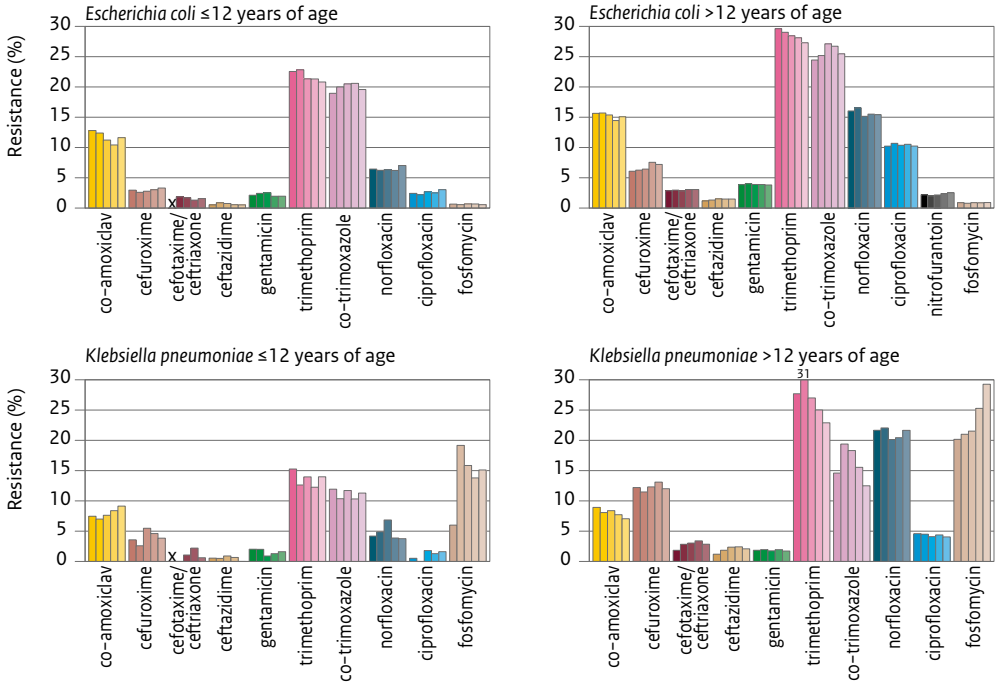
- Resistance not calculated

**Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_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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Figure 4.2.1. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *E. coli* and *K. pneumoniae* from general practitioners, presented per age category.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



Key results

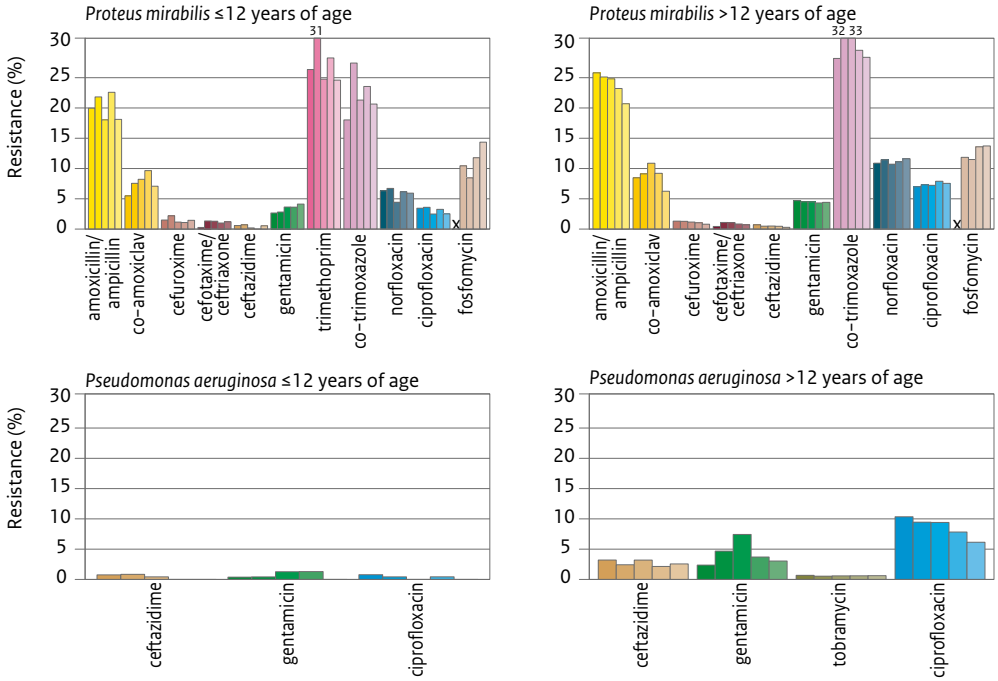
- In general, resistance levels in selected GP patients aged >12 years were higher than in patients aged ≤12 years, in particular for the fluoroquinolones. Only in *K. pneumoniae* resistance among selected GP patients ≤12 years was higher for co-amoxiclav (9% versus 7%), when compared to patients aged >12 years.

Enterobacteriaceae

- Resistance levels were low for cefotaxime/ceftriaxone (≤3%), ceftazidime (≤2%), gentamicin (≤4%), and ciprofloxacin (≤10%) in all Enterobacteriaceae. Resistance levels were also low for nitrofurantoin (≤3%) and fosfomycin (1%) in both age categories in *E. coli*, although resistance to nitrofurantoin increased significantly in patients aged >12 since 2009, from 2% to 3% in 2013. Resistance to cefuroxime remained low (≤7%), except in *K. pneumoniae* in patients aged >12 (12%). Finally, resistance to co-amoxiclav remained low (≤9%) in *K. pneumoniae* and *P. mirabilis*.
- High levels of resistance were found for amoxicillin/ampicillin, norfloxacin and co-trimoxazole (≥12%) in patients aged >12 years.

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Figure 4.2.1. (continued) Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *P. mirabilis* and *P. aeruginosa* from general practitioners, presented per age category.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



- There was a significant decrease in resistance to amoxicillin/ampicillin, co-amoxiclav and trimethoprim in patients aged >12 years for all Enterobacteriaceae. However, resistance to amoxicillin/ampicillin and trimethoprim remained high (above 20%).
- Fosfomycin resistance significantly increased in *K. pneumoniae* and *P. mirabilis*. In *K. pneumoniae*, resistance levels rose from 20% in 2009 to 29% in 2013 in patients aged >12 years and from 6% in 2011 to 15% in 2013 in patients aged ≤12 years. In *P. mirabilis*, resistance was 12% in 2010 and 14% in 2013 in patients aged >12 years.
- Overall, the percentage of highly resistant micro-organisms (HRMO) (≤5%) and multidrug-resistance (≤3%) remained low over time. However, there was a slight increase in percentage of HRMO among *E. coli* (4% in 2009 to 5% in 2013) and K. pneumoniae (2% in 2009 to 3% in 2013).

P. aeruginosa

- Resistance levels for all tested agents were low (≤6%).
- Resistance to ciprofloxacin in patients aged >12 years showed a decreasing trend from 10% in 2009 to 6% in 2013.

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4.2.2 APRES-study

Resistance in *Streptococcus pneumoniae*

The data presented are part of the APRES project (Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance).(1)
In short: the APRES study compared the prevalence and antibiotic resistance of *Streptococcus pneumoniae* and *Staphylococcus aureus* among healthy patients visiting general practitioners in nine European countries for a non-infectious complaint. The results of the *S. pneumoniae* are reported, those of *S. aureus* have been reported previously (2).

Materials and Methods

Twenty general practitioners of the NIVEL network participated in the study, each of them taking nasal swabs from 200 patients (aged >3 years). To be included in the study, patients should not have used antibiotics or been hospitalized in the three months prior to the sampling. Immunocompromised patients and nursing home residents were excluded as well. Isolation and identification were performed using optochine susceptibility, bile solubility and PCR.
Antibiotic susceptibility was determined using micro dilution according to the EUCAST guidelines for the following antibiotics: cefuroxime, cefaclor, ceftazidime, clarithromycine, clindamycin, ciprofloxacin, moxifloxacin, penicillin, tetracycline and trimethoprim-sulfamethoxazole. *S. pneumoniae* ATCC 49619 was used as control. The EUCAST epidemiological cut-offs were used as breakpoints for resistance.
Multidrug resistance was defined as resistance to three or more classes of antibiotics: cefaclor, cefuroxime and ceftazidime were grouped into one group in the calculation of the number of antibiotic classes to which a strain is resistant.

Results

In total 3873 patients from Dutch general practitioners participated. Of the total population, 46.1% was between 30-60 years of age, 34,2% more than 60 years.
The prevalence of *S. pneumoniae* nasal carriage was 27%(19.1- 34.9, 95% confidence interval) among children < 10 years, and 2.5% (2.0-3.0, 95% confidence interval) among those >10 years of age.
A total of 129 *S.pneumoniae* were available for antibiotic resistance testing. The resistance ranged from 3.1% for clindamycin and tetracycline to 34.15% for cefaclor. The percentages for the other antibiotics were 3.9 % for clarythromycin, cefuroxime and ceftazidime , 4.7% for penicillin and 7.0% for trimethoprim- sulfametoxazole. No isolates with increased MICs were found for ciprofloxacin and moxifloxacin.
Multi drug resistance was observed in six isolates.

References

1. Van Bijnen EME et al.
   The appropriateness of prescribing antibiotics in the community in Europe: study design, BMC infectious Diseases 2011; 11, 293
2. Den Heijer CDJ et al.
   Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin resistant *Staphylococcus aureus*; a European cross-sectional study, The Lancet Infectious Diseases 2013, 13 409-4156

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4.3 Hospital departments

Surveillance data on resistance in patients attending outpatient and hospital departments is only available from the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) database. For the outpatient and hospital departments (unselected hospital departments and ICU departments), the antimicrobial susceptibility results are from blood, cerebrospinal fluid, wound, lower respiratory tract and urinary isolates combined. Additionally, we conducted a separate analysis for blood isolates in non-ICU hospital departments and ICU departments combined (chapter 4.3.3). For the urology departments only urinary isolates were included.

4.3.1 Outpatient departments

Table 4.3.1.1 shows the distribution of pathogens from clinical specimens (blood, cerebrospinal fluid (CSF), wound or pus, lower respiratory tract, urinary, and other sterile isolates) of patients attending outpatient departments. The resistance levels for the outpatient departments are shown in tables 4.3.1.2 -4.3.1.3 and figures 4.3.1.1 and 4.3.1.2 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*, separately.

Table 4.3.1.1. Distribution of isolated pathogens N (%) in clinical specimens from outpatient departments, ISIS-AR 2013

Pathogen	Blood N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or Pus N (%)	Other sterile specimens N (%)
E. coli	1431 (26)	560 (9)	21821 (46)	1608 (8)	0 (0)
K. pneumoniae	211 (4)	218 (4)	3317 (7)	287 (1)	1 (1)
P. mirabilis	118 (2)	178 (3)	2552 (5)	907 (4)	1 (1)
P. aeruginosa	73 (1)	1158 (19)	1519 (3)	1396 (7)	0 (0)
E. faecalis	156 (3)	4 (0)	4235 (9)	683 (3)	5 (6)
S. aureus	501 (9)	1300 (21)	1442 (3)	9362 (44)	3 (4)
Other Enterobacteriaceae*	250 (5)	805 (13)	4601 (10)	2152 (10)	1 (1)
Other non-fermenters**	20 (0)	514 (8)	586 (1)	519 (2)	1 (1)
Other *Enterococcus spp.*	61 (1)	4 (0)	1995 (4)	235 (1)	3 (4)
Other gram-positives	2646 (48)	1423 (23)	5003 (11)	4141 (19)	65 (81)

* *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* in outpatient departments, ISIS-AR 2013

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ ampicillin	47	-	24	-
co-amoxiclav	19	9	8	-
imipenem/ meropenem	0	0	0	3
cefuroxime	12	11	1	-
cefotaxime/ ceftriaxone	5	4	1	-
ceftazidime	3	3	0	3
gentamicin	6	3	6	4
tobramycin	6	4	4	1
trimethoprim	31	22	37	-
co-trimoxazole	29	14	30	-
norfloxacin	23	19	14	-
ciprofloxacin	17	6	10	7
nitrofurantoin	4	-	-	-
colistin	-	-	-	2
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	6	3	0
multidrug-resistance**	5	2	1	-

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

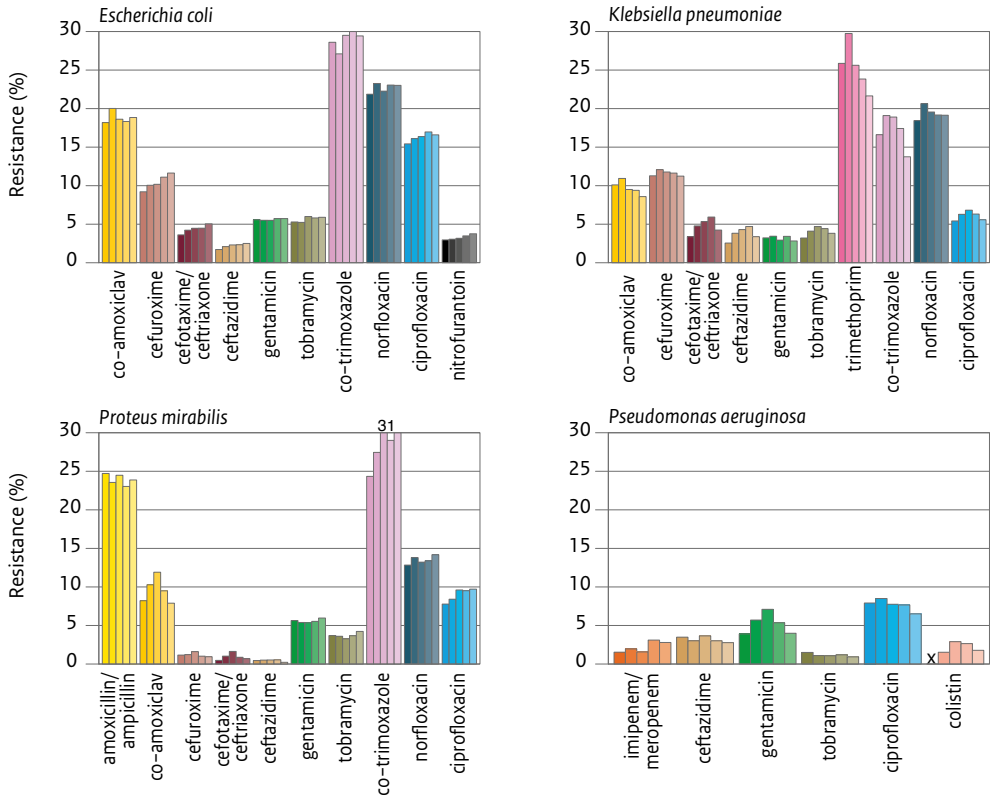
- *Resistance not calculated*

* *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_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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Figure 4.3.1.1. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* from outpatient departments.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



Key results

Enterobacteriaceae

- Low resistance levels were found for cefotaxime/ceftriaxone (≤5%), ceftazidime (≤3%), gentamicin (≤6%), tobramycin (≤6%) and imipenem/meropenem (≤0.5%) in all Enterobacteriaceae. Also, low resistance was found for nitrofurantoin (4%) in *E. coli*, for co-amoxiclav (≤9%) and ciprofloxacin (≤10%) in *K. pneumoniae* and *P. mirabilis*, and for cefuroxime (1%) in *P. mirabilis*.
- Amoxicillin/ampicillin, trimethoprim, co-trimoxazole and norfloxacin resistance was high for all tested compound-pathogen combinations (≥14%). Additionally, resistance to ciprofloxacin was high in *E. coli* (17%).
- Multidrug resistance to all of the following three oral agents, co-trimoxazole, co-amoxiclav and ciprofloxacin, was ≤5%.

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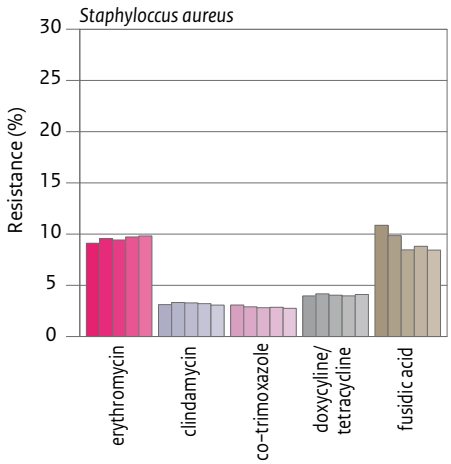
Table 4.3.1.3. Resistance levels among clinical isolates of *S. aureus* from outpatient departments, ISIS-AR 2013

	S. aureus
Antibiotic	
MRSA*	2
erytromycine	10
clindamycine	3
co-trimoxazole	3
doxycycline/ tetracycline	4
ciprofloxacin	8
fusidic acid	8

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

**The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2) or, if these tests were lacking, resistance to flucloxacillin, methicillin, oxacillin, or cefoxitin screentest.*

Figure 4.3.1.2. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *S. aureus* from outpatient departments.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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E. coli

- Resistance to most tested agents, including empiric therapy combinations, significantly increased since 2009, especially to cefuroxime (from 9% in 2009 to 12% in 2013), cefotaxime/ceftriaxone (from 4% to 5%), ceftazidime (from 2% to 3%), ciprofloxacin (from 15% to 17%) and nitrofurantoin (from 3% to 4%).
- The increased resistance to 3rd generation cephalosporins also resulted in an increased percentage HRMO from 7% in 2008 to 8% in 2013.

K. pneumoniae

- Resistance to 3rd generation cephalosporins increased since 2009, although resistance remained below 7% and was lower in 2013 compared to 2012 (4% in 2013 versus 6% in 2012 for cefotaxime/ ceftriaxone and 3% in 2013 versus 5% in 2012 for ceftazidime). This trend was reflected in the percentage of HRMOs (6% in 2013 versus 7% in 2012).
- There was a sharp decline in resistance seen to trimethoprim (30% in 2010 to 22% in 2013), and co-trimoxazole (19% in 2010 to 14% in 2013) since 2010.

P. mirabilis

- There was a significant increase in resistance to co-trimoxazole (24% in 2009 to 30% in 2013) and ciprofloxacin (8% in 2009 to 10% in 2013), which resulted in an increase in HRMO (2% in 2009 to 3% in 2013).

P. aeruginosa

- Resistance to all tested agents remained low (≤7%).
- Resistance to ciprofloxacin decreased from 8% in 2009 to 7% in 2013.
- Resistance to imipenem/meropenem increased from 2% in 2009 to 3% in 2013.

S. aureus

- Resistance to all tested agents remained low (≤10%).
- The proportion of MRSA isolates remained below 2%.
- Resistance to fusidic acid decreased since 2009 from 11% to 8% in 2013.

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4.3.2 Unselected hospital departments

Table 4.3.2.1 shows the distribution of pathogens from clinical specimens (blood, CSF, wound or pus, lower respiratory tract, urinary, and other sterile isolates) of patients admitted at unselected hospital departments. The resistance levels for hospital departments are shown in table 4.3.2.2 and figure 4.3.2.1 for *E. coli*, *K. pneumoniae*, *E. cloacae, P. mirabilis*, *P. aeruginosa*, and *Acinetobacter* spp, in table 4.3.2.3 for *Enterococcus* spp (table only), and in table 4.3.2.4 and figure 4.3.2.2 for *S. aureus*. In Dutch hospital departments, the main part of infections is cultured for susceptibility testing. Therefore, bias because of selective culturing will be limited or non-existing.

Table 4.3.2.1. Distribution of isolated pathogens N (%) in clinical specimens from unselected hospital departments, ISIS-AR 2013

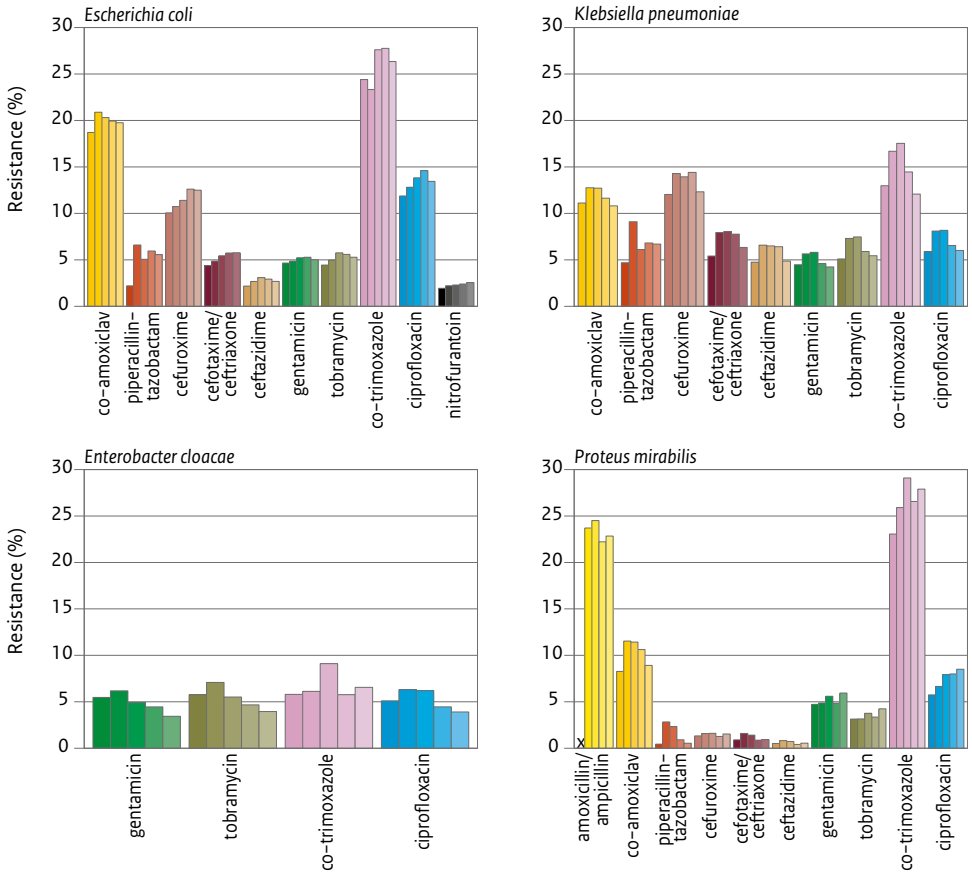
Pathogen	Blood N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or Pus N (%)	Other sterile specimens N (%)
E. coli	3041 (24)	1276 (15)	16099 (45)	3902 (16)	10 (4)
K. pneumoniae	449 (4)	449 (5)	2550 (7)	655 (3)	1 (0)
P. mirabilis	200 (2)	251 (3)	2625 (7)	882 (4)	0 (0)
E. cloacae	181 (1)	439 (5)	830 (2)	855 (4)	0 (0)
P. aeruginosa	287 (2)	1352 (15)	1752 (5)	1340 (6)	3 (1)
Acinetobacter spp	49 (0)	104 (1)	178 (1)	197 (1)	0 (0)
E. faecalis	383 (3)	50 (1)	3706 (10)	1484 (6)	8 (3)
E. faecium	253 (2)	35 (0)	1134 (3)	870 (4)	3 (1)
S. aureus	1484 (12)	1605 (18)	1082 (3)	6259 (26)	31 (13)
CNS	4020 (32)	30 (0)	872 (2)	2370 (10)	111 (46)
Other Enterobacteriaceae*	490 (4)	1150 (13)	2876 (8)	1949 (8)	3 (1)
Other non-fermenters**	21 (0)	504 (6)	123 (0)	216 (1)	1 (0)
Other gram-positives	1787 (14)	1509 (17)	1739 (5)	3141 (13)	72 (30)

* *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*

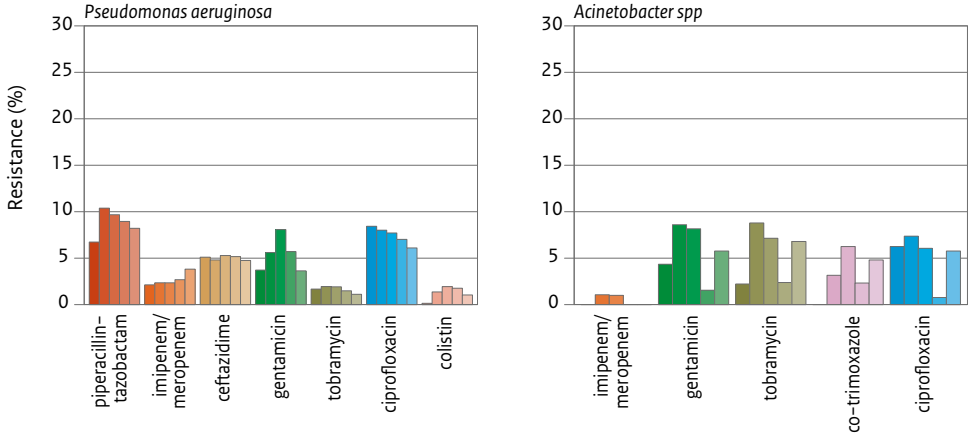
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Figure 4.3.2.1. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis* from unselected hospital departments.
An 'X' indicates no data available in that 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 2009 to 2013) among clinical isolates of *P. aeruginosa* and *Acinetobacter spp* from unselected hospital departments.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Table 4.3.2.2. Resistance levels among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *Acinetobacter* spp. from unselected hospital departments, ISIS-AR 2013

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter spp.*
Antibiotic						
amoxicillin/ ampicillin	47	-	-	23	-	-
co-amoxiclav	20	11	-	9	-	-
piperacillin-tazobactam	6	7	-	1	8	-
imipenem/ meropenem	0	0	0	0	4	0
cefuroxime	12	12	-	2	-	-
cefotaxime/ ceftriaxone	6	6	-	1	-	-
ceftazidime	3	5	-	1	5	-
gentamicin	5	4	3	6	4	6
tobramycin	5	5	4	4	1	7
co-trimoxazole	26	12	7	28	-	5
ciprofloxacin	13	6	4	9	6	6
nitrofurantoin	3	-	-	-	-	-
colistin	-	-	-	-	1	-
Empiric therapy combinations						
gentamicin + amoxicillin/ ampicillin	5	-	-	5	-	-
gentamicin + co-amoxiclav	3	3	-	2	-	-
gentamicin + cefuroxime	2	3	-	0	-	-
gentamicin + cefotaxime/ ceftriaxone	1	3	-	0	-	-
gentamicin + ceftazidime	1	2	-	0	1	-
gentamicin + piperacillin-tazobactam	1	2	-	0	2	-
Multi-drug resistance						
HRMO*	8	8	2	4	1	4

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

- *Resistance not calculated*

* *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_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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Table 4.3.2.3. Resistance levels among clinical isolates of *E. faecalis* and *E. faecium* from unselected hospital departments, ISIS-AR 2013

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

Table 4.3.2.4. Resistance levels among clinical isolates of *S. aureus* from unselected hospital departments, ISIS-AR 2013

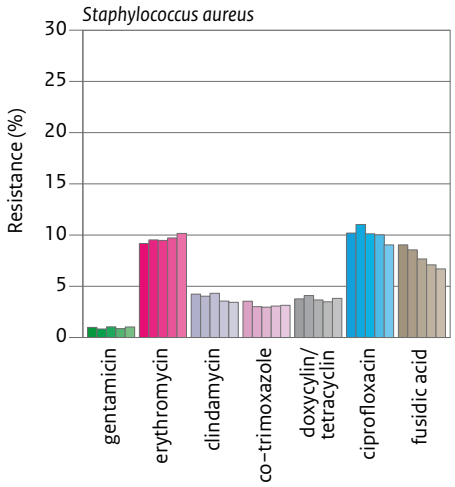
	S. aureus	
Antibiotic		
MRSA*	2	
gentamicin	1	
erythromycin	10	
clindamycin	3	
co-trimoxazole	3	
doxycyclin/ tetracyclin	4	
ciprofloxacin	9	
rifampicin	0	
fusidic acid	7	

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

* *The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2) or, if these tests were lacking, resistance to flucloxacillin, methicillin, oxacillin, or cefoxitin screentest.*

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Figure 4.3.2.2. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *S. aureus* from unselected hospital departments.



Key results

Enterobacteriaceae

- Overall, resistance to imipenem/meropenem (<0.5%), cefotaxime/ceftriaxone (≤6%), ceftazidime (≤5%), gentamicin (≤6%), tobramycin (≤5%), and nitrofurantoin (*E. coli* only; 3%) remained low.
- Resistance to amoxicillin/ampicillin remained high for *E. coli* and *P. mirabilis* (>20%).
- Resistance to co-amoxiclav was high in 2013 and there was no significant time trend over 5 years (2009 to 2013). However, resistance levels to co-amoxiclav decreased since 2010 (for all Enterobacteriaceae: $p_{trend\ 2010\text{-}2013}$ <0.01).
- Resistance to co-trimoxazole was high and increasing for *E. coli* and *P. mirabilis*. However, resistance to co-trimoxazole strongly decreased for *K. pneumoniae* since 2011 (from 18% in 2011 to 12% in 2013).
- Resistance to most common empiric therapy combinations remained low (≤5%), although for *E. coli* there was a significant increasing trend for almost all tested combinations.

E. coli

- There was a significantly increasing time trend in resistance to almost all tested agents, that was not visible in such extent among the other Enterobacteriaceae.
- Although the percentage of HRMO significantly increased since 2009, it remained stable at ~8% since 2011.

K. pneumoniae

- In contrast to the increasing time trends over 2008 to 2012 described in Nethmap 2013, resistance to most agents showed no significant time trend over the years 2009 to 2013. Additionally, resistance was lower in 2013 than in 2012.

E. cloacae

- Resistance to most tested agents (gentamicin, tobramycin, and ciprofloxacin) showed a decreasing time trend (all p_{trend}<0.05).

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P. mirabilis

- Resistance to most agents, including empiric therapy combinations, remained low (≤5%), although resistance to gentamicin, tobramycin, co-trimoxazol, and ciprofloxacin showed a significantly increasing trend.

P. aeruginosa

- Resistance to all tested agents was below 9%.
- Although there was no significant time trend over 5 years, resistance to piperacilline-tazobactam decreased from 10% in 2010 to 8% in 2013 ($p_{trend\ 2010\text{-}2013}$<0.001).
- Resistance to imipenem/meropenem increased from 2% in 2009 to 4% in 2013.
- Resistance to ciprofloxacin significantly decreased since 2009 (8% in 2009 to 6% in 2013).

Acinetobacter spp.

- Resistance to all tested agents remained low (≤7%).

***Enterococcus* spp.**

- Resistance to vancomycin remained rare (<0.5%).

S. aureus

- Resistance to all tested antibiotics was low (≤10%).
- The percentage of MRSA positive isolates remained stable around 1.8%.
- Resistance levels to erythromycin increased since 2009 (from 9% in 2009 to 10% in 2013), whereas resistance levels to clindamycin, ciprofloxacin, and fusidic acid showed a significant decrease (all p_{trend}<0.01).

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4.3.3 Intensive care units

Table 4.3.3.1 shows the distribution of pathogens from clinical specimens (blood, CSF, wound or pus, lower respiratory tract, urinary, and other sterile isolates) of patients admitted at intensive care units. The resistance levels for intensive care units are shown in table 4.3.3.2 and figure 4.3.3.1 for *E. coli*, *K. pneumoniae*, *E. cloacae, P. mirabilis*, and *P. aeruginosa*, in table 4.3.3.3 for *Enterococcus* spp (table only), and in table 4.3.3.4 and figure 4.3.3.2 for *S. aureus* and coagulase negative staphylococci. In Dutch intensive care units, pathogens from almost all infections are cultured for susceptibility testing. The occurrence of selection bias in the results below is therefore unlikely.

Table 4.3.3.1. Distribution of isolated pathogens N (%) in clinical specimens from intensive care units, ISIS-AR 2013

Pathogen	Blood N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or Pus N (%)	Other sterile specimens
E. coli	391 (14)	746 (16)	884 (42)	720 (19)	0 (0)
K. pneumoniae	82 (3)	260 (6)	119 (6)	120 (3)	1 (1)
P. mirabilis	32 (1)	151 (3)	141 (7)	101 (3)	1 (1)
E. cloacae	39 (1)	269 (6)	57 (3)	141 (4)	0 (0)
P. aeruginosa	62 (2)	381 (8)	131 (6)	248 (7)	1 (1)
Acinetobacter spp.	9 (0)	67 (1)	11 (1)	24 (1)	1 (1)
E. faecalis	119 (4)	100 (2)	214 (10)	409 (11)	0 (0)
E. faecium	251 (9)	210 (5)	183 (9)	531 (14)	3 (4)
S. aureus	211 (7)	878 (19)	75 (4)	313 (8)	9 (13)
CNS	1226 (43)	32 (1)	59 (3)	449 (12)	24 (34)
Other Enterobacteriaceae*	120 (4)	834 (18)	153 (7)	362 (10)	4 (6)
Other non-fermenters**	8 (0)	223 (5)	8 (0)	33 (1)	2 (3)
Other gram-positives	289 (10)	479 (10)	55 (3)	292 (8)	25 (35)

* *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 2013

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ ampicillin	48	-	-	24	-
co-amoxiclav	22	13	-	12	-
piperacillin-tazobactam	8	9	-	0	12
imipenem/ meropenem	0	0	0	0	6
cefuroxime	16	20	-	2	-
cefotaxime/ ceftriaxone	8	11	-	2	-
ceftazidime	3	8	-	2	9
gentamicin	5	9	9	6	5
tobramycin	6	10	11	4	2
co-trimoxazole	26	16	8	26	-
ciprofloxacin	13	7	7	6	7
colistin	-	-	-	-	1
Empiric therapy combinations					
gentamicin + amoxicillin/ ampicillin	5	-	-	3	-
gentamicin + co-amoxiclav	3	7	-	1	-
gentamicin + cefuroxime	3	7	-	0	-
gentamicin + cefotaxime/ ceftriaxone	2	7	-	0	-
gentamicin + ceftazidime	1	4	-	0	2
gentamicin + piperacillin-tazobactam	1	5	-	0	3
tobramycin + ciprofloxacin	-	-	-	-	2
tobramycin + ceftazidim	-	-	-	-	2
Multi-drug resistance					
HRMO*	10	13	3	5	1

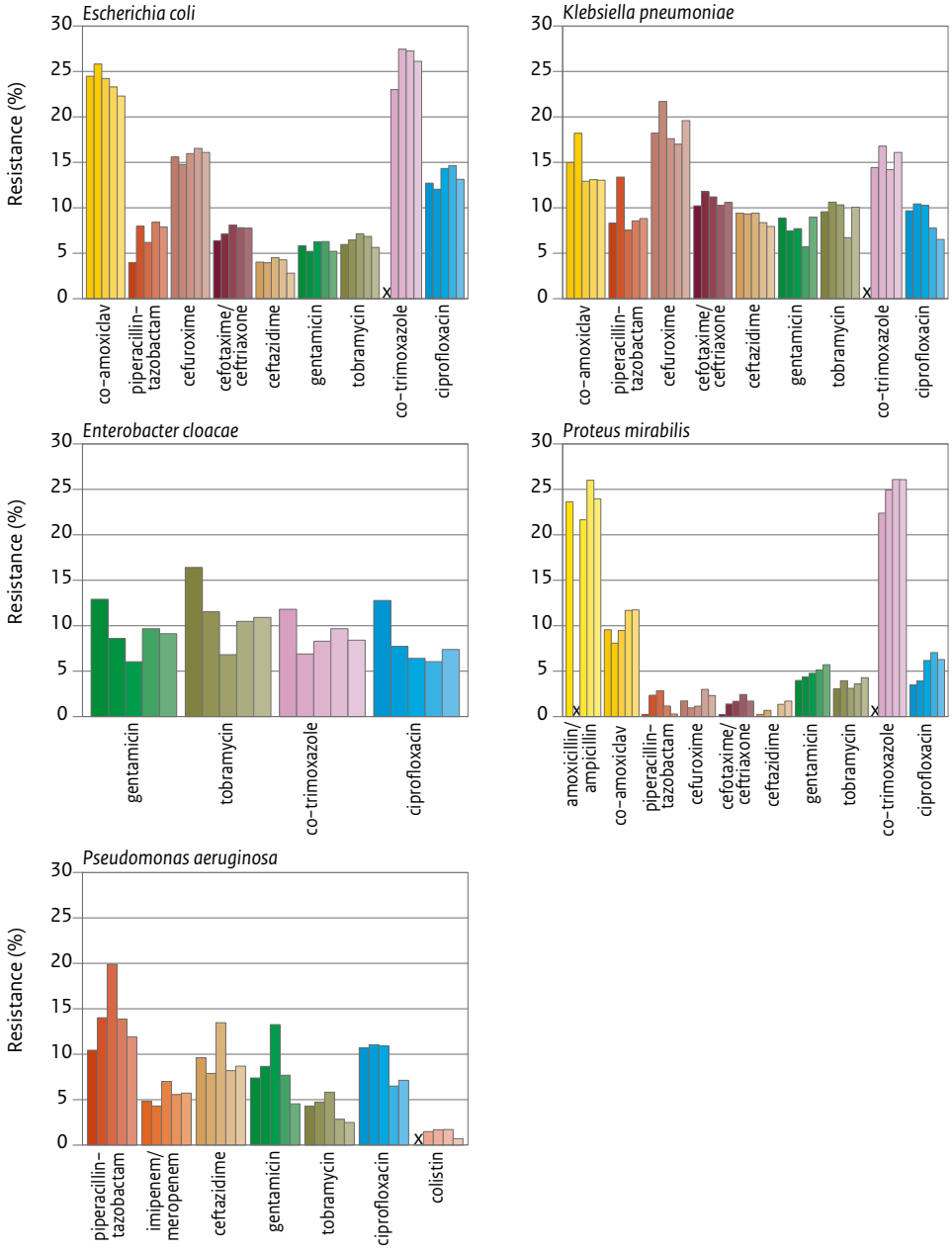
red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

- *Resistance not calculated*

* *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_WIP); for all Enterobacteriaceae except E. cloacaeas resistant to cefotaxim/ceftriaxone or ceftazidim as indicator compounds for the production of Extended-Spectrum Beta-Lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides. For E. cloacae as resistant to both fluoroquinolones and aminoglycosides. For P. aeruginosa as resistant ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

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Figure 4.3.3.1. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from intensive care units.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Table 4.3.3.3. Resistance levels among clinical isolates of *E. faecalis* and *E. faecium* from intensive care units, ISIS-AR 2013

	E. faecalis	*E. faecium*	
Antibiotic			
amoxicillin/ ampicillin	-	91	
vancomycin	0	1	

Table 4.3.3.4. Resistance levels among clinical isolates of *S. aureus* and coagulase negative staphylococci from intensive care units, ISIS-AR 2013

	S. aureus	*CNS*	
Antibiotic			
MRSA*	3	-	
gentamicin	2	-	
erytromycine	10	-	
clindamycine	3	-	
co-trimoxazole	3	-	
doxycycline/ tetracycline	4	-	
ciprofloxacin	7	-	
rifampicine	0	-	
linezolid	0	0	
vancomycin	-	0	

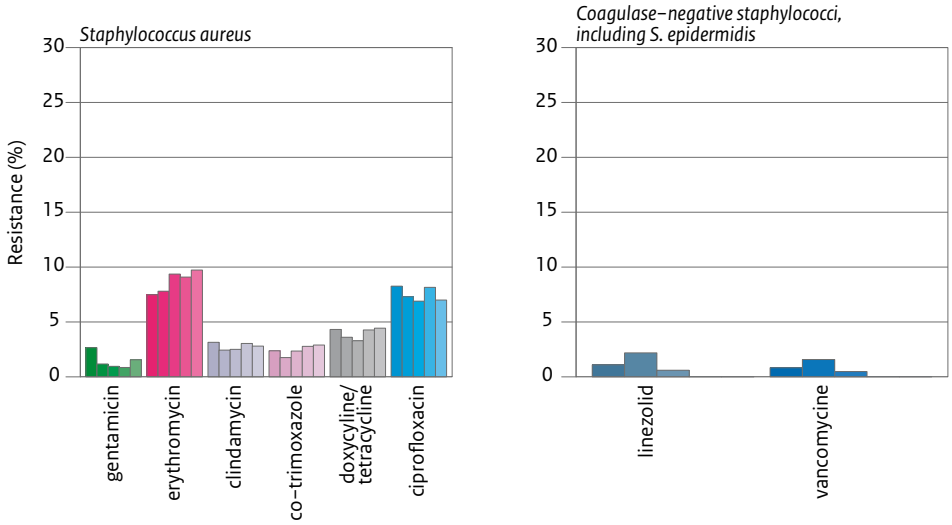
red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

- *Resistance not calculated.*
  CNS = Coagulase-negative staphylococci, including S. epidermidis

* *The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2) or, if these tests were lacking, resistance to flucloxacillin, methicillin, oxacillin, or cefoxitin screentest.*

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Figure 4.3.3.2. Trends in antibiotic resistance (2009-2013) among clinical isolates of *S. aureus* and coagulase negative staphylococci from intensive care units.



Key results

Enterobacteriaceae

- Overall, resistance to imipenem/meropenem (<0.5%), ceftazidime (≤8%), gentamicin (≤9%) and the empiric therapy combinations remained low.
- Resistance to amoxicillin/ampicillin(>20%) and co-trimoxazol (except for *E. cloacae*) was high (>16%).

E. coli

- Resistance to piperacillin-tazobactam significantly increased from 4% in 2009 to 8% in 2013.
- Resistance to most other tested agents and the empiric therapy combinations did not show a significant time trend. Additionally, the percentage of HRMO (10%) was comparable to previous years.

K. pneumoniae

- Although resistance to gentamicin had decreased from 2008 to 2012, the resistance percentage of 2013 was higher than in 2012 (9% in 2013 compared to 6% in 2012).
- Resistance to ciprofloxacin significantly decreased from 10% in 2009 to 7% in 2013.
- The percentage of HRMO (13%) was comparable to previous years.

E. cloacae

- There was a decrease in resistance to ciprofloxacin from 13% in 2009 to 7% in 2013.
- The percentage of HRMO strongly decreased from 11% in 2009 to 3% in 2013.

P. mirabilis

- Resistance to 3rd generation cephalosporins showed a significant increasing trend (from 0.2% in 2009 to 1.7% in 2013). Additionally, resistance to ciprofloxacin increased from 3% in 2009 to 6% in 2013, which was reflected by an increase in HRMOs from 1% in 2009 to 5% in 2013.

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P. aeruginosa

- Resistance to all tested antibiotics was low (≤9%), except for piperacillin-tazobactam, for which the level of resistance was 12%.
- Resistance to tobramycin and ciprofloxacin significantly decreased since 2009 (from 4% in 2009 to 2% in 2013 for tobramycin, and from 11% to 7% for ciprofloxacin).
- The percentage of HRMO was low (1%).

***Enterococcus* spp.**

- Resistance to vancomycin remained rare (<0.5%).

S. aureus

- Resistance to all tested agents was below 10%.
- The percentage of MRSA remained stable at 3%.
- Resistance to erythromycin increased from 7% in 2009 to 10% in 2013.

Coagulase-negative staphylococci

- Resistance to both linezolid and vancomycin remained rare (<0.5%).

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4.3.4 Blood isolates in unselected hospital departments and intensive care units

Table 4.3.4.1 shows the distribution of pathogens from blood of patients admitted at unselected hospital departments and intensive care units. The resistance levels for blood isolates are shown in table 4.3.4.2 and figure 4.3.4.1 for *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa*, in table 4.3.4.3 for *Enterococcus* spp (table only), and in table 4.3.4.4 and figure 4.3.4.2 for *S. aureus* and coagulase negative staphylococci. In most hospitals blood specimens are cultured from patients with a body temperature of >38.5. Selection bias of the results presented below by selective sampling is therefore highly unlikely.

Table 4.3.4.1. Distribution of pathogens N (%) in clinical blood isolates from unselected hospital departments and intensive care units, ISIS-AR 2013

Pathogen	Blood N (%)
E. coli	3397 (22)
K. pneumoniae	519 (3)
P. mirabilis	230 (2)
E. cloacae	217 (1)
P. aeruginosa	344 (2)
Acinetobacter spp.	57 (0)
E. faecalis	497 (3)
E. faecium	495 (3)
S. aureus	1660 (11)
CNS	5206 (34)
Other Enterobacteriaceae*	602 (4)
Other non-fermenters**	29 (0)
Other gram-positives	2071 (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 unselected hospital departments and intensive care units, ISIS-AR 2013

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic					
amoxicillin/ ampicillin	48	-	-	21	-
co-amoxiclav	20	10	-	7	-
piperacillin-tazobactam	6	6	-	0	7
imipenem/ meropenem	0	0	0	0	4
cefuroxime	13	14	-	3	-
cefotaxime/ ceftriaxone	6	7	-	1	-
ceftazidime	3	5	-	0	5
gentamicin	5	6	4	4	3
tobramycin	6	7	5	4	1
co-trimoxazole	28	13	5	22	-
ciprofloxacin	15	8	3	8	6
colistin	-	-	-	-	0
Empiric therapy combinations					
gentamicin + amoxicillin/ ampicillin	5	-	-	3	-
gentamicin + co-amoxiclav	2	4	-	1	-
gentamicin + cefuroxime	2	4	-	0	-
gentamicin + cefotaxime/ ceftriaxone	2	4	-	0	-
gentamicin + ceftazidime	1	3	-	0	1
gentamicin + piperacillin-tazobactam	1	2	-	0	1
tobramycin + ciprofloxacin	-	-	-	-	0
tobramycin + ceftazidim	-	-	-	-	0
Multi-drug resistance					
HRMO*	9	9	1	4	1

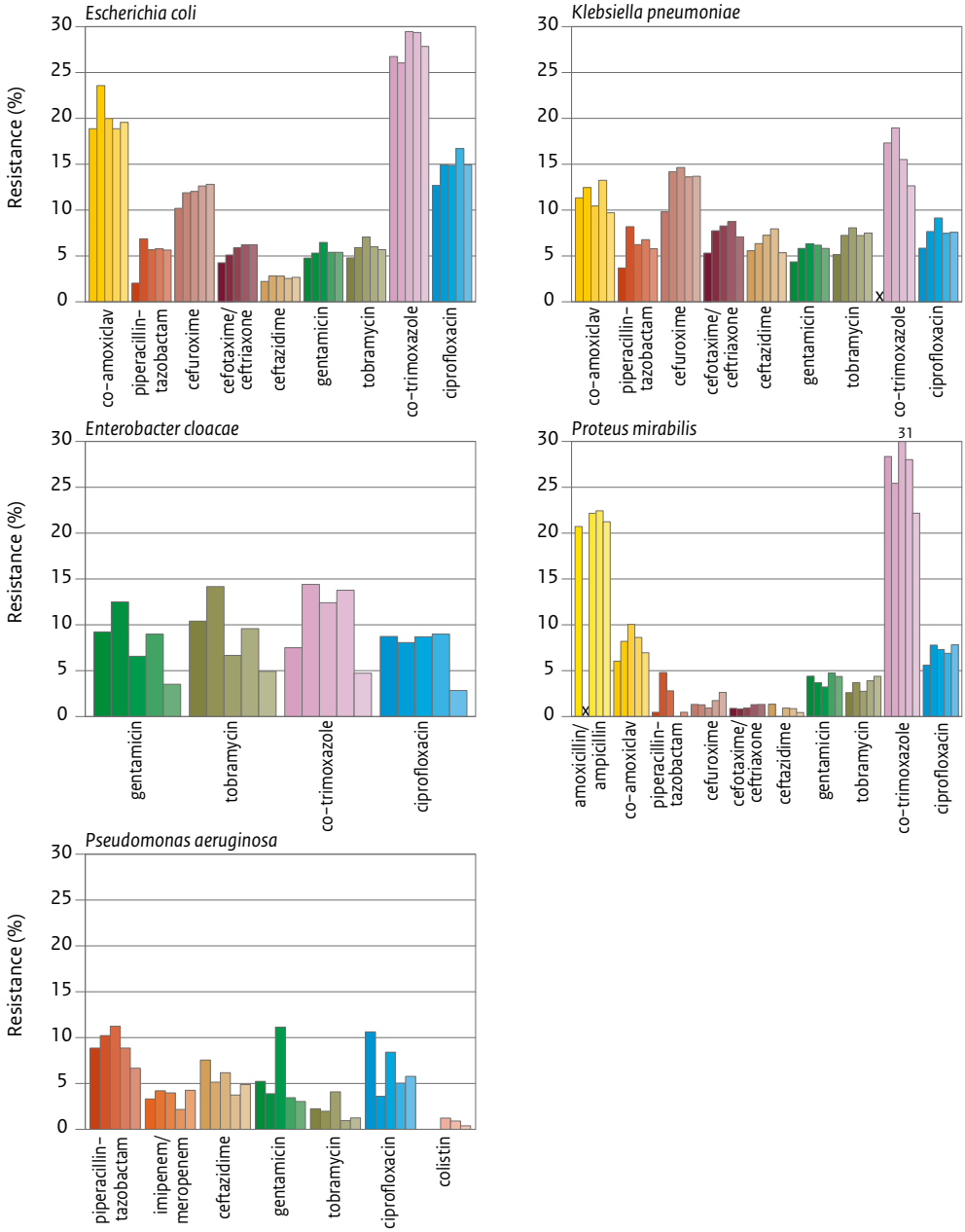
red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

- *Resistance not calculated*

* *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_WIP); for all Enterobacteriaceae except E. cloacaeas resistant to cefotaxim/ceftriaxone or ceftazidim as indicator compounds for the production of Extended-Spectrum Beta-Lactamase (ESBL) or resistant to both fluoroquinolones and aminoglycosides. For E. cloacae as resistant to both fluoroquinolones and aminoglycosides. For P. aeruginosa as resistant ≥3 agent per category/agent of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime and piperacillin/piperacillin-tazobactam.*

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Figure 4.3.4.1. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from clinical blood isolates from unselected hospital departments and intensive care units.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Table 4.3.4.3. Resistance levels among clinical blood isolates of *E. faecalis* and *E. faecium* from unselected hospital departments and intensive care units, ISIS-AR 2013

	E. faecalis	*E. faecium*	
Antibiotic			
amoxicillin/ ampicillin	-	90	
vancomycin	0	2	

Table 4.3.4.4. Resistance levels among clinical blood isolates of *S. aureus* from unselected hospital departments and intensive care units, ISIS-AR 2013

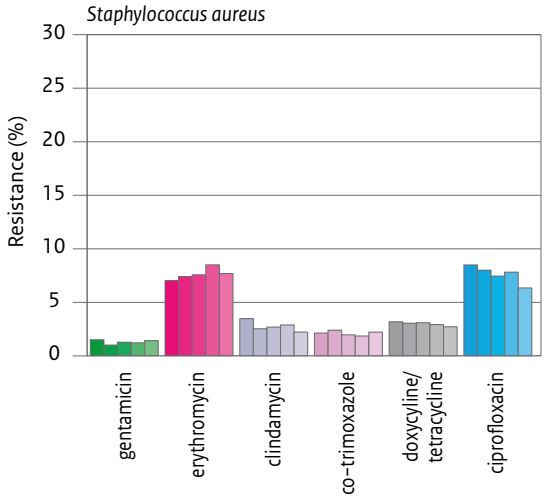
	S. aureus
Antibiotic	
MRSA*	1
gentamicin	1
erytromycine	8
clindamycine	2
co-trimoxazole	2
doxycycline/ tetracycline	3
ciprofloxacin	6
rifampicine	0
linezolid	0

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

* *The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2) or, if these tests were lacking, resistance to flucloxacillin, methicillin, oxacillin, or cefoxitin screentest.*

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Figure 4.3.4.2. Trends in antibiotic resistance (from left to right 2009 to 2013) among clinical blood isolates of *S. aureus* from unselected hospital departments and intensive care units.



Key results

Enterobacteriaceae and *P. aeruginosa*

- Resistance levels were similar to resistance levels as described in 4.3.2 and 4.3.3 for all materials combined in unselected hospital departments and intensive care units. There were some small differences, such as for co-trimoxazol and ciprofloxacin where resistance is somewhat higher in *E. coli* isolated from blood (28% in blood versus 26% in all materials for co-trimoxazol and 15% versus 13% for ciprofloxacin).
- There is an increasing trend in resistance for most agents in *E. coli*, while resistance remained stable among the other Enterobacteriaceae and *P. aeruginosa*.

Enterococci

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

S. aureus

- Resistance levels and time trends 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 hospital departments (HD). The resistance levels for the outpatient departments are shown in tables 4.3.5.2 and 4.3.5.3 and figure 4.3.5.1 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *E. faecalis* (table only), separately.

Table 4.3.5.1. Distribution of isolated pathogens N (%) in urine from urology outpatient departments (OPD) and urology hospital departments (HD), ISIS-AR 2013

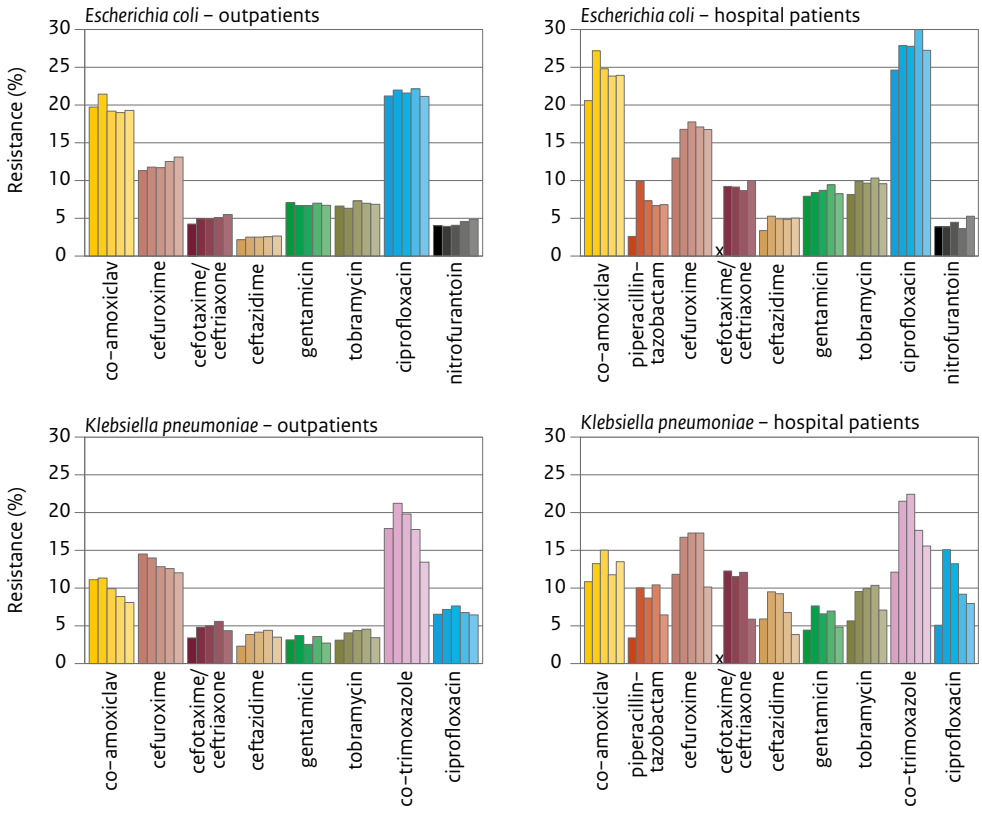
Pathogen	OPD N (%)	HD N (%)
E. coli	10811 (44)	1384 (34)
K. pneumoniae	1801 (7)	264 (6)
P. mirabilis	1307 (5)	234 (6)
P. aeruginosa	802 (3)	220 (5)
E. faecalis	2485 (10)	515 (13)
Other Enterobacteriaceae*	2693 (11)	598 (15)
Other non-fermenters**	354 (1)	93 (2)
Other *Enterococcus spp.*	955 (4)	220 (5)
Other gram-positives	3500 (14)	543 (13)

* *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*

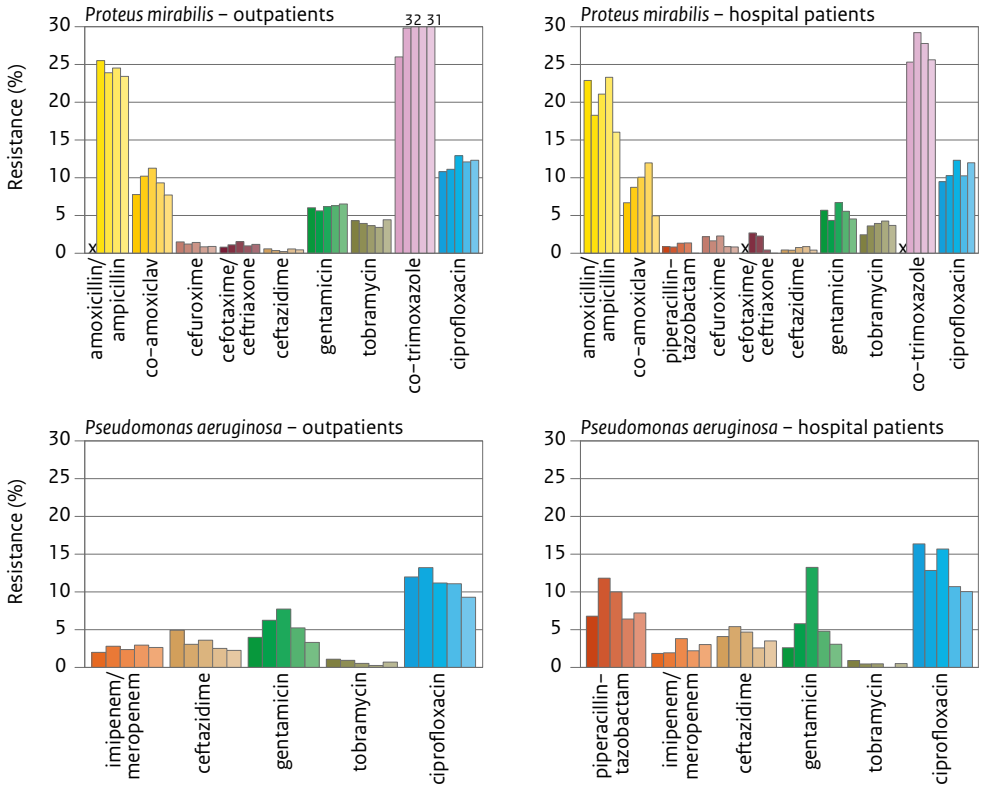
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Figure 4.3.5.1. Trends in antibiotic resistance (from left to right 2009-2013) among urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments and urology hospital departments.
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 2009-2013) among urinary isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments and urology hospital departments.
An 'X' indicates no data available in that year or a percentage of interpretable reported MICs below 80%.



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Table 4.3.5.2. Resistance levels among urinary isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from urology outpatient departments (OPD) and urology hospital departments (HD), ISIS-AR 2013

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	HD	OPD	HD	OPD	HD	OPD	HD
Antibiotic								
amoxicillin/ ampicillin	49	54	-	-	23	16	-	-
co-amoxiclav	19	24	8	13	8	5	-	-
piperacillin-tazobactam	-	7	-	6	-	0	-	7
imipenem/ meropenem	0	0	0	0	0	0	3	3
cefuroxime	13	17	12	10	1	1	-	-
cefotaxime/ ceftriaxone	6	10	4	6	1	0	-	-
ceftazidime	3	5	3	4	0	0	2	4
gentamicin	7	8	3	5	7	5	3	3
tobramycin	7	10	3	7	4	4	1	1
co-trimoxazole	32	36	13	16	31	26	-	-
ciprofloxacin	21	27	6	8	12	12	9	10
nitrofurantoin	5	5	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ ampicillin	6	8	-	-	5	3	-	-
gentamicin + co-amoxiclav	3	4	1	4	2	2	-	-
gentamicin + cefuroxime	3	4	2	3	0	0	-	-
gentamicin + cefotaxime/ ceftriaxone	2	3	1	3	0	0	-	-
gentamicin + ceftazidime	1	2	1	2	0	0	0	0
gentamicin + piperacillin-tazobactam	-	1	-	2	-	0	-	0
Multi-drug resistance								
HRMO*	10	15	6	8	4	3	0	0
multidrug-resistance**	6	-	2	-	2	-	-	-

red	Significantly increasing since 2009
green	Significantly decreasing since 2009
black	No significant time trend or no test for trend conducted

- *Resistance not calculated*
* *Highly Resistant Micro-Organism (HRMO), defined according to HRMO guideline of the WIP (http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectiepreventie_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 to ≥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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Table 4.3.5.3. Resistance levels among urinary isolates of *E. faecalis* from urology outpatient departments (OPD) and urology hospital departments (HD), ISISAR 2013

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

Key results

Enterobacteriaceae

- In general, resistance to all tested agents was higher in patients of urology hospital departments than in patients of urology outpatient departments.
- Many tested agents showed low resistance levels: piperacillin-tazobactam (≤7%), imipenem/meropenem (0%), cefotaxime/ceftriaxone (≤10%), ceftazidime (≤5%), gentamicin (≤8%), and tobramycin (≤10%).
- Low resistance was also found for nitrofurantoin (5%) in *E. coli,* for ciprofloxacin (≤8%) in *K. pneumoniae,* and for co-amoxiclav (≤8%), and cefuroxime (1%) in *P. mirabilis.*
- For *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*, all resistance levels were stable or decreased over time in hospital departments.
- Multidrug resistance to all of the following three oral agents, co-trimoxazole, co-amoxiclav and ciprofloxacin, was ≤6% among OPD.

E. coli

- Resistance levels in outpatient departments for several antibiotics significantly increased, whereas for co-amoxiclav resistance significantly decreased. However, there were only small differences in resistance percentages over time (<2%).
- In hospital departments, there was an increasing trend in resistance to piperacillin-tazobactam, cefuroxime, co-trimoxazole, and ciprofloxacin.
- Resistance to piperacillin-tazobactam decreased from 10% in 2010 to 7% in 2013.
- The percentage of HRMO increased from 11% in 2009 to 15% in 2013 in hospital departments.

K. pneumoniae

- Resistance levels were in general lower in 2013 than in 2012. There was a substantial decrease among isolates of hospitalized patients for the cephalosporins and co-trimoxazole (1-3% absolute difference between 2012 and 2013).

P. mirabilis

- Resistance to co-amoxiclav decreased from 12% in 2012 to 5% in 2013 among patients of hospital departments.

P. aeruginosa

- Resistance to all tested agents was below 10%.
- Resistance to ciprofloxacin in both patients of outpatient- and hospital departments decreased (from 12% to 9% in OPD and from 16% to 10% in HD).
- The HRMO percentage remained low (≤0.5%).

Enterococcus spp

- Resistance to all tested agents was rare (≤1%).

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4.3.6. Respiratory pathogens

For the analyses of respiratory pathogens, resistance levels were analysed separately for general practitioners and hospitals. Table 4.3.6.1 shows the distribution of respiratory pathogens from all clinical specimens (blood, CSF, higher respiratory tract, and lower respiratory tract isolates combined) of patients from general practitioners. The resistance levels for general practitioners are shown in table 4.3.6.2. Table 4.3.6.3 and table 4.3.6.4 show the distribution and resistance levels for patients from outpatient departments, unselected hospital departments, and intensive care units combined.

Although patients from general practitioners are assumed to reflect the general resistance in the community, general practitioners do not routinely culture when lower respiratory tract infection is suspected. Therefore, the results may be biased towards a higher resistance by more severe or more resistant cases of respiratory tract infections. In Dutch hospitals, pathogens from respiratory tract infections are routinely cultured when lower respiratory tract infection is suspected. However, patients in hospitals may be more severely ill than those in the community and patients with chronic obstructive pulmonary diseases (COPD) may be overrepresented. Therefore, resistance levels may be biased towards a higher resistance and may not be representative for the community.

Table 4.3.6.1. Distribution of isolated respiratoiry pathogens N (%) from clinical specimens of general practitioners, ISIS-AR 2013

Pathogen	Blood N (%)	Lower respiratory tract N (%)
S. pneumoniae	0 (0)	92 (21)
H. influenzae	1 (100)	268 (60)
M. catarrhalis	0 (0)	88 (20)

Table 4.3.6.2. Resistance levels among isolated respiratory pathogens from general practitioners, ISIS-AR 2013

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
penicilline	0	-	-
amoxicillin/ ampicillin	-	15	-
co-amoxiclav	-	9	2
erytromycine	9	-	2
doxycycline	13	1	0
co-trimoxazole	-	15	5

\- *Resistance not calculated*

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Table 4.3.6.3. Distribution of isolated respiratoiry pathogens N (%) from clinical specimens of outpatient departments, unselected hospital departments and intensive care units, ISIS-AR 2013

Pathogen	Blood N (%)	Lower respiratory tract N (%)	Other sterile specimens N (%)
S. pneumoniae	525 (92)	1268 (28)	22 (85)
H. influenzae	43 (8)	2574 (56)	3 (12)
M. catarrhalis	3 (1)	756 (16)	1 (4)

Table 4.3.6.4 Resistance levels among isolated respiratory pathogens from clinical specimens of outpatient departments, unselected hospital departments and intensive care units, ISIS-AR 2013

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
penicilline	0	-	-
amoxicillin/ ampicillin	-	18	-
co-amoxiclav	-	7	1
erytromycine	9	-	4
doxycycline	8	3	2
co-trimoxazole	-	19	3

\- *Resistance not calculated*

Key results

S. pneumoniae

- Resistance to penicillin (0.4%) was still rare in the Netherlands.
- Resistance levels to erythromycin (9%) and doxycycline (8%) in hospitals were similar as reported in previous years, but resistance to doxycycline in patients from general practitioners was higher (13%).

H. influenzae

- Resistance to amoxicillin (18% in hospitals and 15% in GP) and co-trimoxazole (19% in hospitals and 15% in GP) remained high, whereas resistance to doxycycline (3% in hospitals and 1% in GP) remained low.
- Resistance to co-amoxiclav (7% in hospitals and 9% in GP) is higher than reported in Nethmap 2013 (4%).

M. catarrhalis

- Resistance to all tested agents was lower than 5% in hospitals and in patients from general practitioners.

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4.4 BRMO

4.4.1 Carbapenemase producing *Enterobacteriaceae* (CPE)

Hester Bootsma, Kim van der Zwaluw, Ellen Stobberingh, Leo Schouls

Carbapenems are broad spectrum β-lactam antibiotics that are highly resistant to hydrolysis by most β-lactamases. These antibiotics often are the last resort for treatment of bacterial infections with Gram-negative bacteria, particularly if these bacteria are producers of extended-spectrum β-lactamases (ESBL). However, in recent years Gram-negative bacteria producing β-lactamases that can hydrolyze carbapenems (carbapenemases) have been emerging. The worldwide spread of carbapenemase-producing bacteria may pose a considerable health threat.
In the majority of the cases, the gene encoding the carbapenemase is located on a plasmid together with other resistance genes. Consequently, a carbapenem-resistance gene together with its neighboring resistance genes can be easily exchanged between bacteria, even if they are of different species. As a result, carbapenem-resistant Gram-negative strains are often multi-drug resistant, leaving the nephrotoxic colistin as the only antibiotic for treatment.
There is a considerable number of different carbapenemases and allelic variants thereof. Some of these enzymes require metal ions and are therefore designated as metallo-β-lactamases such as IMP, VIM and NDM. Other well-known carbapenemases that do not require metals are the serine beta-lactamases OXA-48 and KPC.

The degree in which carbapenem-resistant Gram-negatives are circulating and the predominant classes and variants found among these strains in the Netherlands are unclear, although it is believed that their prevalence is still low. In addition, little is known about the transmission routes and potential reservoirs. For this reason, the National surveillance of Carbapenemase producing *Enterobacteriaceae* (CPE) in The Netherlands was started in 2010. The Dutch Society for Medical Microbiology (NVMM) advised medical microbiological laboratories to confirm the presence of carbapenemases in *Enterobacteriaceae* with a meropenem MIC >0.25 mg/L or an imipenem MIC >1 mg/L and to submit these isolates to a reference centre for phenotypic and genotypic confirmation.

The RIVM performed classical phenotypic assays to detect carbapenemase-activity and a multiplex-PCR targeting genes encoding IMP, VIM, NDM, OXA-48 and KPC carbapenemases and collected epidemiological data of the patients from whom the isolates were obtained.

In 2013, the RIVM received a total of 841 isolates, of which 366 were *Enterobacteriaceae* isolates, while the majority of the submitted isolates (475) consisted of non-fermenter isolates (although the surveillance aimed to collect *Enterobacteriaceae*) (Table 4.4.1.1). A large proportion of the *Enterobacteriaceae* isolates (119/366, 33%) had MICs for meropenem ≤0.25 mg/L and only 1.7% of these isolates yielded a PCR product. The proportion of PCR-positives increased with MIC, with 51.7% for isolates with MICs for meropenem >1 mg/L. The proportion of PCR-positive non-fermenter isolates was considerably lower: 13.1% for isolates with MICs for meropenem >1 mg/L.

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Table 4.4.1.1 Proportion of PCR-positive isolates among isolates received.

Meropenem MIC (mg/L)	n	Number PCR-pos. (%
Enterobacteriaceae		
≤ 0.25	119	2 (1.7)
>0.25 - ≤ 1	75	17 (22.7)
>1	172	89 (51.7)
All	366	108 (29.5)
Non-fermenters		
≤ 0.25	7	
>0.25 - ≤ 1	9	1 (11.1)
>1	459	60 (13.1)
All	475	61 (12.8)

The predominant species among the *Enterobacteriaceae* isolates with MICs for meropenem >1 mg/L were *K. pneumoniae* and *E. coli*, and OXA-48 was the most frequently found carbapenemase (Table 4.4.1.2).

Table 4.4.1.2 Percentage of PCR-positive isolates of the predominant *Enterobacteriaceae* and non-fermenter species with MICs for meropenem of >1 mg/L submitted during 2013.

	KPC	OXA-48	NDM	VIM	Totaal
K. pneumoniae	12	35	6	1	54
K. oxytoca				1	1
Escherichia coli		20	8	2	30
E. cloacae		4		2	6
E. aerogenes	1				1
C. freundii			1	1	2
Totaal	13	59	15	7	94

From all isolates received, 293 unique isolates were received from the same number of patients.. In 94 isolates we were able to demonstrate the presence of a carbapenemase producing enzyme: KPC (n=13), VIM(n=7) , OXA-48(n=59) , NDM(n=15), no IMP were found.

To obtain insight in the risk factors and spread of carbapenemase producing Enterobacteriaeciae additional epidemiological data is collected within the surveillance. Not all questions in the questionnaires were answered for each patient. For 8 patients hospitalization abroad was mentioned. The regions that were visited are often associated with the occurrence of carbapenemase-producing *Enterobacteriaceae* (India, Egypt, Morocco, Curacao, Jordania).

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In conclusion, as in 2012, OXA-48 was the most prevalent carbapenemase (63%). However, the analyses of the carbapenemase resistant isolates the RIVM received in 2013 show that we could not detect a carbapenemase gene by PCR in a considerable number of isolates with MICs for meropenem well above the epidemiological cut-off. The RIVM currently conducts next generation sequencing to identify the resistance mechanism in these isolates. This could be a carbapenemase gene or gene variant not detected by our PCR or other mechanisms such as porin-loss or up-regulated efflux pumps.
The increase in the number of isolates send in to the RIVM might be due to the increased awareness of the microbiologist to send in meropenem resistant isolates and /or a real increase in meropenem resistant isolates. Careful monitoring of meropenem resistant isolates remains important and all medical microbiologists are requested to send isolates for characterization of the mechanism of resistance to the RIVM/IDS.

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4.4.2 Vancomycin Resistant Enterococci in Dutch hospitals

Ellen Stobberingh and Rob Willems

As in previous years VRE outbreaks in various Dutch hospitals were frequently reported in 2013. There is no national, representative surveillance for VRE in the Netherlands. However, since May 2012 the UMC Utrecht offers molecular diagnostics on clinical VRE-isolates. From then on, 34 hospitals have sent 426 VRE isolates to the UMC Utrecht (status of June 1st 2014). These represented 218 strains carrying the *vanA* gene cluster, 205 the *vanB* gene cluster, 1 strain carried both the *vanA* and the *vanB* gene cluster and two isolates carried the *vanD* gene cluster. VRE positive for *vanD* have not been reported before in the Netherlands. Increasing numbers of *vanD* positive VRE could compromise proper molecular-based diagnostics of VRE since PCR-based diagnostics so far only include *vanA* and *vanB* specific primers. Of the 426 VRE, 385 were typed by MLST. This revealed a total of 26 different Sequence Types (STs), suggesting that at least 26 VRE clones circulated in Dutch hospitals. The sudden increase of VRE in Dutch hospitals can therefore not be attributed to the 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. These highly frequent STs include ST117 (17 hospitals), ST203 (15 hospitals), ST18 (11 hospitals) and ST78 (6 hospitals).

To investigate whether strains from different Dutch hospitals with the same ST and isolated within a period of 20 months were indeed clonally related, whole genome sequences of 23 ST117 isolates from 11 different hospitals were determined. Phylogenetic analysis using both a SNP-based (762 SNPs in a total alignment of 1.2 Mb) and an allele-based (inferring allelic differences using 2727 loci) approach revealed 4 distinct ST117 subclones. Subclone 1 included 14 isolates from 9 hospitals, subclone 2, 5 isolates from 3 hospitals, subclone 3, 2 isolates from a single hospital and subclone 4, one isolate. These data strongly indicate cross-transmission of strains (ST117 subclone 1 and 2) between different hospitals.

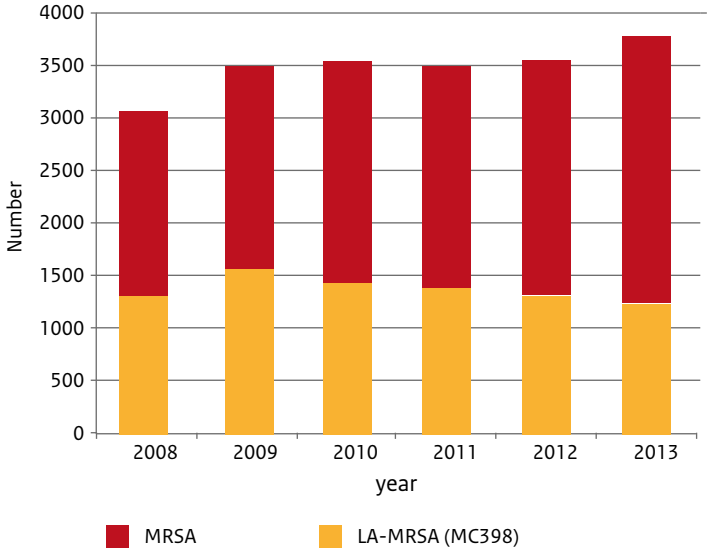
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4.4.3 Methicillin resistant *Staphylococcus aureus* (MRSA)

Thijs Bosch, Max Heck, Ellen Stobberingh, Leo Schouls

Despite the fact that the Netherlands is surrounded by countries with much higher MRSA rates, our country has retained its low MRSA prevalence, underlining the success of the Dutch 'search and destroy' policy. However, there has been a slight increase in the number of isolates sent to the RIVM for typing in the Dutch National MRSA surveillance program in 2013. This increase is remarkable as the number livestock associated MRSA (LA-MRSA) is slowly declining (Figure 1). The RIVM is studying the dynamics of LA-MRSA circulating in Dutch patients using both *spa* typing and MLVA typing.

Figure 4.4.3.1. Distribution of MRSA and LA-MRSA isolates submitted to the National MRSA surveillance in the years 2008-2013. LA-MRSA was defined as isolates belonging to the MLVA-complex MC398.



There were no remarkable changes in the distribution of the *spa*-types and MLVA-types compared to 2012. MLVA was a more discriminatory technique for MRSA and the top-10 MLVA-types comprise 29.7% of all MRSA in 2013 (Table 4). In contrast, the top-10 *spa*-types comprise 50.9% of the MRSA isolates. For LA-MRSA there was no difference in discriminatory power between both methods and in fact they lack sufficient resolution to type LA-MRSA.
MLVA-type MT1352 was the most prevalent MRSA type in 2013. This MLVA-type represents the dominant MLVA-type found among *spa*-type t1081 isolates, a type that has caused an outbreak among nursing homes. Despite its high prevalence, MT1352 was found predominantly as carriage isolate and has rarely been involved in disease.

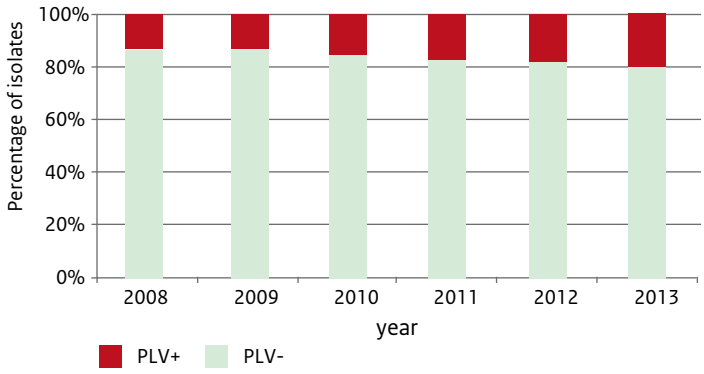
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Table 4.4.3.1 Distribution of the top-10 *spa*-types and MLVA-types among the MRSA and LA-MRSA isolates received for the Dutch National MRSA surveillance.

MRSA (n = 2640)				LA-MRSA (n = 1235)			
spa-type	Freq. %	MLVA-type	Freq. %	*spa*-type	Freq. %	MLVA-type	Freq. %
t008	14.6	1352	5.3	t011	59.8	398	56.3
t002	9.8	314	4.4	t034	13.4	569	12.5
t1081	7.6	240	4.1	t108	11.4	572	12.1
t019	3.5	212	2.9	t899	2.4	567	4.6
t127	3.2	489	2.2	t1457	2.1	565	2.6
t179	2.8	37	2.1	t1451	1.5	564	2.5
t223	2.8	195	2.1	t571	1.1	555	1.1
t032	2.3	491	2.1	t1456	0.8	566	1.1
t064	2.3	265	2.0	t1255	0.7	588	0.7
t437	2.0	22	1.5	t588	0.7	589	0.7
Other	49.1	Other	71.3	Other	5.9	Other	5.7

In recent years, there has been a significant increase of MRSA carrying the *lukF* gene (Figure 4.4.3.2). This gene, detected by PCR in the MLVA, is part of the gene cluster involved in production of the toxin Panton-Valentine leukocidin (PVL). MRSA expressing PVL are considered to be more virulent. Therefore, the RIVM is currently investigating the nature of and reasons for this increase.

Figure 4.4.3.2. Increase of PVL-positive MRSA isolates received for the Dutch National MRSA surveillance during 2008 - 2013.



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4.5. Resistance in specific pathogens

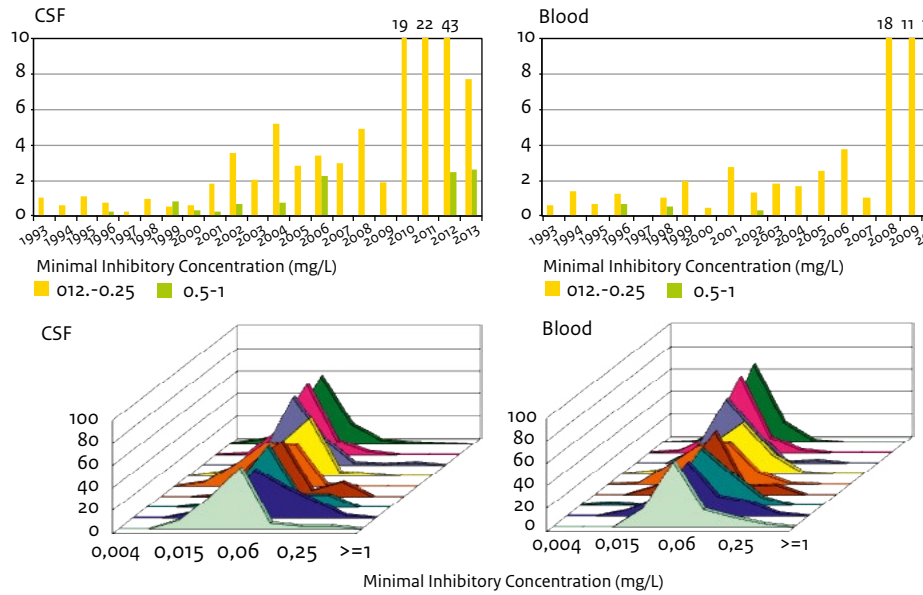
4.5.1. *Neisseria meningitidis*

Lodewijk Spanjaard and Arie van der Ende

From 1994-2013 a total of 4736 strains from cerebrospinal fluid (CSF) and 2972 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 and in 2013 in one strain from CSF and one from blood.
- 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; in 2013 these figures were 25% and 8%, respectively (figure 4.5.1).
- In 2013, a total of 14 moderately susceptible strains from blood and/or CSF belonged to serogroup B, one to serogroup C, four to serogroup Y and two to serogroup W. The two resistant strains belonged to serogroup B.
- One strain was resistant to rifampicin (also penicillin-resistant); no resistance to ceftriaxone was found.

Figure 4.5.1. Trends in penicilin resistance and MIC distributions of penicilin for *Neisseria meningitidis* from CSF (N = 4,736) and blood (N = 2,972). MIC data for 2007 are incomplete.



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- The interpretation of the phenotypic susceptibility testing might not be fully reliable, because the susceptible/moderately susceptible breakpoint is exactly at the peak of the susceptibility distribution (0.064 mg/l). As E-test, like most assays, is not 100% reproducible, this can give rise to a considerable number of minor and major interpretation errors. Therefore, the *penA* gene of the isolates from 2013 was sequenced.
- Alterations in the *penA* gene, associated with non-susceptibility to penicillin, were detected in 11 (10%) of the 106 strains.
- Apparently, E-test with EUCAST criteria yields more strains (21%) non-susceptible to penicillin than *penA* genotyping does (10%).
- 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.

***Neisseria meningitidis* - Conclusion**

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

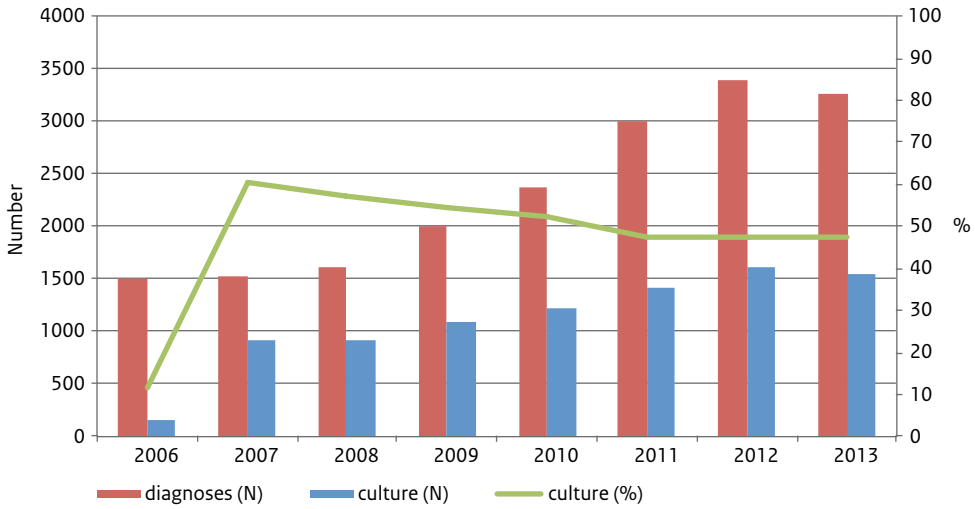
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4.5.2. *Neisseria gonorrhoeae*

Loes Soetens, Alje van Dam, Birgit van Benthem

The national project Gonococcal Resistance to Antimicrobials Surveillance (GRAS) started in 2006, collecting epidemiologic data on gonorrhea and resistance patterns of isolated strains from STI centers. The participating STI centers represent 89% of the total population of STI center attendees. Diagnosis of gonorrhea 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 8950 isolates was performed by E-test for penicillin, tetracycline, ciprofloxacin and cefotaxime; in 2011, ceftriaxone, azithromycin and spectinomycin were added to the panel and testing for penicillin and tetracyclin became optional. Resistance levels were calculated using the EUCAST breakpoints for resistance.

Figure 4.5.2.1. Diagnoses of gonorrhea in STI centers in the Netherlands since 2006.

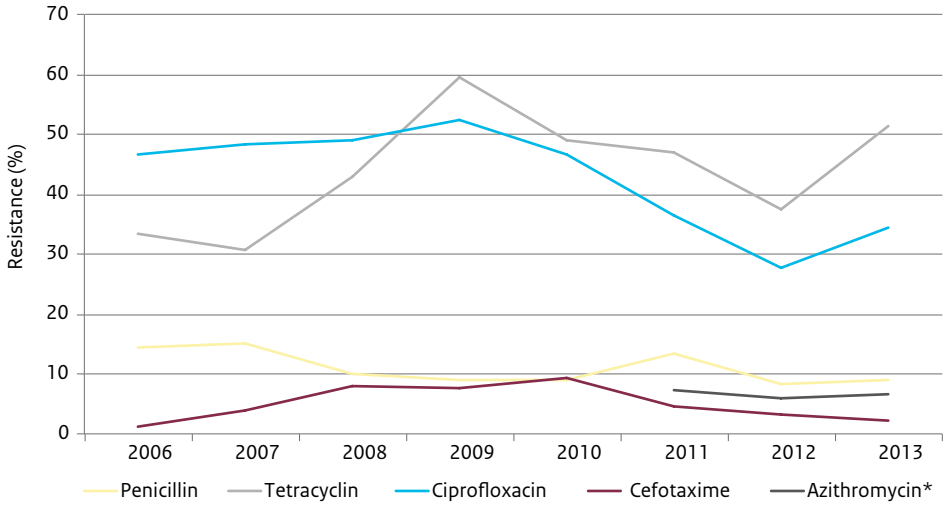


Results

- Resistance to tetracycline (51%) and ciprofloxacin (34%) decreased since 2009, but showed a sharp increase since last year. Resistance to cefotaxime (2%) decreased since 2010 and resistance to penicillin (9%) and azithromycin (7%) increased slightly since 2012. (Figure 4.5.2.2)
- No resistance was found to ceftriaxone and spectinomycin. (Figure 4.5.2.2)
- Cefotaxime resistance in 2013 was highest among heterosexual men (2.5%), patients who had sexual contact with commercial sex workers in the last 6 months (6.6%), and patients from Latin American (4.8%) or Turkish (7.1%) origin.
- MIC distributions of cefotaxime and ceftriaxone were both highly skewed to the right and showed a unimodal shape. (Figure 4.5.2.3a and b)

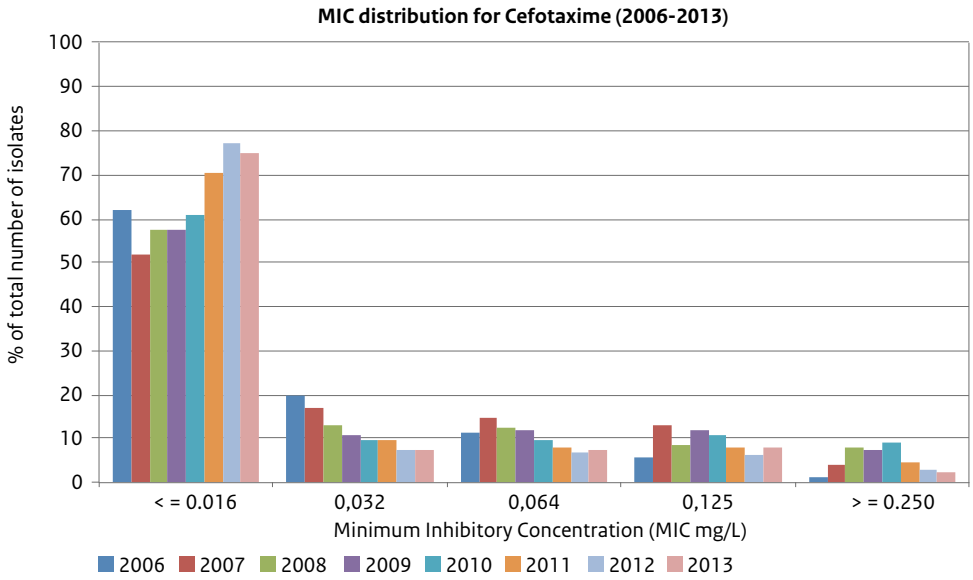
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Figure 4.5.2.2. Trends in antibiotic resistance among *Neisseria gonorrhoeae* (N = 8,950)
* Ceftriaxone, azithromycin and spectinomycin were added to the panel in 2011 and testing for penicillin and tetracycline became optional.



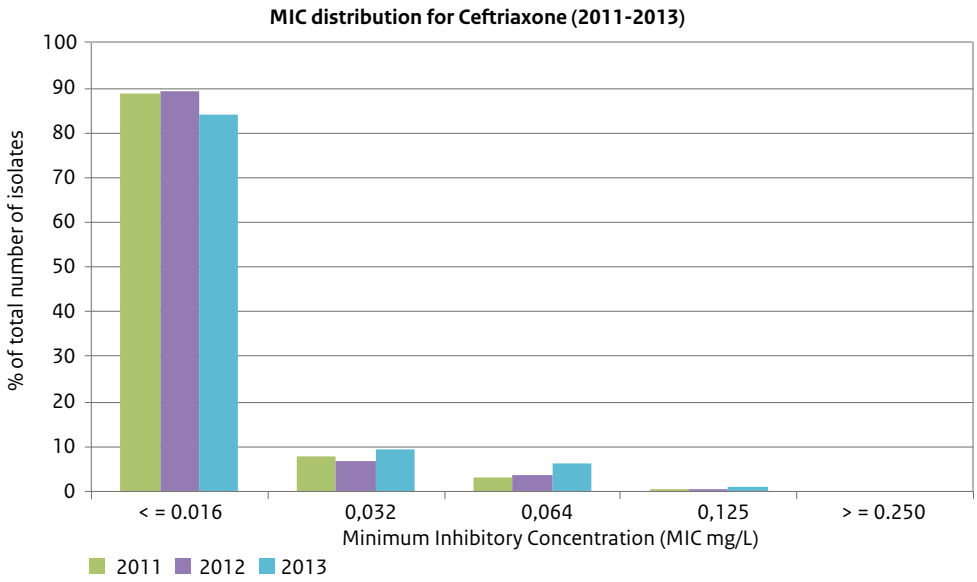
Footnote: No resistance was found for ceftriaxone and spectinomycin.

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



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Figure 4.5.2.3b. MIC distribution of cetriaxone for *Neisseria gonorrhoeae*



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4.5.3. *Mycobacterium tuberculosis*

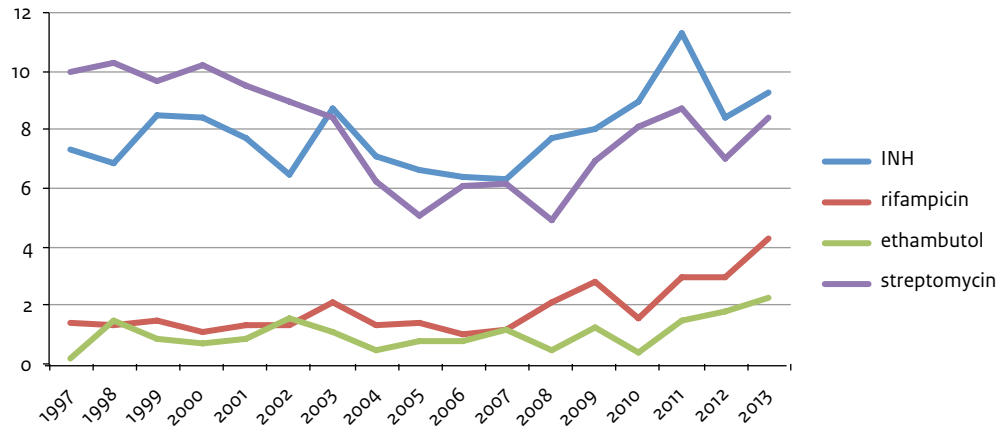
Miranda Kamst and Dick van Soolingen

- A total of 13544 strains of *M. tuberculosis* complex were obtained during 1998-2013. In 2013 we received 610 *M. tuberculosis* complex strains.
- INH resistance increased since 2008 to 11.3% in 2011, but decreased to 8.4% in 2012. In 2013 there was a small increase to 9.3 %. (figure 4.5.3.1).
- Rifampicin resistance increased from 1.2 % in 2007 to 4.3 % in 2013.
- Resistance to ethambutol remained low, fluctuating between 0.2% and 1.8%. In 2013 resistance increased to 2.3%.
- Streptomycin resistance decreased from 10.2% in 2000 to 4.9% in 2008, but has raised since then to 8.4 % in 2013.
- Combined resistance to more than one drug increased from 3.5% in 2010 to 5.7% in 2014 (figure 4.5.3.2), of which multidrug (MDR) resistance, at least to INH + rifampicin, was found in 3.9 % of the isolates and resistance to all four antimicrobial agents in 1.8 % in 2013. XDR-TB was not found.

***Mycobacterium tuberculosis* - Conclusion**

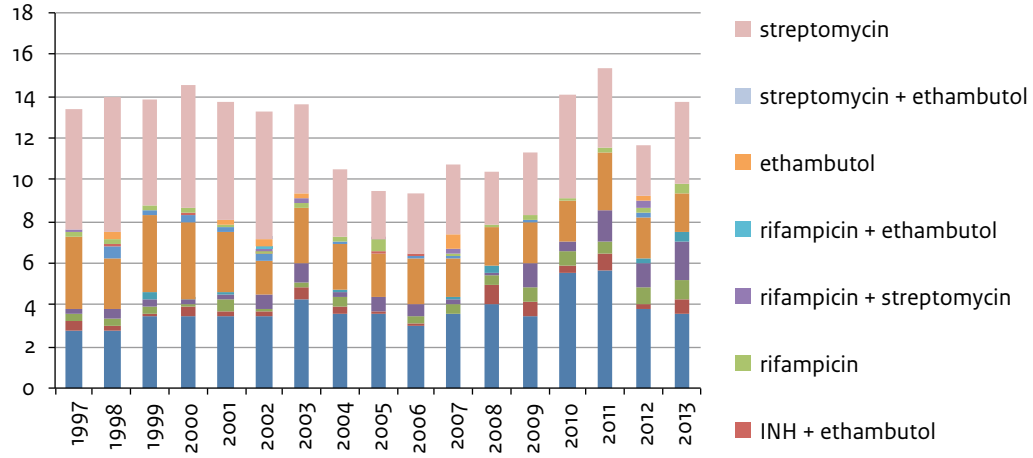
- Small increase resistance to INH (from 8.4% to 9.3%)
- Varying and low resistance to ethambutol (2.3% in 2013).
- MDR resistance increased to 3.9% in 2013 due to the increase of rifampicine resistance. (2.4% in 2012)

Figure 4.5.3.1. Trends in antibiotic resistance TB.



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Figure 4.5.3.2. Trends in combined resistance TB



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4.5.4. Resistance to influenza antiviral drugs

Adam Meijer

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). 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 National Influenza Centre. 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 2012/2013 season not reported in the 2013 NETHMAP report are highlighted here. The National Influenza Centre received an A(H1N1)pdm09 positive specimen that was collected from a patient in March 2013, which appeared to comprise the NA H275Y oseltamivir 'highly reduced inhibition' amino acid substitution. The virus isolate showed a 850-fold increase in IC50 for oseltamivir compared to wild type NA 275H A(H1N1)pdm09 virus. None of the A(H1N1)pdm09, A(H3N2) and B influenza viruses analysed so far for the 2013/2014 season showed reduced or highly reduced inhibition against the neuraminidase inhibitors. All A(H1N1)pdm09 and A(H3N2) influenza viruses since the 2008/2009 season for M2B susceptibility showed 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 susceptibility of influenza viruses to NAIs and M2Bs in the Netherlands, 2005/2006 - 2013/2014[a].

Season	A(H3N2)		A(H1N1) seasonal		A(H1N1)pdm09		B
	NAI	M2B	NAI	M2B	NAI	M2B	NAI
	1/39						2/48
2005/2006	(3%)[b]	29/39 (74%)	NA	NA	NA	NA	(4%)[c]
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%)[d]	0/49	NA	NA	1/81 (1%)[b]
2008/2009	5/74 (7%)[e]	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%)[f]	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%)[g]	7/7 (100%)	0/10
2012/2013	0/156	15/15 (100%)	NA	NA	3/125 (2.4%)[h]	10/10 (100%)	0/8
2013/2014[i]	0/137	13/13 (100%)	NA	NA	0/118	11/11 (100%)	0/2

a 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.

b The virus with reduced susceptibility had an extreme outlier IC_{50} for oseltamivir and mild outlier IC_{50} for zanamivir.

c Both viruses with reduced susceptibility had outlier IC_{50} values for oseltamivir as well as zanamivir.

d Viruses with highly reduced susceptibility for oseltamivir only. Viruses were sensitive to zanamivir and M2Bs.

e The 5 viruses had mild outlier IC_{50} values for oseltamivir but normal IC_{50} values for zanamivir.

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

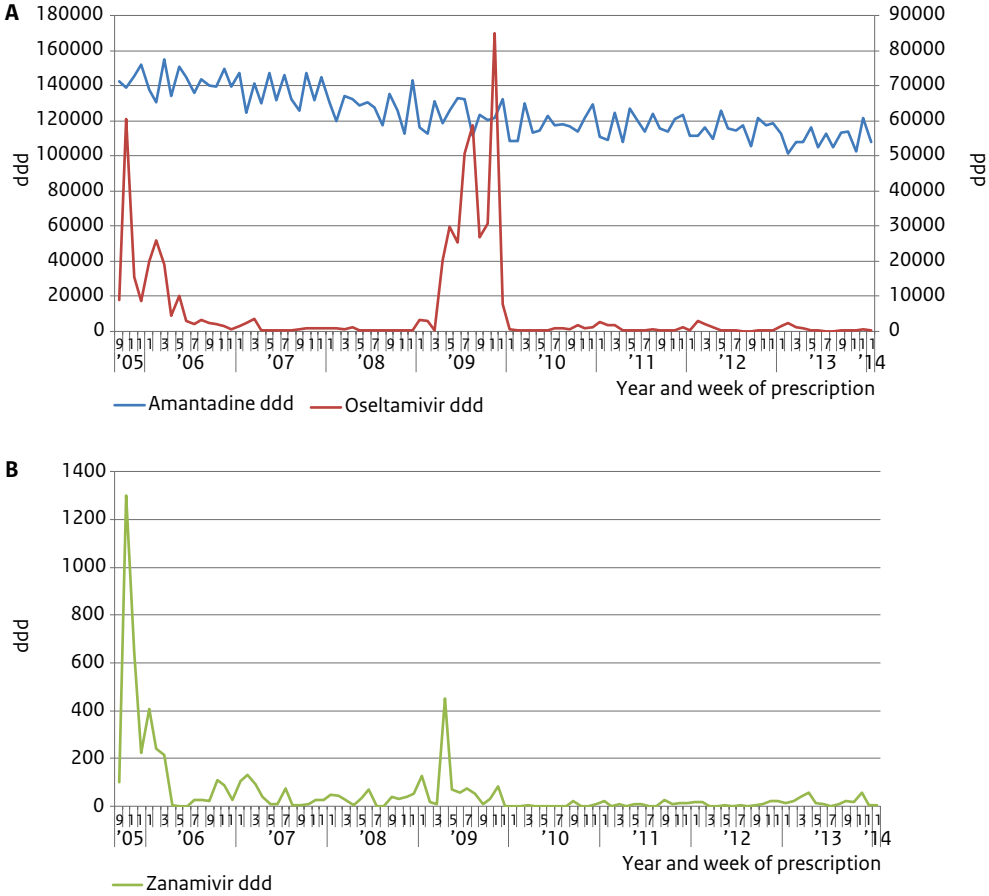
g Two viruses with highly reduced susceptibility for oseltamivir due to the H25Y amino acid substitution, isolated from two epidemiological unlinked not treated patients returning from holiday at the Spanish coast.

h Three viruses with highly reduced susceptibility for 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.

i 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 anaerobic pathogens

Linda Veloo and Arie Jan van Winkelhoff

Anaerobic bacteria isolated from patients hospitalized at the University Medical Center Groningen in 2013 were included in the study. Susceptibility was determined by Etest for amoxicillin, co-amoxiclav (only gram-negative anaerobic bacteria), clindamycin and metronidazole. EUCAST criteria were used to determine the percentage of resistant strains.

Gram-negative anaerobes

Resistance for amoxicillin was found for the genera *Bacteroides fragilis* sp. (91%), *Parabacteroides* sp. (60 %), *Fusobacterium* sp. (16 %), *Prevotella* sp. (60 %) and *Bilophila* sp. (100%). No resistance was encountered for *Campylobacter ureolyticus* and *Veillonella* sp. As in previous years, the MIC distribution of the *B. fragilis* group for amoxicillin in bimodal, with two subpopulations of 12-96 mg/L and >256 mg/L. The distribution of *Fusobacterium* sp. in unimodal with a main population of <0.016-0.064 mg/L. The MIC's of *Prevotella* sp. cover a wide range, from <0.016 to 256 mg/L.
Compared to 2013, an increase in resistance for amoxicillin is observed for *Fusobacterium* sp. (from 9% to 16%) and *Prevotella* sp. (from 33% to 60%). Co-amoxiclav resistance was encountered for one isolate of *Fusobacterium* sp.

Clindamycin resistance was encountered for *B. fragilis* sp. (20 %), *Parabacteroides* sp. (60 %) and *Prevotella* sp. (4 %). Among the fusobacteria, *Bilophila* sp., *C. ureolyticus* and *Veillonella* sp. no resistance for clindamycin was observed. Compared to 2013, the frequency of resistance was lower for the *B. fragilis* sp. and *Prevotella* sp.

Metronidazole resistance was only encountered within the genus *Prevotella*. Two strains (4 %) were found resistant. These two strains belonged to the *P. bivia* species (data not shown).

Gram-positive anaerobes

Among the tested gram-positive anaerobes of the gram-positive anaerobic cocci and the genera *Clostridium* sp., P*ropionibacterium* sp. and *Actinomyces* sp. no resistance for amoxicillin was observed.

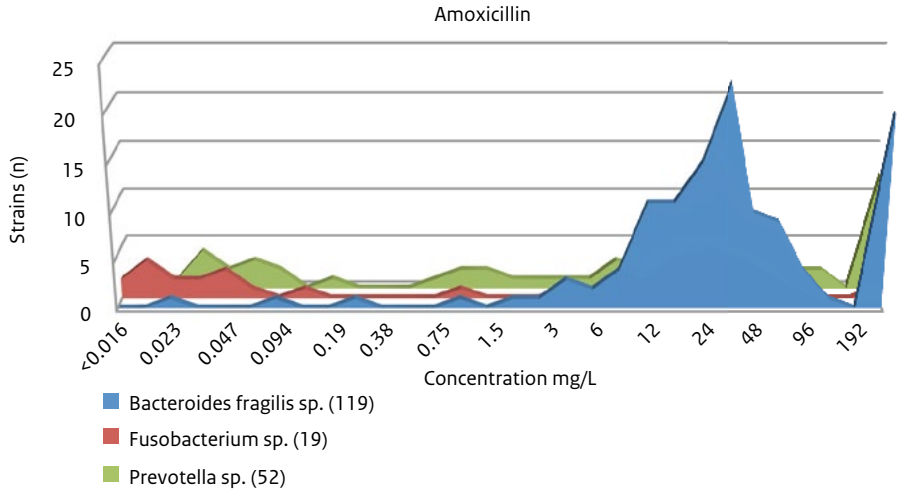
Clindamycin resistance was observed for the gram-positive anaerobic cocci (10 %), *Clostridium* sp. (27 %) and *Propionibacterium* sp. (3 %). The amount of resistance is only slightly different than that of 2013. Metronidazole resistance was only observed for one of the gram-positive anaerobic cocci strains. All other tested strains were sensitive.

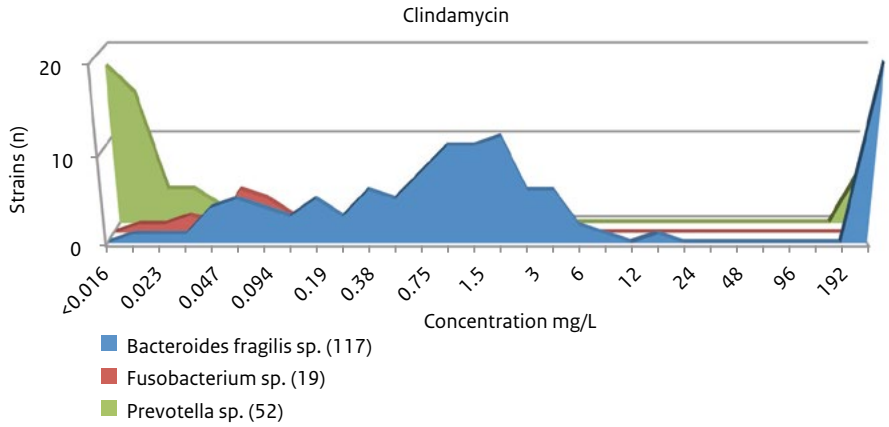
Anaerobic bacteria – Conclusion

- Amoxicillin resistance among the *B. fragilis* sp. was high. Resistance of co-amoxiclav was observed in one of the *Fusobacterium* sp. strains.
- Metronidazole resistance among anaerobes is rare, but was first observed in the *Prevotella* genus.

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Figure 4.5.5.1 MIC distribution of amoxicillin and clindamycin for clinical strains of Gram-negative bacteria





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Table 4.5.5.1 Resistance among anaerobic bacteria.

Species (N)	Antibiotic resistance N (%) amoxicillin	co-amoxiclav	clindamycin	metronidazole
Gram-negative bacteria				
Bacteroides fragilis sp. (116-119)*	108 (91)	0	23 (20)	0
Parabacteroides sp. (5)	3 (60)	0	3 (60)	0
Fusobacterium sp. (19)	3 (16)	1 (5)	0	0
Prevotella sp. (52)	31 (60)	0	2 (4)	2 (4)
Bilophila sp. (2)	2 (100)	0	0	0
Campylobacter ureolyticus (3)	0	0	0	0
Veillonella sp. (10)	0	0	0	0
Gram-positive bacteria				
Gram-positive anaerobic cocci (98-101)*	0	NT	11 (10)	1 (1)
Clostridium sp. (22)	0	NT	6 (27)	0
Propionibacterium sp. (73-75)*	0	NT	2 (3)	NA
Actinomyces sp. (5)	0	NT	0	NA

* not all strains were tested for all antibiotics

NT, not tested

NA, not available

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4.5.6. *Clostridium difficile*

D.W. Notermans (RIVM, Bilthoven), S. van Dorp, I. Sanders, E.J. Kuijper (LUMC, Leiden)

C. difficile infections (CDI) are of increased interest since the recognition in 2005 of outbreaks caused by the hypervirulent PCR ribotype 027. As of that moment, ad hoc typing in case of possible outbreaks was made available to all microbiological laboratories in the Netherlands. As of May 2009, sentinel surveillance was started, with continuous monitoring of CDI in approximately 20 hospitals. Isolates of *C. difficile* are characterized at the Reference Laboratory at LUMC, Leiden and a minimum of clinical and epidemiological data are collected. The principle characterization of isolates is PCR ribotyping. Resistance measurements are not part of routine characterization. Yearly reports of the surveillance are available.[1]
For the 19 hospitals participating in the sentinel surveillance in the period May 2012-May 2013, the mean incidence of CDI was 14.7 per 10,000 hospital admissions, varying from 5 to 27 per 10,000 admissions. Among the 911 *C. difficile* isolates, the most frequent encountered PCR ribotypes were Type 014 (16%), Type 001 (14%), Type 078 (12%), Type 002 (6%) and Type 005 (5%). The hypervirulent Type 027 was found 28 (3%) times.
In the ad hoc typing in outbreaks, Type 027 appeared to be re-emerging, with a proportion increasing to 20% in the period May 2012-May 2013. Spread of Type 027 occurred both in hospitals as in nursing homes.

Resistance measurements
As part of a large European study of CDI, the European *Clostridium difficile* infection surveillance network (ECDIS-net), 25 isolates from Dutch hospitals were tested (table 1). A standardized agar dilution assay was used. [2, 3]

Results
See table 4.5.6.1.

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Table 4.5.6.1. MIC (mg/L) values for 25 at random selected strains of 911 patients with CDI in Dutch hospitals in 2013.

Strain / faeces no.	PCR ribotype	metro-nidazole	vanco-mycin	fidaxo-micin	rifam-picin	moxi-floxacin	clinda-mycin	imi-penem	chloram-phenicol	tige-cycline
1	029	2	1	0.015	< 0.001	1	8	4	8	≤ 0.03
2	001/072	2	0.5	0.015	< 0.001	32	>64	4	8	0.06
3	020	2	1	0.03	< 0.001	2	8	4	8	0.06
4	013	2	1	0.015	< 0.001	1	8	4	8	0.06
5	003	0.25	0.5	0.03	0.002	2	4	2	8	≤ 0.03
6	265	2	0.5	0.06	0.002	2	8	2	8	0.06
7	078	0.5	0.5	0.015	< 0.001	1	2	2	8	≤ 0.03
8	043	≤ 0.125	1	0.06	< 0.001	2	8	4	8	≤ 0.03
9	001/072	2	0.5	0.015	< 0.001	32	>64	4	8	0.06
10	014	2	0.5	0.015	0.002	2	32	16	8	≤ 0.03
11	017	≤ 0.125	0.5	0.015	>16	32	>64	8	8	0.06
12	002	0.5	2	0.06	0.002	2	16	4	8	0.06
13	475	2	0.5	0.06	< 0.001	2	4	4	4	≤ 0.03
14	001/072	0.5	≤ 0.125	0.03	< 0.001	32	>64	8	8	≤ 0.03
15	012	0.25	1	0.06	< 0.001	2	>64	4	32	≤ 0.03
16	087	0.25	1	0.06	0.002	1	16	4	4	0.06
17	014	0.125	1	0.06	< 0.001	16	16	4	8	≤ 0.03
18	081	0.25	0.5	0.03	0.002	0.5	4	4	8	0.06
19	001/072	0.5	0.5	0.03	< 0.001	32	>64	4	32	0.06
20	003	0.25	1	0.06	< 0.001	32	2	4	8	≤ 0.03
21	001/072	≤ 0.125	1	0.03	< 0.001	16	>64	4	32	≤ 0.03
22	127	0.5	0.5	0.015	< 0.001	4	>64	4	8	0.06
23	001/072	≤ 0.125	0.5	0.008	0.002	32	>64	4	32	≤ 0.03
24	265	0.5	0.5	0.25	0.002	2	4	4	4	0.06
25	Sporadic type	0.25	0.5	0.25	0.002	2	4	4	32	0.06

Conclusions

The incidence of CDI in the hospitals participating in the sentinel surveillance has remained stable around 15 per 10,000 admissions in the period May 2012-May 2013. The most frequently encountered PCR ribotypes remained stable as well. Among isolates for ad hoc typing in outbreaks, Type 027 appeared to be re-emerging. Resistance data is available from a limited number of isolates, showing all isolates are susceptible to vancomycin and metronidazole. For fidaxomycin, two isolates (8%) revealed a slightly elevated MIC value of 0.25 mg/L (normal MIC_{90}= 0.06 mg/L), but no official breakpoints have been established yet.

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4.5.7. Azole resistance in *Aspergillus fumigatus*

P.E. Verweij, T. Leenstra

Aspergillus fumigatus is an important cause of fungal diseases in humans. The fungus may cause a spectrum of diseases ranging from allergic conditions to acute invasive disease. The azoles play an important role in the management of aspergillus diseases, most notably itraconazole, voriconazole and posaconazole. Very soon a fourth azole with activity against *Aspergillus* species, isavuconazole, will most likely receive approval for treatment of invasive aspergillosis.
Since 2007, the emergence of azole resistance has been reported and two Dutch surveillance studies have been published,[1,2] which have investigated the prevalence and spread of azole resistance in clinical *A. fumigatus* isolates. Both studies indicated that azole resistance is endemic in the Netherlands, and that around 90% of resistance mechanisms found in azole-resistant clinical isolates were also recovered from the environment indicating that resistance selection takes place through environmental exposure to azole compounds rather than through patient treatment. Azole resistance was associated with changes in the *Cyp51A*-gene, which is the target for antifungal azoles, with two dominant mutations: the TR_{34}/L98H and the TR_{46}/Y121F/T289A.
Several University Medical Centers contribute to resistance surveillance by subculturing any clinical isolate, irrespective of its clinical relevance, on a four-wells screening plate that has azoles added to three wells (itraconazole, voriconazole and posaconazole) and includes a growth control. If isolates are able to grow on any of these azole-supplemented agars, the isolates are sent to the Radboudumc for MIC determination and analysis of resistance mechanism. In 2013 for the first time the laboratory information systems (LIS) in the different centers was used to derive a more exact estimate of the prevalence of resistance. Azole screening agar results are captured in the LIS and therefore can be used to determine the exact number of isolates that have been screened.

Epidemiology
In 2013 1,089 *A. fumigatus* isolates were screened, recovered from 626 patients, in four UMCs (ErasmusMC, Rotterdam; LUMC, Leiden; UMCG, Groningen; Radboudumc, Nijmegen), of which 78 isolates were confirmed to be azole-resistant (7.2%) (Table 4.5.7.01). The overall prevalence of azole resistance in patients was 8.1%, and varied between the different University Medical Centers, with the highest prevalence in LUMC (19.2%) and the lowest in ErasmusMC (4.1%).

Patient populations
It is important to further analyse the patient populations from which these isolates were cultured from. In Radboudumc and LUMC a detailed analysis was made based on underlying disease of the patients

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Table 4.5.7.1. Overview of number of screened *A. fumigatus* isolates and patients in four UMCs.

	# isolates screened	# patients screened	#Confirmed azole resistant isolates (%)	#Patients with confirmed azole resistant isolates(%)
ErasmusMC	358	231	11 (3.1)	10 (4.3)
LUMC	145	99	29 (20)	19 (19.2)
Radboudumc	215	123	16 (7.4)	6 (4.9)
UMCG	371	194	22 (5.9)	16 (8.2)
Total	1,089	626	78 (7.2)	51 (8.1)

from whom the screened isolates were recovered. In both centers most *A. fumigatus* isolates were recovered from patients with pulmonary diseases, followed by hematology/oncology in Radboudumc and ICU in LUMC. In the Radboudumc 15 patients were identified with probable or proven invasive aspergillosis in 2013. As clinical isolates were used for resistance screening all of the 15 patients were culture positive. In three patients the diagnosis invasive aspergillosis was proven. An isolate with resistance to at least azole was found in four of these patients (26.7%), which is much higher than the overall prevalence of resistance of 4.9% in the Radboudumc. The mortality in patients with an azole-resistant isolate was 100% (4/4) compared to 45.5% (5/11) in patients with azole-susceptible invasive aspergillosis. A high azole resistance rate was also observed in a recent study among ICU patients in the LUMC.[3] Over the period 2011 to 2013, 146 patients that received antifungal therapy for suspected invasive aspergillosis were analyzed. Thirty-eight patients were culture positive of whom 10 harboured an azole-resistant isolate, indicating an overall prevalence of 26% azole resistance. The crude mortality rate was 75% in azole-susceptible infection compared to 100% in azole-resistant disease.[3]

Cystic fibrosis

A National study investigating fungal colonisation in patients with cystic fibrosis was recently completed. Between 2010 and 2013, 2,890 *A. fumigatus* were analysed of which 192 were found to be azole resistant (6.6%) (J. Meis, unpublished).

Analysis of the isolates

Overall, 103 *A. fumigatus* isolates sent to the Radboudumc were found to be azole-resistant, including 78 from the four UMCs that performed systematic surveillance. MICs were determined using the EUCAST microbroth dilution reference method.[4] Clinical breakpoints have been established, with a MIC of >2 mg/l for itraconazole and voriconazole, and a MIC of > 0.25 mg/l for posaconazole indicating resistance. In contrast with epidemiological cut-off (Ecoff), the clinical breakpoints take into account pharmacokinetic and pharmacodynamic characteristics of the drugs. Overall over 90% of the 103 tested isolates were resistant to itraconazole (92%), voriconazole (94%) or posaconazole (96%). The TR_{34}/L98H resistance mutation was found in 60 isolates (58%) and the TR_{46}/Y121F/T289A resistance mechanism in 25 (24%). In three isolates other cyp51A-mediated mutations were found (G54W, G54V and P216L). In the remaining 14 azole resistant isolates no mutations were found in the Cyp51A-gene, thus indicating other yet unknown resistance mechanisms.

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Overall 82.5% of isolates harboured an environmental resistance mechanism, which is a similar percentage compared with previous years (Figure). Genetic analysis of the 192 azole-resistant isolates from CF patients showed TR_{34}/L98H in 117 (61%) isolates and TR_{46}/Y121F/T289A in 52 (27%). In total 88% of resistance mechanisms were from environmental origin.

Discussion

In order to obtain a more exact estimation of the prevalence of azole resistance in 2013 a transition was made from a web-based database to information routinely collected through the Laboratory Information Systems (LIS). The web-based database relied on entering data in the surveillance centers, while the use of screening agar for detection of resistance is captured automatically in the LIS. As screening has become routine, these procedures are now captured in many LIS of the contributing UMCs thus enabling the use of these data. We believe we have more precise estimate of the prevalence of azole resistance in four UMCs. Since we followed a new procedure comparisons with previous years were not made. One factor that might influence the prevalence is the performance of the screenings agars. It is known that the presence of azole resistance might be overestimated. However, since the isolates that grow on the screening agar are further analysed, false positive growth will be detected. Resistant isolates that fail to grow on the screening plates are not detected. Therefore in 2014 the frequency of this occurring will be further investigated.

Significant variation of the prevalence in different UMCs was observed. This was observed previously in surveillance studies, the highest prevalence was then also found in the LUMC.[1] There are several possible explanations for this phenomenon. The prevalence of resistance might be influenced by the patient population that is screened. The case mixes in the different UMCs will differ, for instance depending on the number of patients cared for with chronic lung diseases, such as cystic fibrosis. The frequency of culturing patients, the number of colonies cultured differs for different patient groups and might have implications for the frequency that an azole-resistant isolate is encountered. Another reason for the variable prevalence of azole resistance might be geographical variability in exposure. Although azole-resistant isolates can readily be cultured from soil, ambient air and seeds, it is unknown if the burden in the environment differs. It is not well known where azole-resistant A. fumigates has its niche in the environment and if this is influenced by the presence of azole fungicides or certain practices of azole fungicide use. However, one can imagine that in areas of high exposure, the probability of humans acquiring an azole-resistant isolate will increase. As observed in previous years, the environmental route of resistance remains the highly dominant route, accounting for over 80% of resistance. This percentage was also found in the study in CF patients, and has remained stable over the past years. The proportion of TR_{46}/Y121F/T289A resistance mechanisms that has showed increase over the past years, appears to stabilize at 24%. In 2013 only 3 isolates with other Cyp51A-mutations were found, consistent with resistance selection through patient treatment. In 14 azole-resistant isolates there we no mutations present in the Cyp51A-gene, which suggests that other resistance mechanisms may be present. It is important to identify these mechanisms as this will make it possible to follow trends in its prevalence and migration.

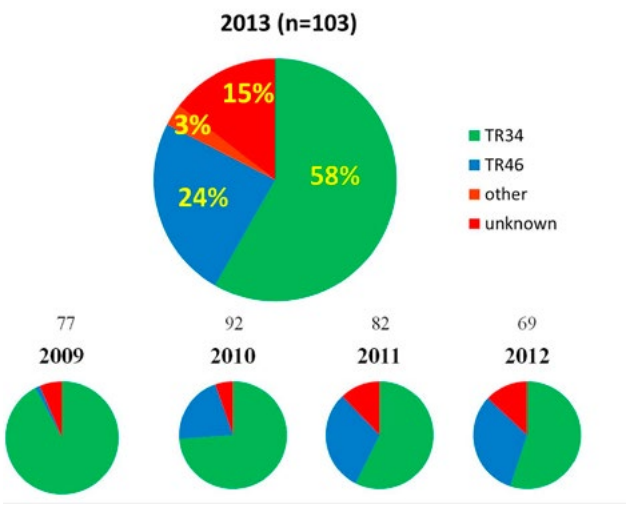
In addition to the overall prevalence of azole resistance, more detailed information collected in the Radboudumc and the LUMC indicates that in certain patient populations the prevalence of azole-resist-

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ance might be higher than found through general surveillance. Both in the Radboudumc and LUMC a prevalence of around 26% was found in patients with invasive aspergillosis. A high number of azole-resistant cases in the ICU might be due to the fact that patients with azole-resistant aspergillosis fail to (azole) therapy when treated in the ward, and are transferred to the ICU due to respiratory failure. Clearly these observations implicate that more detailed information needs to be collected, i.e. to determine resistance risk in various patient populations.
The presence of a Cyp-mutation was associated with resistance to the three mould-active azoles, itraconazole, voriconazole and posaconazole. For all three azoles a resistant phenotype was found in over 90% of isolates, indicating that any role of azoles in infected patients will be very limited. Early studies indicate that cross-resistance will be present in the new azole isavuconazole, which has a molecule structure similar to that of voriconazole. Clearly, the use of azole should be avoided when resistance is detected, leaving combination therapy (azole plus an echinocandin) or a lipid-formulation of amphotericin B as alternative treatment options.

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Figure 4.5.7.1. Distribution of resistance mechanisms in 103 *A. fumigatus* isolates collected in 2013, and compared with 2009 to 2012 (numbers given above the year indicate the number of azole-resistant isolates that were analysed).



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MARAN 2014

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013

June 2014

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Colophon

This report is published under the acronym MARAN-2014 by the Central Veterinary Institute of Wageningen University and Research Centre in collaboration with the Food and Consumer Product Safety Authority (NVWA), and the National Institute for Public Health and the Environment (RIVM). The information presented in MARAN-2014 is based on total sales data of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
MARAN-2014 is published in a combined back-to-back report with NETHMAP-2014. 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-2014 can be ordered from the secretariat of CVI-Lelystad, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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Contents

Colophon 2
Acknowledgements 4
Contents 5
1 Summary 7
2 Usage of antibiotics in animal husbandry in the Netherlands 11
2.1 Total sales of veterinary antibiotics in the Netherlands 2013 11
2.1.1. Analysis of sales data 11
2.1.2. Trends in total sales 12
2.2. Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands, 2012-2013 15

3 Resistance data 21
3.1 Food-borne pathogens 21
3.1.1 Salmonella 21
3.1.2 Campylobacter 33
3.1.3 Shiga-toxin producing *E. coli* (STEC) 40
3.2 Commensal indicator organisms 43
3.2.1 *Escherichia coli* 44
3.2.2 *E. coli* in raw meat products of food-animals 49
3.2.3 *Enterococcus faecalis* and *E. faecium* in faeces of food-animals 52
3.2.4 *Enterococcus faecalis* and *E. faecium* in raw meat products of food-animals 55

4 Appendix I 57
Results of the screening for ESBL, AmpC and carbapenemase-producing Enterobacteriaceae in food producing animals in the Netherlands in 2013 57
4.1 ESBL-producing bacteria 57
4.2 Carbapenemases 66

5 Appendix II 68
Materials and methods 68

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1 Summary

Antibiotic Usage

Total sales of antibiotics licensed for therapeutic usage in The Netherlands decreased by 63% since 2007, to 209 tons in 2013. The reduction in sales from the National authority defined index year, 2009, is 58%. This means that the reduction target defined by the authorities for 2013 (50% reduction) is abundantly reached. Relatively largest reductions were realized for cephalosporin 3rd and 4th generation (-76%) en fluoroquinolones (-50%), which is in accordance with Dutch antimicrobial formularies and stimulated by new legislation limiting the use of these (third choice) antimicrobial drugs to bacterial culture proven infections.

One sector was added to the monitoring program (turkeys), resulting in a further narrowing down of discrepancies between sales data and consumption data, although differences are still recognizable due to unmonitored sectors like companion animals and horses. In all major livestock producing sectors a steady decrease in use of antimicrobials is observed since 2009.

Antimicrobial resistance

S. Enteritidis or *S.* Typhimurium was most frequently isolated from human clinical infections. In 2013 *S.* Typhimurium (N = 214) in combination with the monophasic variant of Typhimurium: *S. enterica* subspecies *enterica* 1,4,5,12:i:- (N = 182), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (N = 314) in second place. The relative contribution of different animal species to infections in humans varied by serovar. *S.* Typhimurium and its monophasic variant were predominantly associated with pigs and to a lesser extend with cattle and poultry. *S.* Enteritidis was mainly associated with poultry and more specifically layers and contaminated eggs. In pigs, next to *S.* Typhimurium and its monophasic variant, *S.* Derby dominated. 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 *S.* Heidelberg was isolated frequently in poultry. This was mainly due to extra sampling of contaminated poultry meat imported from Brazil, which did not result in human cases.

S. Typhimurium and the monophasic variants have acquired resistance against a number of antimicrobials. The most common resistance pattern was ASSuT (resistant to ampicillin, streptomycin, sulfonamides

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and tetracycline). Resistance levels for ciprofloxacin and nalidixic acid were highest in S. Heidelberg, Typhi and Paratyphi A (humans only), Infantis and Enteritidis. Partly this reflects the usage of quinolones in poultry production. ESBL producing strains (cefotaxime R) dominated in S. Heidelberg from imported poultry products.

In *C. jejuni* isolates from broiler feces for all antibiotics tested the resistance levels determined in 2013 were lower than those of 2012. The resistance level determined in 2013 for ciprofloxacin was 17% lower than that of 2011. Although the ciprofloxacin resistance level is still quite high, the tendency to decrease is a positive signal that the measures initiated in livestock production to reduce total antibiotic use and the use of third-choice drugs, show an effect on the levels of resistance. In *C. jejuni* from poultry meat no decreasing trends were observed. This suggests that part of the meat that was collected at retail, originated from non-domestic sources.

Also in human *C. jejuni* in 2013 the resistance level for ciprofloxacin was slightly lower in 2013 compared to 2012. Resistance rates for macrolides in *C. coli* isolates from pigs show a clear decreasing trend from 26% in 2010 to 7% in 2013. This may reflect the decreased use of macrolides (tylosin, tilmicosin, and tulathromycin) in these animals.

Over the last ten years, MIC profiles of STEC isolates show a tendency to increase. Most striking was the increases in resistance to tetracycline, streptomycin, sulfamethoxazole, kanamycin and ampicillin. In 2013 (4%) of the isolates tested were resistant to the quinolones (ciprofloxacin and nalidixic acid). This was never seen in former years, in which resistance levels to quinolones were always below 1%.

Among indicator *E. coli* from meat and animals, resistance to ampicillin, streptomycin, tetracyclines, sulfonamides and trimethoprim was commonly detected in all host species except dairy cattle. Resistance to antimicrobials recognised as critically important in human medicine, such as the fluoroquinolones and third generation cephalosporins, was predominantly observed in the indicator *E. coli* of poultry sources. In isolates from most animal species a continuous decrease in resistance levels was observed in 2013, most likely as a result of the reductions in antibiotic usage. Also resistance to third-generation cephalosporins decreased in most animal species, most likely the result of the vast limitations in usage of cephalosporins in food producing animals. Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested. Levels of resistance in *E. coli* from organic broilers were substantially lower than those from conventional broilers for almost all antibiotics tested. Reduced susceptibility to ciprofloxacin was highest for *E. coli* isolates from broilers.

In 2013 for the first year, enterococci isolates only from poultry were included in the monitoring program. The reason is that 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.
Highest resistance levels were observed for tetracycline (80.5% in *E. faecalis* and 53.7% in *E. faecium*), erythromycin (68.8% in *E. faecalis* and 47.3% in *E. faecium*), and streptomycin (42.5% in *E. faecalis* and 29.8% in *E. faecium*). In *E. faecium*, additional high levels of resistance were observed for quinu/dalfopristin

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(72.3%), salinomycin (38.5%) and to a lesser extent to ampicillin (21.5%).
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. As a result MIC data for isolates from meat may not reflect the data on isolates from live animals. Vancomycin resistant enterococci were not detected in animals in 2013.

Since 1998, cefotaxime reduced susceptibility, indicative of ESBL/AmpCs, was observed at low levels in *E. coli* from all animal species. 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 decreased in 2010, and declined even further in 2011, 2012 to 2.7% in 2013. This is most likely the result of decreased usage of antibiotics in broilers and the fact that since spring 2010 off label use of ceftiofur at Dutch hatcheries was stopped. In all years $bla_{\text{CTX-M-1}}$ was predominantly found. $Bla_{\text{CTX-M-9}}$ and $bla_{\text{TEM-20}}$ (both found in *E. coli* from broilers) were only sporadically found and do not seem to play a role in the spread of ESBL enzymes in food-producing animals. On the other hand, next to $bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-52}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{CMY-2}}$ were collected almost every year and are still collected in 2013, indicating successful spread of these resistance genes among food-producing animals.
Since 2011, an active surveillance using selective media, was performed for ESBL/AmpC-producing *E. coli* in Dutch food-producing animals (veal calves, dairy cows and pigs) in close collaboration between the Dutch Food and Consumer Product Safety Authority (NVWA) and the Central Veterinary Institute (CVI). At Dutch slaughterhouses a faecal sample was taken from ten (apparently healthy) animals per slaughter batch of animals. In 2013, 93 batches of slaughter pigs were sampled, 89 batches of veal calves and 93 individual dairy cows, each representing a different farm. Moreover, 1932 meat samples were analysed for ESBL/AmpC-producing *E. coli*. In 46.1% of the veal calves batches examined and in 57% of slaughter pigs batches ESBL-producing *E. coli* were detected. The number of positive animals varied from 1 to 10 all animals per slaughter batch. In individually sampled dairy cows the prevalence of animals positive for ESBL-producing *E. coli* in their faeces was 7%. Since the start of this surveillance program in 2011, batch prevalence in pigs was highest in 2012 (75%) and decreased in 2013 to 57%. In veal calves the batch prevalence decreased in 2013 from 70% in 2011 and 2012 to 46% in 2013. In individual dairy cows the prevalence seems stable in 2012 and 2013 (between 7 and 8 %). Future sampling will reveal if the decreasing trends will continue.
In 23% of the raw meat samples ESBL/AmpCs were confirmed to be present. Highest prevalence was observed in poultry meat (83%), this was somewhat higher than found in 2012 (73%).Thirty five percent of turkey meat was found positive (in 2012 this was 29%) while in beef and pork the prevalence of confirmed ESBLs was comparable to 2012 (respectively 5% in 2013 versus 6% in 2012 in pork and 2% versus 1% in beef). Surprisingly, in crocodile meat 4/10 (40%) of the isolates were confirmed ESBL producers. In kangaroo meat (n=11) no ESBLs were detected. The differences in prevalences in meat between 2012 and 2013 may be due to sampling bias that varies between years.

The prevalence of ESBL-producing *Salmonella* was in 2013 4%, which is more than two times as high as in previous years. This can mainly be attributed to an extra import project in which poultry meat from South America was over sampled. This was done according to article 24 of Counsil Directive 97/78/EC for re-enforced sampling of suspected batches. These samples were often positive for CMY-2-producing

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S. Heidelberg isolates. Next to this serovar, a wide variation of 10 other serovars was identified to carry ESBLs.

From 2012 onwards *E. coli* and *Salmonella* were screened for susceptibility to the carbapenems: ertapenem, meropenem and imipenem by disk diffusion. As carbapenemase producing *Enterobacteriaceae* are almost always also ESBL-producers, the screening included all *E. coli* and *Salmonella* isolates displaying reduced susceptibility to cefotaxime (N > 100/year). In 2012 and 2013, all isolates tested were susceptible to these carbapenems and no further analysis was performed.
In 2013 an active surveillance for carbapenemase-producing *Enterobacteriaceae* was initiated using a commercial RT-PCR on broth cultures of > 1000 faecal samples. All samples were negative for isolates with plasmid mediated carbapenemase genes.
In 2014 this active surveillance is continued in all faecal samples from food animals. An active surveillance for carbapenemases in food products will only be conducted on samples that are considered to be at risk to be positive (fresh herbs from South-East Asia and North Africa).
It can be concluded that antibiotic sales data show a steady and very substantial decrease since the top year 2007. Hence, the policies initiated in 2008 to limit antibiotic usage were highly successful. In 2013 in organisms from all animal species the resistance levels have decreased including a substantial decrease in the occurrence of cefotaxime resistance in *E. coli* from broilers. In 2013 the prevalence of ESBL/AmpC-producing *E. coli* was lower in faecal samples of veal calves and pigs at herd level than in 2012. In meat the prevalence of ESBL/AmpC-producers remained stable. This suggests that the reduction of the quantity of antibiotic use in the Netherlands and those to reduce the use of third-generation cephalosporins have resulted in this reverse of trends. This is a very important signal for policy makers, veterinarians and animal producers, that all their constraints to reduce antibiotic use and at the same time maintain animal health in food producing animals does improve the resistance situation in the food chain.

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2
Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2013

2.1.1. Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data of all antimicrobial veterinary medicinal products on package level sold in the Netherlands in 2013, 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 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 EMA method for all antimicrobial veterinary medicinal products, but including (unlike the ESVAC reports) topical applications like ointments, eye drops and sprays. The sales data in this report gives information about the total sales for all animals, not per individual animal species. Detailed information about antibiotic usage per animal species in the Netherlands is reported on in the next chapter.

The average number of food-producing animals present in Dutch livestock farming sector (pigs, poultry, veal calves, other cattle and sheep) shows annual variations (Table ABuse 01). Overall, the total live weight of livestock produced in The Netherlands has remained stable, 2.5-2.6 million tons. This indicates that the reported reduction in sales of antimicrobials can be interpreted as true reductions in usage.

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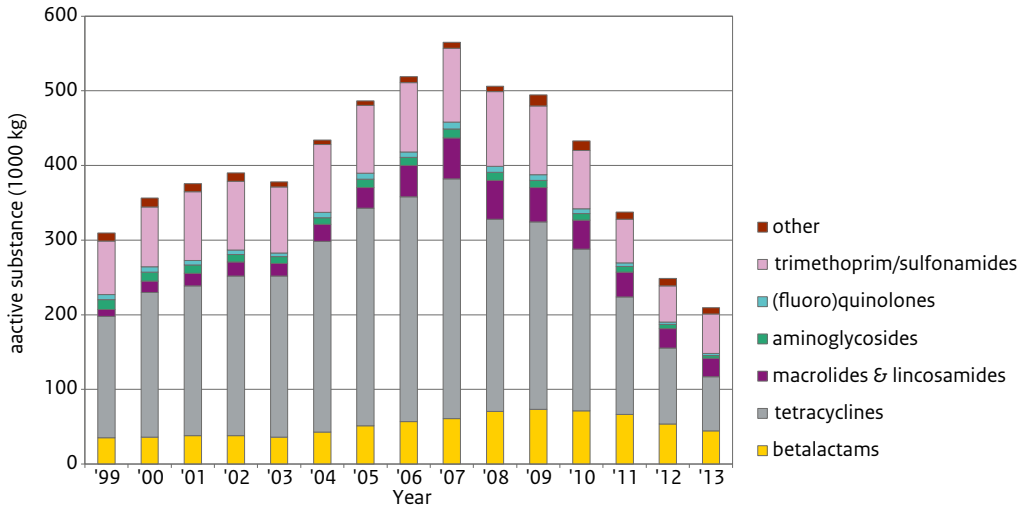
Table ABuse 01. Trends in livestock in the Netherlands in numbers (thousands)

Number of animals * 1000	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Piglets (less than 20 kg)	4.225	3.896	4.300	4.170	4.470	4.680	4.555	4.809	4.649	4.797	4.993	4.920
Sows	1.140	1.052	1.125	1.100	1.050	1.060	1.025	1.100	1.098	1.106	1.081	1.095
Fattening pigs	5.789	5.818	5.715	5.730	5.700	5.970	6.155	6.199	6.459	6.200	4.189	4.209
Other pigs	1.876	1.883	1.865	1.900	1.660	1.960	2.050	2.100	2.040	2.021	1.841	1.789
Turkeys	1.451	1.112	1.238	1.245	1.140	1.232	1044	1060	1036	990	827	841
Other poultry	62.066	42.991	43.854	45.525	42.529	44.487	50.270	52.323	54.367	57.811	43.912	44.242
Veal calves	692	748	775	813	824	860	913	886	921	919	940	1.026
Cattle	3.088	2.986	2.984	2.933	2.849	2.960	3.083	3.112	3.039	2.993	3.045	3.064
Sheep	1.300	1.476	1.700	1.725	1.755	1.715	1.545	1.091	1.211	1.113	1.093	1.074

2.1.2. Trends in total sales

Figure ABuse 01 and Table ABuse 02 show the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands.

Figure ABuse 01. Antimicrobial veterinary medicinal product sales 1999-2013 in kg (thousands)



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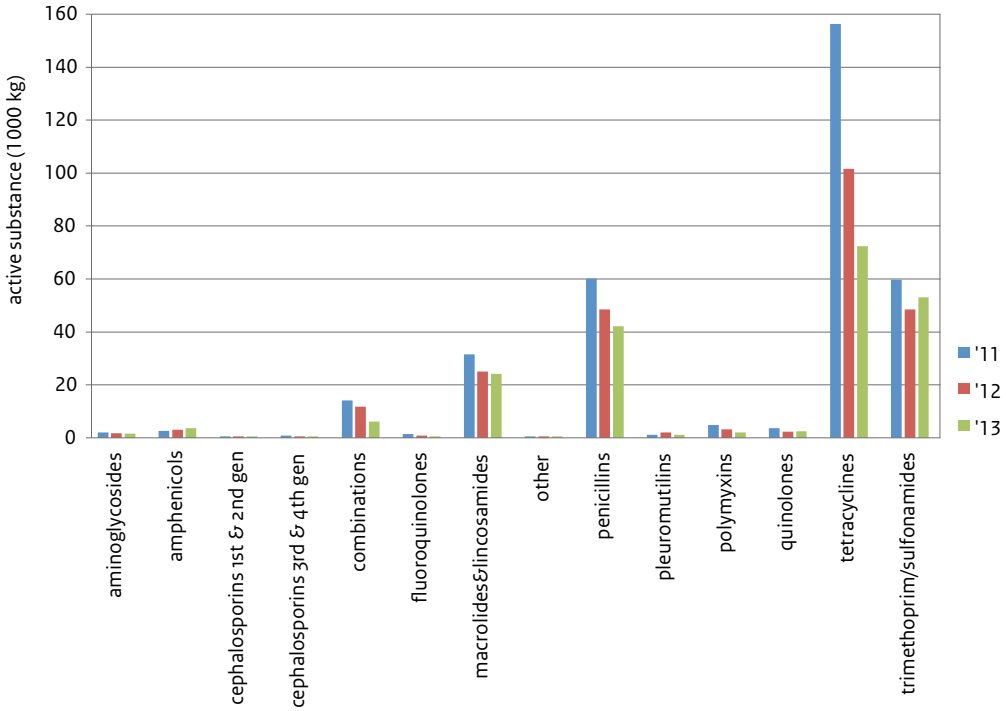
Table ABuse 02. Antimicrobial veterinary medicinal product sales from 1999-2013 in kg (thousands) (FIDIN, 2014)

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13
betalactams	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	72
macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25
aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	8,6	7,3	5,8	3,4
(fluoro) quinolones	7	7	6	6	5	7	8	7	9	8	8	6,6	5,1	3,1	2,8
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53
other	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8,1
total therapeutic sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	209

2013 resulted in a reduced sales of antimicrobial veterinary medicinal products. The total sales in the years 2009-2013 decreased by 57.7%, to a total of 209 tonnes in 2013. This means that the policy objective for 2013 – a 50% reduction – is accomplished. Compared to 2007 as year with the highest antibiotic usage, the decrease in kg was 63%, sold up to 2013.
Almost all classes of antibiotics showed a decrease in 2013, except for the trimethoprim/sulfonamides combinations (+9%) (Figure ABuse 02). When studied more in detail increases in sales were additionally noted for amphenicols (+18%), cephalosporins 1st and 2nd generation (+10%), quinolones (+7%). Relatively largest reductions were realized for cephalosporin 3rd and 4th generation (-76%) en fluoroquinolones (-50%).

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Figure ABuse 02. Antimicrobial veterinary medicinal product sales by pharmacotherapeutic class 2011-2013 in kg (thousands)



Tetracyclines

The tetracyclines contributed the most to the 2012-2013 reduction with 29 tonnes (75% of total reduction, 29% decrease for tetracyclines compared to 2012). This year doxycycline sales were reduced (-47%) more pronounced than the overall sales of tetracyclines, resulting in a contribution of 31% to this group (41% in 2012 and 34% in 2011). This might be an indicator for a shift from oral (partly group) treatment to individual treatment by injection. Furthermore, the sharp decrease for doxycycline in mass attributes to a higher impact on the reduction of total treatments due to its higher potency than the other representatives of this group.

Trimethoprim-sulfonamides

The trimethoprim-sulfonamides combinations are now the second contributor in mass sold. The relatively high doses compared to penicillins will result in a third place in therapeutic treatments.

Penicillins

Third in mass, the penicillin sales were reduced with 13%. 90% of this group is represented by amoxicillin (40%), ampicillin and benzylpenicillin.

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(Fluoro)quinolones

The sales of fluoroquinolones halved in 2013 (0.19% of total sales), whereas the sales of quinolones increased slightly with 150 kg (data not shown).

Cephalosporins

The cephalosporins represent 0.05% (100 kg) of the total sales. Like in 2012, the sales of 1st and 2nd generation cephalosporins increased marginally with 9 kg, while the sales of 3rd and 4th generation cephalosporins further diminished with 43 kg (0.006% of total sales). 90% 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 therapy in the Netherlands is still continuing since signing of memoranda of understanding in 2008 between national authorities, private parties involved in animal production and the Dutch Royal Veterinary Association, although some flattening in the curve is notable. The measures that were implemented were aimed at creating maximal transparency through benchmarking of antibiotics use by veterinarians and farmers. The use of antibiotics of critical importance to humans has been reduced to indications without alternative treatments.

2.2. Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands, 2012-2013

Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands by LEI Wageningen UR for sentinel farms. These data were, in cooperation with Utrecht University, converted to the number of defined doses per animal year (DD/AY). The calculation method is similar to the method applied in human drug use. Applied antimicrobial veterinary medicinal products are converted to treatable animal mass*days and related to animal mass present on a farm. Results are calculated for a period of a year and expressed as the number of days an average animal is treated in that year on that particular farm. The sentinel data are weighted by farm related variables to obtain figures representative for the whole population of farms in a sector.
Since 2011, husbandry related consumption reports are prepared by the Netherlands Veterinary Medicines Authority (SDa) using consumption data from all farms in the largest sectors of food production animals: pigs, veal calves, broilers and (starting 2012) cattle. In 2013 also the turkeys sector provided consumption data. While the calculation method for treatable body mass (numerator) is the same, although aggregated across all farms, the denominator represents animal weight for the whole sector, and this measure is referred to as Defined Daily Doses Animal ($DDDA_{nat}$). Table ABuse 03 shows the animal populations for which veterinary medicinal products consumption data are reported in 2012 and 2013 (pigs, veal calves, broilers and cattle). In Table ABuse 04 the results $DDDA_{nat}$ are shown. For turkeys in 2013 the $DDDA_{nat}$ (total) was 21.9.

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Table ABuse 03. Weight per sector in kg (thousands) for $DDDA_{nat}$ calculation

Sector	2012	2013
pigs*	710.688	710.802
sow/piglets**	363.006	367.708
fatttening pigs**	527.279	521.570
veal calves*	162.056	176.882
cattle*	1.522.500	1.532.000
diary cows*	924.600	958.200
other cattle*	597.900	573.800
broilers*	43.846	44.242
turkeys*	4.961	5.046

* CBS population data; ** sector population data

Table ABuse 04. Trends in $DDDA_{nat}$ in the Netherlands in livestock

	Animalsector							
	Pigs		Veal calfs		Cattle		Broilers	
Number of farms with prescriptions	6425	6713	2175	2125	32254	31650	732	801
Year	2012	2013	2012	2013	2012	2013	2012*	2013
Pharmacotherapeutic group								
Amphenicols	0.06	0.09	1.19	1.11	0.05	0.07	-	-
Aminoglycosides	-	-	0.78	0.48	0.01	0.01	1.91	0.03
Cefalosporins 1st & 2nd generation	-	-	-	-	0.02	0.02	-	-
Cefalosporins 3rd & 4th generation	-	-	-	-	0.03	-	-	-
Combinations	0.27	0.10	0.42	0.08	0.84	0.67	0.81	0.37
Fluoroquinolones	-	-	0.22	0.01	0.01	-	1.16	0.24
Macrolides/lincosamides	1.39	1.02	3.54	3.26	0.08	0.11	-	0.31
Penicillins	2.91	2.17	2.16	1.57	1.20	1.45	7.97	6.34
Pleuromutilins	0.35	0.12	-	-	-	-	-	-
Polymyxins	0.58	0.44	0.69	0.32	0.05	0.02	0.88	0.08
Quinolones	0.03	0.03	0.26	0.27	-	-	0.55	1.65
Tetracyclines	6.79	4.58	10.45	8.64	0.47	0.50	2.52	2.52
Trimethoprim/sulfonamides	1.92	1.40	2.67	1.68	0.19	0.19	2.02	1.46
Other	-	-	-	-	-	-	-	-
Total	14.32	9.97	22.40	17.43	2.97	3.04	18.40	13.01

* 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

In all sectors reduction of use is noted when comparing 2012 and 2013, except for cattle.
For a few years whole sector data based estimates overlap sentinel farm derived data (http://www.wageningenur.nl/en/Research-Results/Projects-and-programmes/MARAN-Antibiotic-usage/Trends-in-use-per-species.htm), for broilers (sentinel number of animals in 2009 n = 2530313, 5%), veal calves (n = 134446, 15%) and dairy cattle (n = 7382, 0.5%) (Figure ABuse 03, Figure ABuse 04). Discrepancies between

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Figure ABuse 03. Consumption of antimicrobial veterinary medicinal products in broilers in sentinel farms for 2004-2012 (DD/AY), and in the whole sector for 2012-2013 ($DDDA_{nat}$)

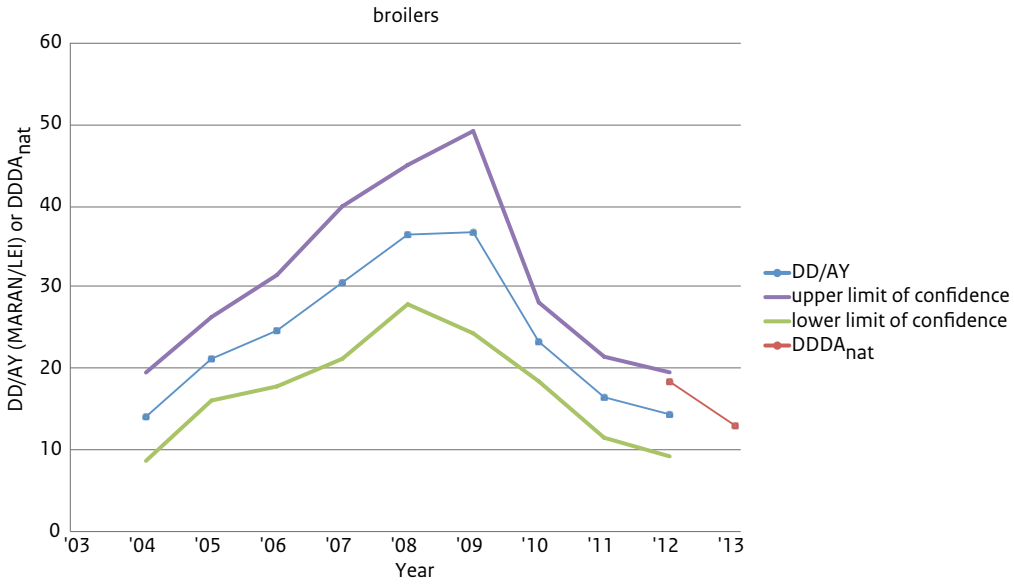
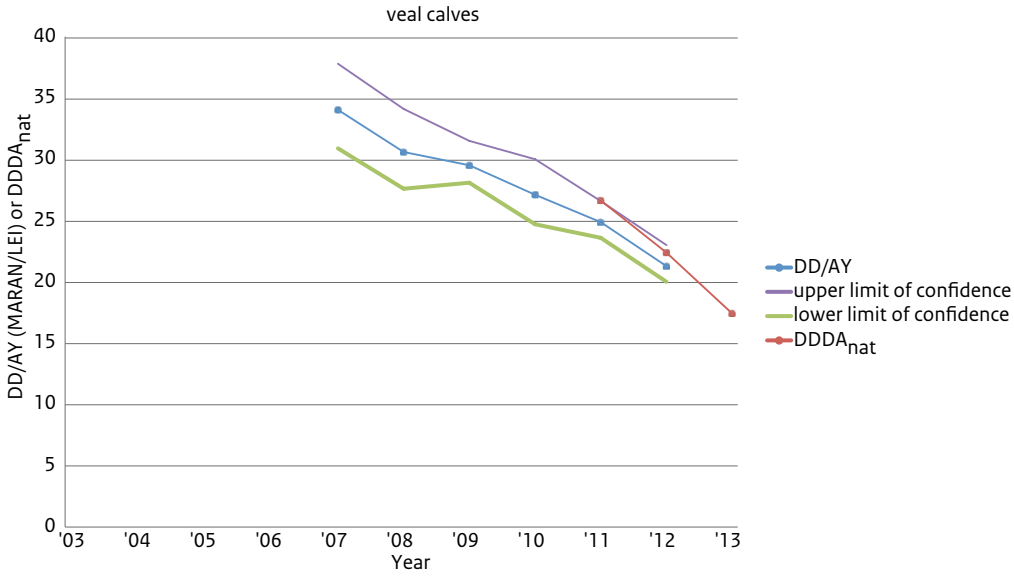


Figure ABuse 04. Consumption of antimicrobial veterinary medicinal products in veal calves in sentinel farms for 2007-2012 (DD/AY), and in the whole sector for 2011-2013 ($DDDA_{nat}$)



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Figure ABuse 05. Consumption of antimicrobial veterinary medicinal products in dairy cattle in sentinel farms for 2004-2012 (DD/AY), and in the whole sector for 2012-2013 ($DDDA_{nat}$)

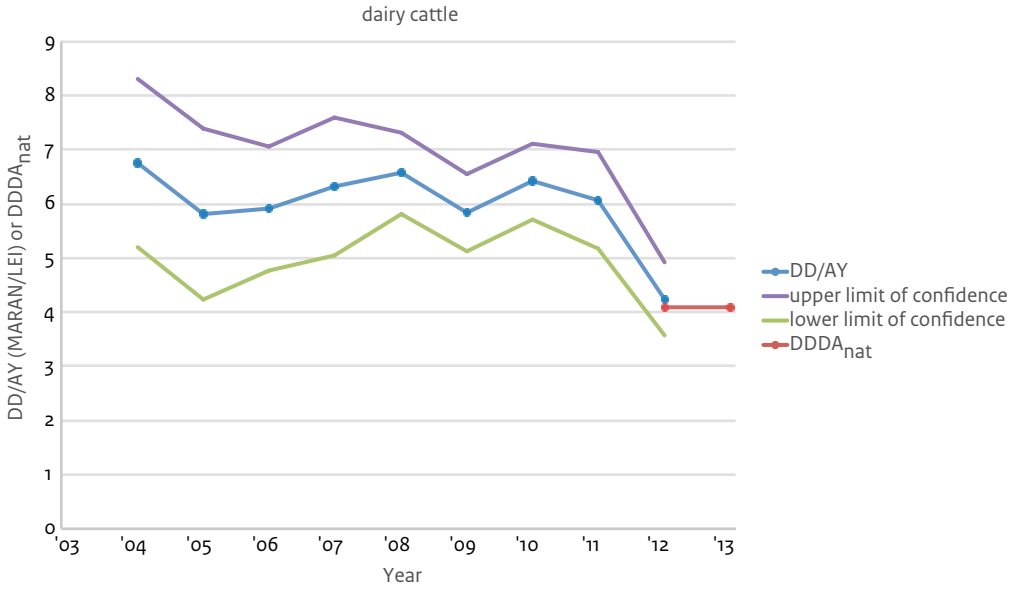
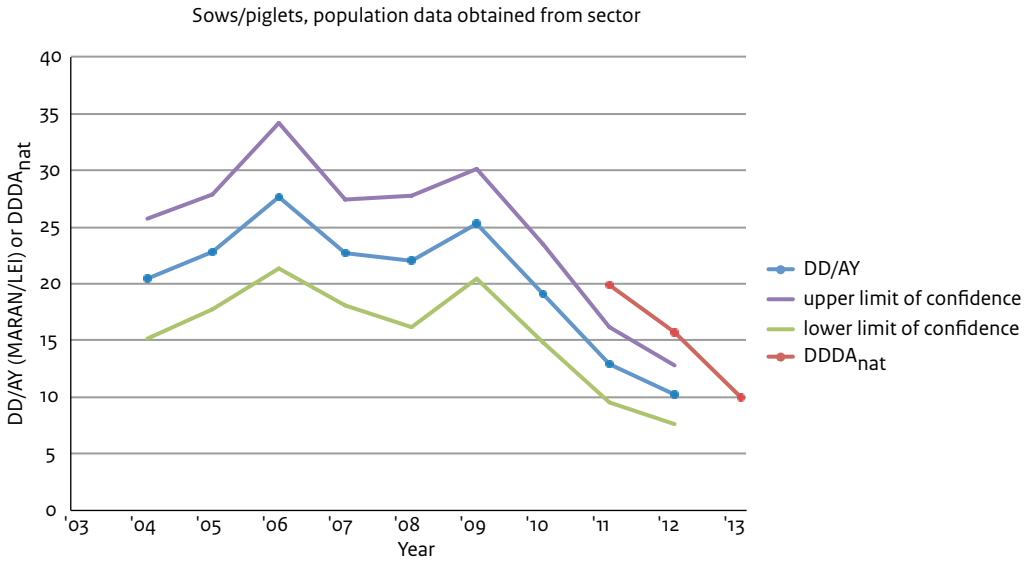
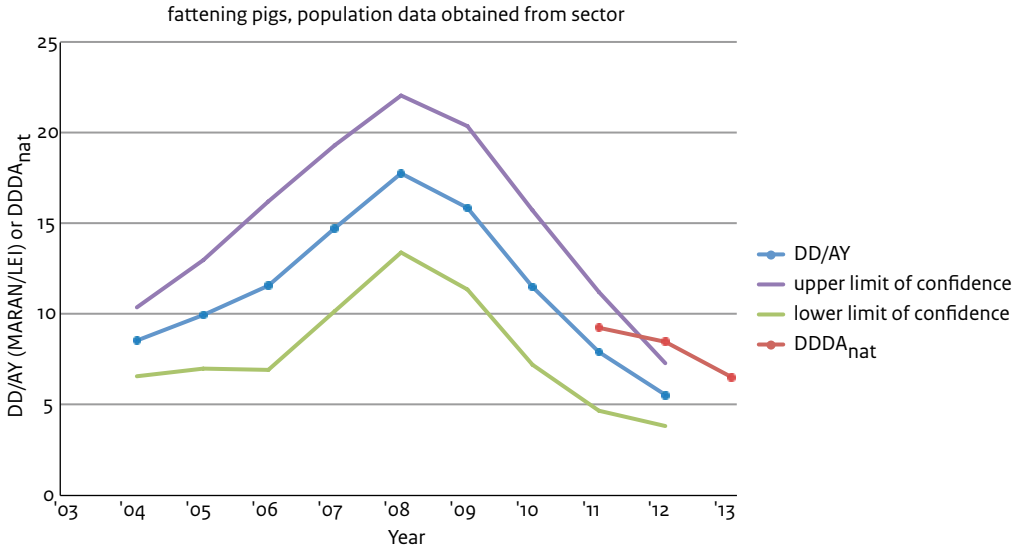


Figure ABuse 06. Consumption of antimicrobial veterinary medicinal products in sows/piglets in sentinel farms for 2004-2012 (DD/AY), and in the whole sector for 2011-2013 ($DDDA_{nat}$)



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Figure ABuse 07. Consumption of antimicrobial veterinary medicinal products in fattening pigs in sentinel farms for 2004-2012 (DD/AY), and in the whole sector for 2011-2013 ($DDDA_{nat}$)



(Figure ABuse 05, Figure ABuse 06, Figure ABuse 07) the sentinel based estimates and whole population antimicrobial consumption data in 2011 are small and likely the result of sampling error in the sentinel data and assumptions related to back-extrapolation to the whole population..
Of note is that de denominator for calculation of the $DDDA_{nat}$ and DD/AY in dairy cows in sentinel and national calculations is different from farm based consumption calculations as for instance used in benchmarking, since only milk producing cows (with a weight of 600 kg) are taken into account, while on farm level other cattle (like heifers and calves) also attribute to the weight (Bos et al., 2013). For the pig sector some difficulties were encountered to report national data differentiated in two subsectors (sows/piglets and fattening pigs) for both 2012 and 2013, as MARAN/LEI did for previous years. Eventually this necessitated a different way to determine the denominator (total mass in bodyweight) than for the other sectors. For sow/piglets and for fattening pigs, the denominator is based on the population data of the sector in 2013, matching the consumption data for both subgroups.

References

Bos ME, Taverne FJ, van Geijlswijk IM, Mouton JW, Mevius DJ, Heederik DJ; Netherlands Veterinary Medicines Authority SDa. Consumption of antimicrobials in pigs, veal calves, and broilers in the Netherlands: quantitative results of nationwide collection of data in 2011. PLoS One. 2013;8; e77525 1-9.

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3 Resistance data

In this chapter susceptibility test results are presented as determined in 2013 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*.

3.1 Food-borne pathogens

3.1.1 Salmonella

Highlights

1. In 2013 *S.* Typhimurium (N = 214) in combination with the monophasic variant of Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (N = 182), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (N = 314) in second place.
2. In pigs, next to *S.* Typhimurium and its monophasic variant, *S.* Derby dominated. 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 *S.* Heidelberg was isolated frequently in poultry. This was mainly due to extra sampling of contaminated poultry meat imported from Brazil, which did not result in human cases.
3. Highest resistance levels were observed in *S.* Heidelberg, the monophasic *S. enterica* subspecies *enterica* 1,4,[5],12:i:- and *S.* Paratyphi B var. Java, and to a lesser extend in *S.* Typhimurium and *S.* Infantis.
4. Resistance levels for ciprofloxacin and nalidixic acid were highest in *S.* Heidelberg, Typhi and Paratyphi A (in humans only), Infantis en Enteritidis. Most probably the result of usage of quinolones in poultry production.
5. ESBL/AmpC producing strains (cefotaxime R) dominated in *S.* Heidelberg from imported poultry products.

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In this paragraph 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.

Salmonella serovar prevalence

In the Netherlands, an extensive monitoring 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 sero- and phage typing results is presented in Table S01 concerning *Salmonella* isolates recovered from humans and farm animals (swine, cattle and poultry).
Human isolates (N = 1201 in 2013) 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 = 90) and cattle (N = 54) were partially sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and clinical *Salmonella* infections. Those from chickens (broilers, including poultry products, N = 202; layers, reproduction animals and eggs, N = 57) 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 have been analysed as well (animal feed and human food products; other animals from animal husbandry and pets, samples from the environment etc.).

Traditionally, *S.* Enteritidis or *S.* Typhimurium was most frequently isolated from human clinical infections. In 2013 *S.* Typhimurium (N = 214) in combination with the monophasic variant of Typhimurium: *S. enterica* subspecies *enterica* 1,4,5,12:i:- (N = 182), were most frequently isolated from humans suffering from salmonellosis, with *S.* Enteritidis (N = 314) in second place.
The relative contribution of different animal species to infections in humans varied by serovar. *S.* Typhimurium and its monophasic variant were predominantly associated with pigs and to a lesser extent with cattle and poultry. *S.* Enteritidis was mainly associated with poultry and more specifically layers and contaminated eggs (Table S01).
In pigs, next to *S.* Typhimurium and its monophasic variant, *S.* Derby dominated. 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 *S.* Heidelberg was isolated frequently in poultry. This was mainly due to contaminated poultry meat imported from Brazil. This meat is not allowed to be sold in the Netherlands as fresh meat. Therefore the presence of these isolates did not result in human cases.
Depending on the sero/phage type, travel contributed up to 53% of the cases of human salmonellosis in 2012/2013. More than 50% contribution was noted for *S.* Paratyphi A, but also for a number of non-typhoidal serovars such as *S.* Kentucky, a serovar known to be associated with travel to Africa (Egypt). 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

Antimicrobial susceptibility testing in 2013 was performed on 1906 isolates. Table S02 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2013. Highest levels of resistance were observed for streptomycin, sulfamethoxazole, tetracycline, ampicillin and to a lesser extent ciprofloxacin, nalidixic acid and trimethoprim. The levels of reduced susceptibility to ciprofloxacin

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Table So1. Most prevalent *Salmonella* serotypes isolated in 2012 and 2013 from humans, pigs, poultry, broilers and layers and the % travel related human infections.

		Travel	Humans		Pigs	
		2010-2013	2012	2013	2012	2013
N Total			2743	1201	362	90
N tested	*Tested*		1495	1103	175	73
Enteritidis	777	17%	467	314		
Typhimurium	714	5%	308	214	129	29
SI 1,4,[5],12:i:2-	585	3%	352	182	91	29
Infantis	150	4%	27	34	5	3
Paratyphi B. var. Java	137	6%	11	17		
Thompson	84	2%	1135	31		
Derby	58	2%	11	12	72	18
(Para)Typhi (A B C)	53	51%	25	26		
Heidelberg	49	2%	3	4		
Newport	47	21%	27	14		
Dublin	45	2%	4	6		
Brandenburg	41	3%	13	16	29	2
Kentucky	34	53%	15	19		
Corvallis	30	29%	16	10		
Agona	29	19%	8	5		
Braenderup	29	8%	8	7		
Stanley	29	41%	17	14		
Virchow	28	45%	10	17		
Hadar	24	39%	11	8		
Livingstone	24	0%	7	1	6	
Montevideo	24	21%	18	5		
Napoli	24	9%	8	17		
Rissen	24	13%	6	11	7	
Mbandaka	22	21%	5	5	1	
Goldcoast	22	0%	15	5	2	1
Bovismorbificans	20	13%	15	6	3	
Anatum	16	17%	3	7		5
Oranienburg	16	39%	11	4		
Panama	16	24%	3	11		1
Minnesota	13	n.a.				
Muenchen	13	20%	7	6		
Indiana	12	0%	3	3		
London	12	3%	3	6	4	
Saintpaul	12	21%	6	3		
Senftenberg	12	14%	3	5		
Kottbus	11	20%	5	2		
Poona	11	27%	5	9		
Javiana	10	6%	6	6		
Mikawasima	10	0%	10	1		
Bareilly	6	25%	3	1		
Gallinarum	4	n.a.				
SI 9,12:l,v:2-	1	0%	5	6	5	
Other	225	19%	128	131	8	2

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Table So1. Continued

	Cattle		Poultry		Broiler		Layer	
	2012	2013	2012	2013	2012	2013	2012	2013
N Total	76	54	379	431	126	202	95	57
N tested	72	52	256	276	97	119	79	5
Enteritidis	1	2	76	43	13	11	43	17
Typhimurium	25	13	6	31	2	4	3	1
SI 1,4,[5],12:i:2-	16	14	16	8	7	3	7	3
Infantis		1	103	63	23	21	9	
Paratyphi B. var. Java	1		79	100	43	54	8	
Thompson			1	1				
Derby			5	5	3	3	1	1
(Para)Typhi (A B C)								
Heidelberg			8	91	4	66	3	3
Newport	5	2	2		2			
Dublin	23	16	1				1	
Brandenburg			3	4	2	2		
Kentucky								
Corvallis			4	3				
Agona			5	9	1	2	4	4
Braenderup			4	9		3	3	6
Stanley			1					
Virchow			1		1			
Hadar				3		3		
Livingstone			7	7	3	5	1	2
Montevideo	1	3						
Napoli								
Rissen			2	1	2			1
Mbandaka			6	5	6	2		1
Goldcoast	1			1		1		
Bovismorbificans		1						
Anatum			2	3	1	3		
Oranienburg			1					
Panama		1						
Minnesota			6	14	3	12	1	1
Muenchen								
Indiana			2	3	1	2		
London	1							
Saintpaul			5	1	1			
Senftenberg			4	3	1	2		
Kottbus			3	1	2			1
Poona				1				
Javiana								
Mikawasima								
Bareilly			5				5	
Gallinarum			3	1			3	
SI 9,12:l,v:2-				1				1
Other	2	1	18	19	5	3	3	6

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Table So2. MIC distribution (in %) and resistance percentages (R%) for all *Salmonella's* (N=1906) tested for antibiotic susceptibility during 2013.

Salmonella	MIC (%) distribution mg/L																		
N = 1906	0.015	0 .03	0 .06	0.125	0 .25	0 .5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin						0.9	41.8	27.5	3.6	0.3			25.9						25.9	24-27.9
Cefotaxime			36.3	49.7	9.7	1.3				2.8									3.0	2.3-3.8
Ceftazidime					60.3	34.1	2.7	0.2	0.4	0.5	0.6	1.3							2.8	2.1-3.6
Gentamicin					13.0	69.0	15.0	0.8	0.2	0.2	0.5	0.8	0.6						2.2	1.5-2.8
Kanamycin									95.2	1.9	0.3	0.2			2.3				2.9	2.2-3.7
Streptomycin								1.6	18.2	22.8	26.8	7.7	3.0	3.0	16.9				30.7	28.6-32.8
Tetracycline							5.9	60.5	5.4	0.3		1.3	1.8	24.8					27.9	25.9-29.9
Sulfamethoxazole										35.6	28.1	7.1	0.2	0.1			0.3	28.6	28.9	26.9-31
Trimethoprim						86.4	1.2						12.4						12.4	11-14
Ciprofloxacin	22.5	59.1	1.8	1.5	7.1	5.0	2.0	0.3		0.3	0.5								16.6	15-18.3
Nalidixic acid									77.7	5.1	2.3	0.6	0.0	14.3					14.9	13.3-16.5
Chloramphenicol								0.2	6.2	77.3	11.0	0.8	0.4	4.0					5.2	4.2-6.2
Florfenicol								0.6	44.6	47.1	4.4	1.0	0.9	1.4					3.3	2.5-4.1

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.

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and cefotaxime/ceftazidime have increased compared to 2012.
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 2013. Highest resistance levels were observed in *S.* Heidelberg, the monophasic *S. enterica subspecies enterica* 1,4,[5],12:i:- and *S.* Paratyphi B var. Java, and to a lesser extent in *S.* Typhimurium and Infantis. Generally, *S.* Typhimurium and the monophasic variants have acquired resistance against a number of antimicrobials. The most common resistance pattern was ASSuT. Resistance levels for ciprofloxacin and nalidixic acid were highest in *S.* Heidelberg, Typhi and Paratyphi A (humans only), Infantis and Enteritidis. ESBL producing strains (cefotaxime R) dominated in *S.* Heidelberg from imported poultry products.

Table S03. Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in the Netherlands in 2013.

	Enteritidis (348)	Typhimurium (346)	1,4,[5],12:i:- (226)	Infantis (82)	Paratyphi B var Java (93)	Thompson (29)	Derby (42)	(Para)typhi (A,B,C) (24)	Heidelberg (43)	Newport (18)	Dublin (21)	Brandenburg (28)
Ampicillin	3.7	43.6	85.4	6.1	33.3	3.4	7.1	8.3	74.4	0	9.5	10.7
Cefotaxime	0	1.2	0	1.2	3.2	0	0	0	74.4	0	0	0
Ceftazidime	0	0.3	0	1.2	3.2	0	0	0	74.4	0	0	0
Gentamicin	0	1.7	3.1	1.2	2.2	0	0	0	9.3	5.6	0	3.6
Kanamycin	0	1.7	4.4	2.4	22.6	0	0	0	4.7	5.6	0	3.6
Streptomycin	1.1	37.6	86.7	46.3	86.0	0	26.2	29.2	25.6	5.6	9.5	10.7
Tetracycline	1.7	42.2	92.0	34.1	14.0	3.4	23.8	4.2	95.3	0	9.5	7.1
Sulfamethoxazole	0.9	42.2	87.2	39.0	48.4	3.4	23.8	16.7	95.3	0	4.8	28.6
Trimethoprim	0.3	14.2	10.6	26.8	86.0	3.4	16.7	8.3	2.3	0	4.8	25.0
Ciprofloxacin	20.1	4.9	6.2	46.3	51.6	0	2.4	66.7	90.7	11.1	0	7.1
Nalidixic acid	19.8	4.3	2.2	46.3	49.5	0	2.4	66.7	90.7	5.6	0	3.6
Chloramphenicol	0.3	13.6	9.3	9.8	5.4	0	0	4.2	2.3	0	0	0
Florfenicol	0	11.3	6.2	3.7	0	0	0	0	0	0	0	0

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, 16.6% of *Salmonella* isolates (N = 317) demonstrated a non-wild type phenotype for ciprofloxacin, while 1.2% showed MICs larger than the clinical breakpoint (1 mg/L). The dominant serovars of these ciprofloxacin reduced susceptible isolates were *S.* Enteritidis (22%) predominantly derived from humans, *S.* Java (15%), *S.* Heidelberg (12%) and *S.* Infantis (12%) mainly from poultry sources, or *S.* Typhimurium (5%) and *S.* Kentucky (4%) mainly from humans of which about one fifth is travel related.

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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 limitation of the possibilities for effective treatment of human infections (WHO, factsheet 139, 2005). In 2013, the total number of cefotaxime reduced susceptible (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 57 (3%), among 11 different serovars. In recent years S. Java (mostly recovered from poultry) was the predominant type in cefotaxime resistant *Salmonella*. This year S. Heidelberg was most prominent (32 isolates). In total, 3% of all S. Java isolates were suspected ESBL-producers. In recent years the resistance percentage for cefotaxime was traditionally higher in S. Heidelberg isolates than in S. Java isolates. This also holds for 2013 in which 74% of S. Heidelberg isolates were ESBL-suspected. This was even higher than in 2010/2011 (33%) and 2012(60%), probably due to extra sampling of contaminated poultry from Brazil (for more detailed information on ESBL/AmpC-producers, see Appendix 1).

S. Typhimurium

As shown in Table S01, *S.* Typhimurium represented 17.8% of all human *Salmonella* isolates as characterized by the RIVM in 2013. This is more than in 2012 (11%). 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 animals like pigs and cattle. In 2013 it was relatively frequent isolated from poultry sources as well.

Resistance in *S.* Typhimurium was very high for ampicillin, tetracycline, sulphonamides and streptomycin (Table S04). Resistance to the fluoroquinolones (ciprofloxacin and nalidixic acid) and third generation cephalosporins (cefotaxime and ceftazidime), regarded as clinically important drugs in human medicine, was moderate (between 0.5 - 2 % and 5 - 7% respectively) in isolates from humans, in pigs one (3.6%) and in poultry two (6.3%) ciprofloxacin/nalidixic acid resistant isolates were found. Resistance to chloramphenicol, florfenicol and trimethoprim was common.

Generally, the typical resistance pattern for *S.* Typhimurium DT104 (ACSSuT) was less frequently observed than in previous years. Apparently this clone is replaced by the monophasic ASSuTvariant.

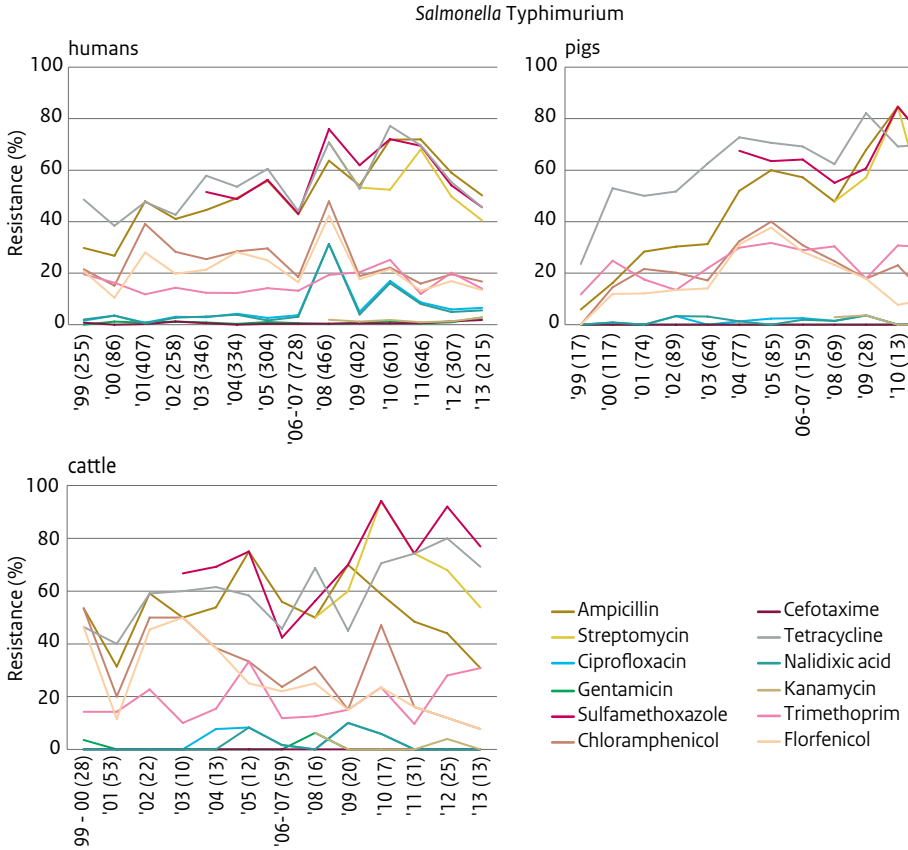
Table S04. Resistance (%) of *S.* Typhimurium isolated from different sources in 2013.

	S. Typhimurium				
	Humans (215)	Cattle (13)	Pigs (28)	Poultry (32)	Food products (57)
Ampicillin	50.2	30.8	60.7	37.5	17.5
Cefotaxime	1.9	0	0	0	0
Ceftazidime	0.5	0	0	0	0
Gentamicin	2.8	0	0	0	0.0
Kanamycin	2.8	0.0	0	0	0.0
Streptomycin	40.5	53.8	35.7	46.9	17.5
Tetracycline	45.6	69.2	57.1	38	17.5
Sulfamethoxazole	45.6	76.9	53.6	37.5	17.5
Trimethoprim	14.0	30.8	25.0	3.1	12.3
Ciprofloxacin	6.5	0	4	6.3	0.0
Nalidixic acid	5.6	0	4	6.3	0.0
Chloramphenicol	16.7	7.7	10.7	9.4	7.0
Florfenicol	13.5	7.7	10.7	9.4	5.3

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With regard to trends, resistance levels in *S.* Typhimurium isolates from human samples have increased over the years until 2010 after which resistance shows a constant decreasing trend (Figure S01). This is probably partially the result of the recent emergence of the monophasic SI 1,4,5,12:i:- variant. 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.

Figure S01. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999 - 2013.



S. Enteritidis

In the Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs. Phage typing, that was used to differentiate between types isolated from Dutch broilers and humans has been replaced by MLVA-typing. The four dominant MLVA-types (03-10-05-04-01, 03-11-05-04-01, 03-09-05-04-01 and 02-10-07-03-02) were found in isolates from humans and poultry of undefined food-products. 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

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infection exist. These are considered to be consumption of contaminated imported eggs and poultry food products and travel abroad (Table S01).
In Dutch broilers the prevalence of *S.* Enteritidis is substantially lower than *S.* Java as shown in Table S01. Although *S.* Enteritidis prevalence varies over the years, it is traditionally much higher in layers than in broiler chickens.

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 in 2013 the level of resistance to quinolones increased in comparison to 2012.

Table S05. Resistance (%) of *S.* Enteritidis isolated from different sources in 2013.

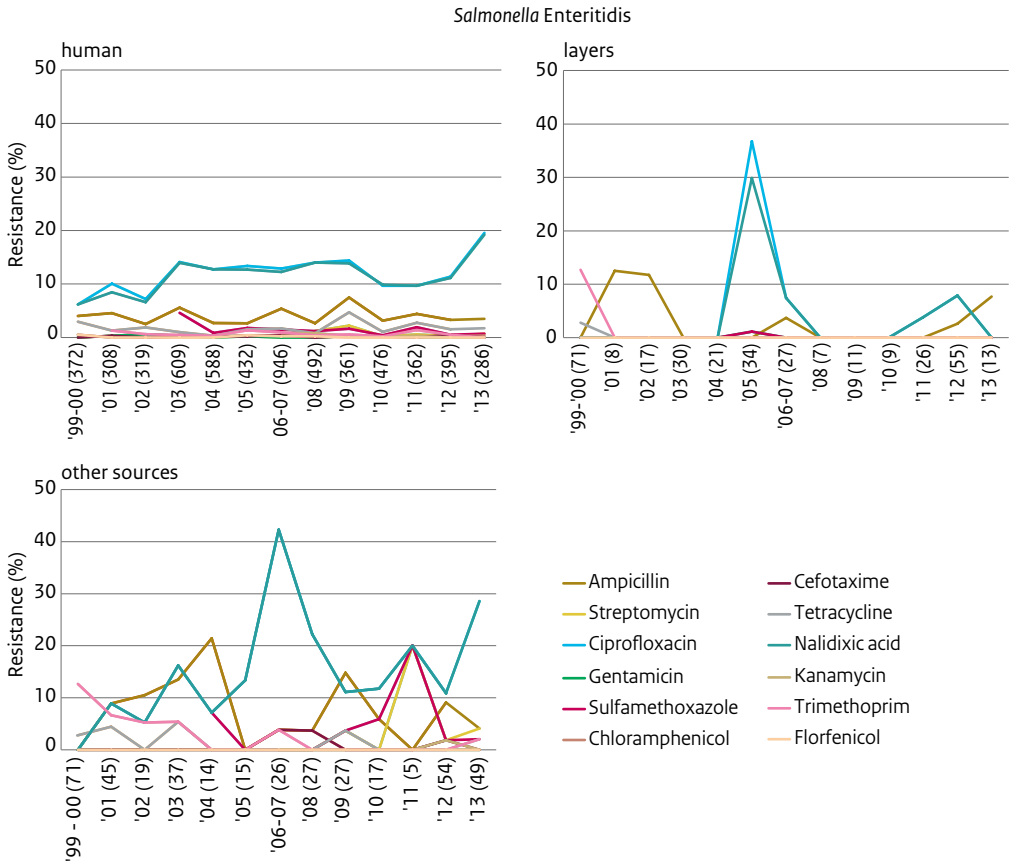
	S. Enteritidis		
	Humans (286)	Laying hens (13)	Other food products (49)
Ampicillin	3.5	7.7	4.1
Cefotaxime	0.0	0	0
Ceftazidime	0.0	0	0
Gentamicin	0.0	0	0
Kanamycin	0.0	0.0	0
Streptomycin	0.7	0.0	4.1
Tetracycline	1.7	0.0	2.0
Sulfamethoxazole	0.7	0.0	2.0
Trimethoprim	0.0	0.0	2.0
Ciprofloxacin	19.6	0	29
Nalidixic acid	19.2	0	29
Chloramphenicol	0.3	0.0	0.0
Florfenicol	0.0	0.0	0.0

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

As in previous years, in 2013 *S.* Java was the most predominant serovar isolated in broiler production. *S.* Heidelberg was recorded more frequently, but this was due to extra sampling of contaminated poultry products from Brazil. (Table S01).
From poultry 56 *S.* Java strains were isolated. 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). The majority of *S.* Java isolates from poultry expressed non-wild type susceptibility to ciprofloxacin (51.8%) and nalidixic acid (48.2%); Resistance to cefotaxime/ceftazidime (ESBL-producers) was detected in 1.8% of the isolates from poultry, which is substantially less than in previous years (11.4%

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Figure So2. Trends in resistance (%) of S. Enteritidis isolated from humans, layers and other poultry sources from 1999 - 2013.



in 2012, 13% in 2010/2011, 22.9% in 2009 and 20.9 in 2008). This mimics the observed decrease of ESBL/AmpC-producing *E. coli* in broilers as a result of the reduction measured implemented on antibiotic use. A number of S. Java strains were isolated from human infections in 2013 (16). All strains tested were trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related.

Salmonella in raw meats from poultry 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 So6, Figure So3). In 2013

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Figure So3. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 - 2013 and humans (Separate data on the right indicate all human *S.* java isolates from 1999-2013)

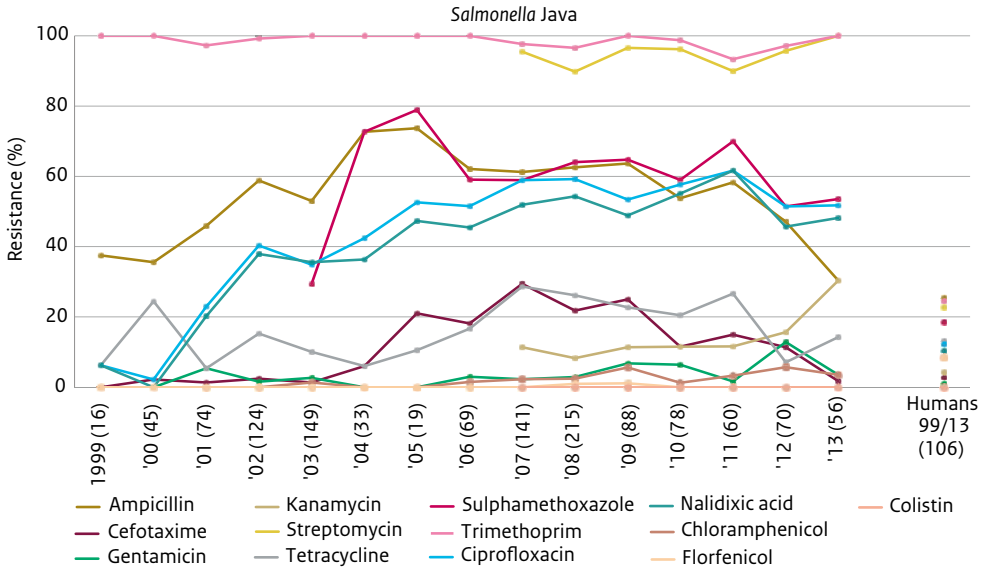


Table So6. Resistance (%) of *Salmonella enterica* isolated from raw meats from poultry and other meat sources in the Netherlands in 2013.

Meat products	poultry S. java	poultry other serovars*	poultry other serovars without S. Heidelberg	other raw meat sources all serovars
	N = 23	N = 114	N = 36	N = 40
Ampicillin	30.4	65.8	25.0	30.0
Cefotaxime	4.3	63.2	16.7	12.5
Ceftazidime	4.3	62.3	16.7	7.5
Gentamicin	0.0	4.4	2.8	5.0
Kanamycin	34.8	4.4	11.1	0.0
Streptomycin	95.7	29.8	30.6	62.5
Tetracycline	8.7	79.8	36.1	45.0
Sulfamethoxazole	60.9	79.8	36.1	35.0
Trimethoprim	95.7	4.4	11.1	10.0
Ciprofloxacin	47.8	85.1	55.6	52.5
Nalidixic acid	39.1	84.2	52.8	50.0
Chloramphenicol	4.3	4.4	11.1	15.0
Florfenicol	4.3	0.9	2.8	12.5

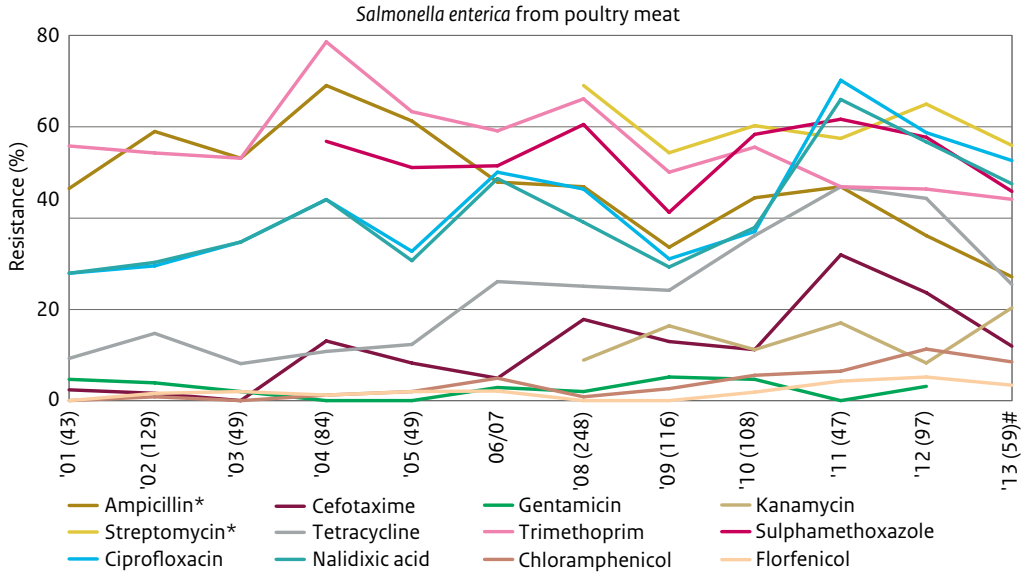
* includes 78 S. heidelberg isolates derived from an import project which includes extra sampling of specific holdings.

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S. Heidelberg was the dominant serovar found in raw meat products (56.9%), followed by S. Java (16.8%) and S. Infantis (7.3%), all from poultry sources. All S. Heidelberg isolates were derived from poultry meat sampled at import (according to article 24 of Counsil Directive 97/78/EC for re-enforced sampling) and were multidrug resistant. Because of the high resistance levels in S. Heidelberg isolates, Table S06 provides resistance data for Salmonella serovars (other than S. Java) with and without S. Heidelbergs. As expected, resistance profiles of S. Java isolates were similar to those from life animals. Noteworthy in poultry meat isolates other than S. Java is the high level of resistance against cefotaxime and ceftazidime, associated with the presence of CMY-2 producing S. Heidelberg (see for details appendix 1). Also resistance to the quinolones in *Salmonella* isolates from raw meat was very high

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 exposure of 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 meats in the Netherlands from 2001-2013.



* *Epidemiological cut off value of ampicillin changed from 4 mg/L to 8 mg/L and the Ecoff of streptomycin from 32 mg/L to 16 mg/L compared to the trend analysis in MARAN 2012.*

\# *S. Heidelberg was excluded from the analysis.*

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3.1.2 Campylobacter

Highlights

As a result of prioritization and changes in legislation, since 2013 the focus of the surveillance of antimicrobial resistance in Campylobacter is solely at poultry and pigs and poultry meat.

1. In *C. jejuni* isolates from broiler's feces for all antibiotics tested the resistance levels determined in 2013 were lower than those of 2012.
2. The resistance level determined in 2013 for ciprofloxacin was 17% lower than that of 2011. Although the ciprofloxacin resistance level is still quite high, the tendency to decrease is a positive signal that the measures initiated in livestock production to reduce total antibiotic use and the use of third-choice drugs, show an effect on the levels of resistance.
3. In *C. jejuni* from poultry meat no decreasing trends were observed. This suggests that part of the meat that was collected at retail, originated from non-domestic sources.
4. Also in human *C. jejuni* in 2013 the resistance level for ciprofloxacin was slightly lower in 2013 compared to 2012. Resistance rates for macrolides in *C. coli* isolates from pigs show a clear decreasing trend from 26% in 2010 to 7% in 2013. This may reflect the decreased use of macrolides (tylosin, tilmicosin, and tulathromycin) in these animals.

This paragraph describes the resistance in *Campylobacter jejuni* and *C. coli* isolated from food animals and from humans suffering from diarrhoea. Samples from food animals (broiler chickens and slaughter pigs), as well as meat samples have been collected by the Dutch Food and Consumer Product Safety Authority (NVWA). MICs have been determined by the Central Veterinary Institute (CVI) for the isolates from live animals, isolates from meat were tested at the NVWA. Data on human isolates were provided by the Dutch National Institute for Public Health and the Environment (RIVM). In previous years also MIC data on isolates from veal calves, dairy cows and turkeys were included. As a result of prioritization and changes in legislation, from 2013 onwards the focus of the surveillance of antimicrobial resistance in Campylobacter is solely at poultry and pigs and poultry meat. Furthermore, in 2013, besides conventionally raised fast growing broilers (fattening period of approximately 42 days), also organic animals (slower growing with a fattening period of approximately 70 days) were included in the surveillance.

In Table C01 the MIC-distributions and resistance percentages are summarized for all *Campylobacter jejuni* and *C. coli* strains isolated at CVI from broilers and pigs in 2013. Table C02 shows the more detailed resistance profiles of *C. jejuni* and *C. coli* according to the different sources (meat as well as from fecal samples from different animal species). Figure C01 and C02 present trends over the last decade in resistance of *C. jejuni* and *C. coli* from the different sampling categories.
National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and Table C03.

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Table C01. MIC distribution (in %) and resistance percentages (R%) for all *Campylobacter jejuni* (N = 167) and C. coli (N = 83) isolated from fecal samples of broilers and pigs in 2013.

C. jejuni	MIC (%) distribution mg/L															R%	95% CI
(N = 167)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin					4.2	28.1	22.2	3.0	9.6	32.9						45.5	37.7-53.3
Gentamicin		100														0.0	0.0
Neomycin			99.4					0.6								0.6	0-1.8
Streptomycin				99.4				0.6								0.6	0-1.8
Tetracycline			47.3	3.6	1.8					13.8	33.5					49.1	41.3-56.9
Sulfamethoxazole							1.8	7.2	26.9	50.3	12.6	0.6			0.6	0.6	0-1.8
Ciprofloxacin	45.5	3.0	2.4	0.6		1.2	20.4	14.4	12.6							49.1	41.3-56.9
Nalidixic acid					3.6	36.5	9.6	0.6	1.2		7.2	41.3				49.7	41.9-57.5
Erythromycin			34.1	45.5	18.0	2.4										0.0	0.0
Clarithromycin			28.1	48.5	18.0	5.4										0.0	0.0
Tulathromycin			96.4	3.6												0.0	0.0
Chloramphenicol					41.9	37.7	15.6	4.8								0.0	0.0

C. coli	MIC (%) distribution mg/L															R%	95% CI
(N = 83)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin					1.2	19.3	48.2	13.3	7.2	10.8						31.3	21.7-41.0
Gentamicin		98.8	1.2													0.0	0.0
Neomycin			96.4								3.6					3.6	0-8.4
Streptomycin				88.0		1.2		3.6	7.2							10.8	4.8-18.1
Tetracycline			44.6	9.6							45.8					45.8	34.9-56.6
Sulfamethoxazole							4.8	9.6	30.1	22.9	3.6		9.6	15.7	3.6	28.9	19.3-38.6
Ciprofloxacin	43.4	7.2	1.2			12.0	9.6	26.5								48.2	37.3-59
Nalidixic acid						33.7	15.7	2.4			37.3	10.8				48.2	37.3-59
Erythromycin			30.1	27.7	16.9	8.4			4.8	3.6	8.4					16.9	9.6-25.3
Clarithromycin			31.3	34.9	9.6	7.2	1.2	2.4	4.8		8.4					8.4	2.4-14.5
Tulathromycin			83.1					1.2	7.2	2.4	6.0					15.7	8.4-24.1
Chloramphenicol					7.2	59.0	31.3		2.4							2.4	0-6

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Table Co2. Resistance (%) of *Campylobacter jejuni* and *C. coli* isolated from raw meat from poultry and from fecal samples of broilers (organic and conventional) and pigs (only C. *coli*) in 2013.

	C. jenuni			*C. coli*			
	Poultry meat	Broilers conven-tional	Broilers organic	Poultry meat	Pigs	Broilers conven-tional	Broilers organic
N	54	113	54	72	214	27	56
Ampicillin	70.4	50.4	35.2	76.4	37.9	40.7	26.8
Gentamicin	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Neomycin	7.4	0.0	1.9	2.8	7.0	0.0	5.4
Streptomycin	5.6	0.9	0.0	11.1	82.7	11.1	10.7
Tetracycline	53.7	49.6	48.1	80.6	85.0	51.9	42.9
Sulfamethoxazole	51.9	0.0	1.9	31.9	54.2	25.9	30.4
Ciprofloxacin	57.4	52.2	42.6	81.9	6.1	48.1	48.2
Nalidixic acid	61.1	53.1	42.6	80.6	8.4	48.1	48.2
Erythromycin	3.7	0.0	0.0	16.7	7.0	18.5	16.1
Clarithromycin	3.7	0.0	0.0	15.3	7.0	11.1	7.1
Tulathromycin	1.9	0.0	0.0	15.3	6.1	18.5	14.3
Chloramphenicol	0.0	0.0	0.0	0.0	0.0	3.7	1.8

It should be noted that data on antimicrobial resistance in isolates from human cases were mainly interpreted using clinical breakpoints, while the quantitative data on antimicrobial resistance in isolates from food and animals were interpreted using epidemiological cut-off values defining the microbiologically resistant isolates. The epidemiological cut-off values discriminate between the wild-type (susceptible) bacterial population and the non-wild type populations which have a decreased susceptibility towards a given antimicrobial. This enables the early detection of developing resistance. However, the use of different thresholds, clinical breakpoints and epidemiological cut-off values, means that resistance data in isolates from humans and in isolates from animals and food may not be fully comparable and interpretation should be done with caution.

Resistance levels

In 2013 the highest resistance levels of *C. jejuni* were observed for ampicillin, tetracycline and the quinolones ciprofloxacin and nalidixic acid. No or very low resistance levels were observed for the aminoglycosides (gentamicin, neomycin and streptomycin), the macrolides (erythromycin, tulathromycin, clarithromycin), sulfamethoxazole and chloramphenicol. The highest resistance levels (> 28%) of *C. coli* were observed for ampicillin, tetracycline, the quinolones and sulfamethoxazole, moderate levels (10 - 20%) for streptomycin and the macrolides and low levels (< 4%) for gentamicin, neomycin and chloramphenicol (Table Co1).

In *C. jejuni* isolates from broilers for all antibiotics tested the resistance levels determined in 2013 were lower than those of 2012. This suggests a positive effect of the reductions in antibiotic use in broilers that were initiated in 2011 and 2012.

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Quinolones

The continuous increasing trend in the percentage of isolates resistant to the quinolones, both in strains from animal origin (Figure Co1 and Co2) and in those from human patients (Figure Co3) has been an increasing public health concern. However, since 2011 *C. jejuni* in isolates from poultry feces show a tendency to decrease from 69.2% in 2011 to 52.2% in 2013 for ciprofloxacin. Although the ciprofloxacin resistance level is still quite high, the tendency to decrease is a positive signal that the measures initiated in livestock production to reduce total antibiotic use and the use of third-choice drugs, show an effect on the levels of resistance. In *C. jejuni* from poultry meat no decreasing trends can be observed. This suggests that part of the meat that was collected at retail, originated from non-domestic sources. Also in human *C. jejuni* in 2013 the resistance level for ciprofloxacin was slightly lower than in 2012 (57.6%). versus 59.4%. However it is too early to conclude that this apparent decrease is associated with the observed trends in isolates from poultry. In *C. coli* from broilers the numbers of isolates tested are too small for trends analysis. In pigs more isolates are tested annually, but in pigs quinolones are not used very frequently. As a result resistance levels in pig isolates are low.

Macrolides

Erythromycin, or other macrolides (clarithromycin (humans), tulathromycin (animals)) it represents, 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 0% of strains from animal origin in 2013 (n=113) and 2.5% of human isolates from 2011-2013 (n=7957) 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), for animal isolates the EUCAST epidemiological cut-off values were used (≥4 for *C. jejuni*, and ≥8 for *C. coli*).

In contrast, in *C. coli* erythromycin resistance levels are much higher. Trends in isolates from poultry and poultry meat are difficult to assess because of the small numbers tested annually. Resistance rates for erythromycin in *C. coli* isolates from pigs show a clear decreasing trend from 26% in 2010 to 7% in 2013. This may reflect the decreased use of macrolides (tylosin, tilmicosin, and tulathromycin) in these animals (Figure Co2).

Broiler chickens (conventional and organic) and poultry meat

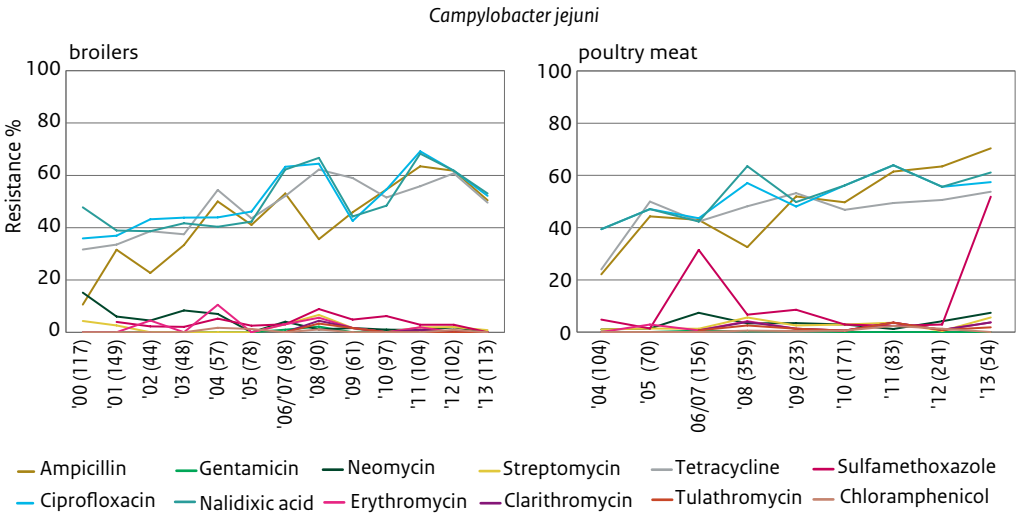
In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from animals as well as from meat samples. This year *Campylobacter* isolated from faeces of both conventional and organic broilers are included. In organic animals the antibiotic use will on average be substantially less than in conventionally raised animals.

As shown in Table Co2, levels of resistance of *C. jejuni* for ampicillin and the quinolones were higher in conventional broilers, whereas tetracycline resistance was equally high and resistance to the other antibiotics included equally low. Apparently the major difference in antibiotic use between the two production systems is use of beta-lactams (such as amoxicillin or phenoxymethyl-penicillin) and quinolones (flumequine and enrofloxacin). In *C. jejuni* isolates from poultry meat the overall resistance rates were higher than in isolates from broilers raised in the Netherlands. This suggests that part of the meat that was collected at retail, originated from non-domestic sources. Specifically the finding of macrolide resistant isolates points towards a foreign source since these isolates are very rarely observed in Dutch broilers. The sudden finding of more than 50% *C. jejuni* isolates from meat resistant to sulfonamides also suggests a foreign origin.

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Resistance rates for most antimicrobials tested in *C. coli* derived from poultry meat were substantially higher than those of *C. jejuni*. The high resistance rates for ampicillin, tetracycline and the quinolones in isolates from poultry meat suggest an also partial foreign source of the samples.

Figure Co1. Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and poultry meat in the Netherlands

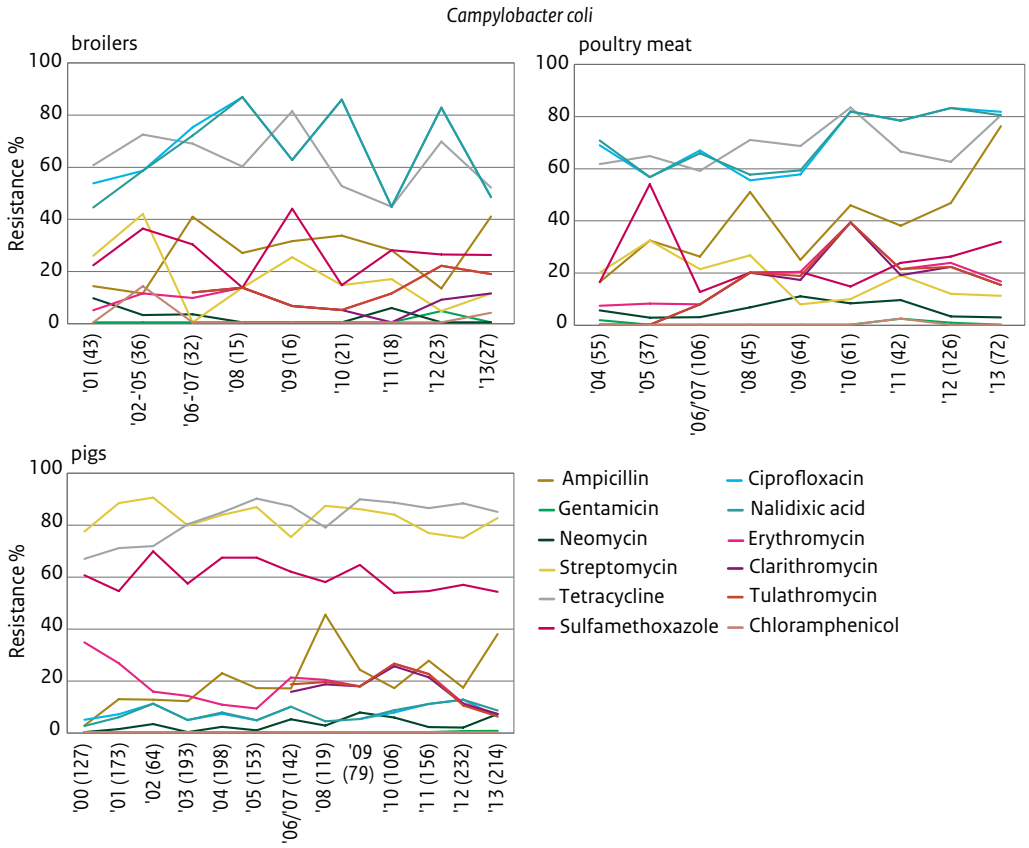


Pigs

In *C. coli* from pigs, as in former years, highest resistance levels were observed for tetracycline (85%), followed by streptomycin (82.7%), and sulfamethoxazole (54%). Resistance to nalidixic acid and ciprofloxacin was relatively low (6.1% and 8.4%, respectively) compared to levels in Dutch broilers (> 80%), probably reflecting the low use of quinolones in swine. Resistance to macrolides was lower than in 2012. Over the last 4 years, these resistance levels have reduced remarkably.

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Figure Co2. Trends in resistance (%) of *Campylobacter coli* isolated from broilers and poultry meat and pigs in the Netherlands



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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 that resistance levels for ciprofloxacin and tetracycline have shown a constant tendency to increase until 2012. However in 2013 slightly less ciprofloxacin resistant *C. jejuni* isolates were detected. Also resistance to erythromycin is slowly increasing, but at much lower levels.

In Table C03 resistance levels, for human isolates 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.

Table C03. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans from 2002 - 2013 from all 16 Public Health Laboratory Services (PHLS) covering >50% of the Dutch population.

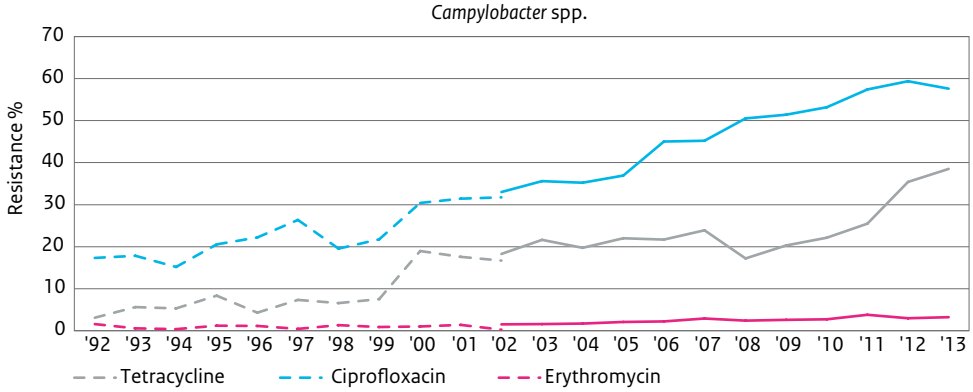
	2002-2005							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	6792	32.7	386	36.3	600	53.5	56	50
Tetracycline	5028	18.5	353	22.7	425	27.1	49	20.4
Erythromycin	5735	1.2	372	3	511	1.6	52	0

	2011-2013							
	Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	8979	57.3	607	59.8	466	69.6	67	67.2
Tetracycline	4505	31.1	304	46.1	101	44.6	20	60
Erythromycin	7603	2.4	465	14	354	4	54	24.1

	C. spp.						
	2013	2012	2011	2010	2009	2008	2002/5
	R%	R%	R%	R%	R%	R%	R%
Fluoroquinolone	57.6	59.4	57	53.3	51.4	50.5	35.2
Tetracycline	38.5	35.4	25.5	22.1	20.3	17.2	20.2
Erythromycin	3.2	3	3.7	2.7	2.6	2.4	1.5

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Figure Co3. Trends in resistance (%) of *Campylobacter spp*. isolated from humans between 1992 and 2013 at the regional PHLS of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900 – 2900.



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

Highlights

1. Over the last ten years, MIC profiles of STEC isolates show a tendency to increase.
2. Most striking was the increases in resistance to tetracycline, streptomycin, sulfamethoxazole, kanamycin and ampicillin.
3. In 2013, 4% of the isolates tested were resistant to the quinolones (ciprofloxacin and nalidixic acid). This was never seen in former years, in which resistance levels to quinolones were always below 1%.
4. In 2013, no ESBL-producing STEC isolates were detected.

In 2013, 143 Shiga-toxin producing *E. coli* O157 (STEC) isolates were tested for susceptibility. Since 2012, isolates were only obtained from human patients and not anymore from cattle. MIC results are presented in Table STEC01 and the trends over time in Figure STEC01.

Trends in resistance

Over the last ten years, MIC profiles of STEC isolates seem to have a tendency to increase as shown in Figure STEC01. Traditionally, resistance levels in *E. coli* O157 have been very low. Most striking increases have been noted over the years for tetracycline streptomycin, sulfamethoxazole, kanamycin and ampicillin. In 2012 resistance levels seemed stable or even decreased, however in 2013 resistance levels for tetracycline and sulfamethoxazole again increased. Remarkable is the occurrence of resistance (4%) to the quinolones (ciprofloxacin and nalidixic acid). This was never seen in former years, in which resistance levels to quinolones were always below 1%.

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Table STEC01. MIC distribution (in %) and resistance percentages (R%) for *E. coli* O157 isolated from humans (N = 143) in the Netherlands in 2013.

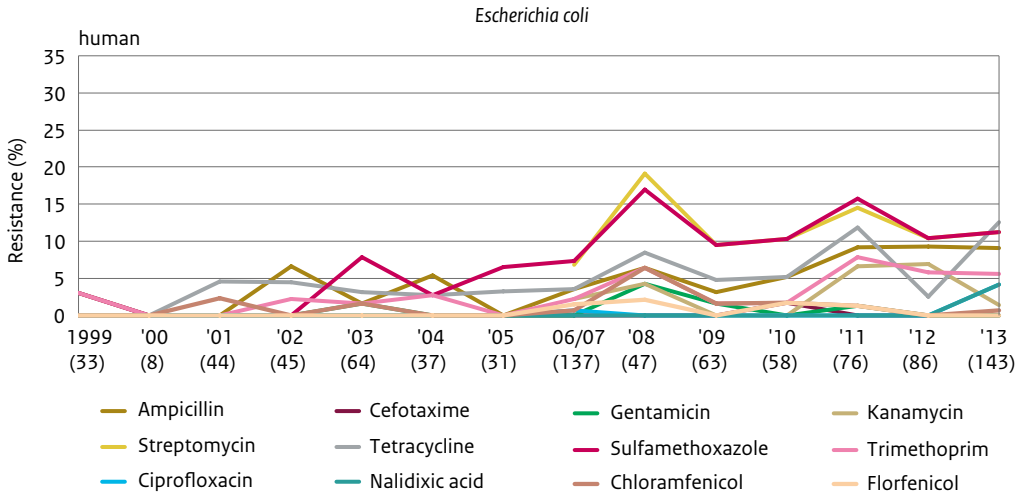
E. coli	MIC (%) distribution mg/L																			
N = 143	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin								4.9	83.2	2.8			9.1						9.1	4.2-13.8
Cefotaxime			79.0	21.0															0.0	0-0
Ceftazidime					94.4	5.6													0.0	0-0
Gentamicin					4.9	76.9	16.1	2.1											0.0	0-0
Kanamycin									94.4	4.2					1.4				1.4	0-3.3
Streptomycin								2.1	50.3	32.9	3.5		2.8	1.4	7.0				11.2	5.9-16.4
Tetracycline								42.7	44.1	0.7			0.7	11.9					12.6	7-18.1
Sulfamethoxazole*										88.8								11.2	11.2	5.9-16.4
Trimethoprim						93.0	1.4						5.6						5.6	1.7-9.4
Ciprofloxacin	57.3	37.8	0.7		1.4	2.8													4.2	0.8-7.5
Nalidixic acid									93.7	1.4	0.7			4.2					4.2	0.8-7.5
Chloramphenicol									4.2	72.0	23.1			0.7					0.7	0-2
Florfenicol									6.3	86.7	7.0								0.0	0-0

** Epidemiological cut off value for sulfamethoxazole changed from 256 mg/L in 2012 to 64 mg/L in 2013.*

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.

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Figure STEC01. Trends in resistance (%) of *E. coli* O157 (STEC) isolated in the Netherlands from humans from 1999-2013.



Beta-lactamases (ESBLs)

In 2010, for the first time resistance to third generation cephalosporins (cefatoxime or ceftazidime) was encountered in one human strain, which is considered to be an indication for the presence of extended spectrum beta-lactamases (ESBLs). In this particular isolate an MIC level of >4 mg/L and >16 was observed for cefotaxime and ceftazidime respectively. In 2013, no ESBL-producing isolates were detected.

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3.2 Commensal indicator organisms

This paragraph describes the susceptibility profiles of commensal micro-organisms of the gastro-intestinal tract. 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 and from 2005 onwards, resistance in isolates from both dairy cattle and veal calves have been included. In the years 2010 and 2011 samples of individual dairy cattle were taken at slaughter houses, in all other years pooled faecal samples were collected at dairy farms. In addition, monitoring programs in veal calves at farms stopped and in 2012 samples of veal calves were taken at slaughterhouses. In 2012 for the first year resistance levels were reported separately for white veal calves and rosé veal calves, respectively. Furthermore, in 2013 besides conventionally raised fast growing broilers (fattening period of approximately 42 days), also organic animals (slower growing with a fattening period of approximately 70 days) were included in the surveillance.

It should be noted, that these sampling strategies imply that these methods 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 percent 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 smaller numbers (< 10^3-10^4 cfu/g faeces) 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. Among indicator *E. coli* from meat and animals, resistance to ampicillin, streptomycin, tetracyclines, sulfonamides and trimethoprim was commonly detected in all host species except dairy cattle.
2. Resistance to antimicrobials recognised as critically important in human medicine, such as the fluoroquinolones and third generation cephalosporins, was predominantly observed in the indicator *E. coli* of poultry sources.
3. In isolates from most animal species a continuous decrease in resistance levels was observed in 2013, most likely as a result of the reductions in antibiotic usage.
4. Resistance to third-generation cephalosporins decreased in most animal species, most likely the result of the vast limitations in usage of cephalosporins in food producing animals.
5. Levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves for almost all antibiotics tested.
6. Levels of resistance in *E. coli* from organic broilers were substantially lower than those from conventional broilers for almost all antibiotics tested.
7. Reduced susceptibility to ciprofloxacin was highest for *E. coli* isolates from broilers.

In this paragraph 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. 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 more non-wild type susceptible isolates being classified as resistant, depending on the MIC distribution and the antibiotic.

Resistance levels

Resistance levels of a total of 1371 *E. coli* isolates obtained from chickens, pigs, dairy cattle, and veal calves, are presented as MIC-distributions in TableEco01 and as resistance percentages per animal species in Table Eco02. Trends in resistance levels from 1998 to 2013 are shown in Figure Eco 01 and information on trends in multidrug resistance is shown in Figure Eco 02.
In addition, resistance levels of 967 *E. coli* isolates collected from meat are presented in Table Eco 03. Trends in resistance of *E. coli* isolated from poultry meat products, beef, pork, veal and lamb in the Netherlands from 2002 to 2013 are presented in Figure Eco 03.
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 conventional broilers, veal calves and slaughter pigs, lowest levels for dairy cattle.
In general, highest resistance is seen for ampicillin, tetracycline, streptomycin, trimethoprim and sulfamethoxazole. These include the drug classes that are most frequently used in veterinary medicine.

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Table Eco 01. MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1371) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2013.

E. coli	MIC (%) distribution mg/L																			
N = 1371	0.015	0 .03	0 .06	0.125	0 .25	0 .5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin						0.1	1.6	18.8	48.4	5.0			26.2						26.2	23.8 - 28.5
Cefotaxime			79.9	18.0	0.7	0.1		0.1	0.1	1.2									1.4	0.7 - 2
Ceftazidime	0.0	0.0	0.0	0.0	94.9	3.9	0.2	0.3		0.1	0.3	0.3							1.2	0.6 - 1.8
Gentamicin					0.6	47.0	43.0	7.2	0.4	0.3	0.7	0.5	0.3						2.2	1.3 - 2.9
Kanamycin									89.4	6.2	1.0				3.4				4.4	3.2 - 5.4
Streptomycin									9.9	48.7	7.6	2.8	6.6	6.6	17.9				33.8	31.2 - 36.3
Tetracycline							6.7	32.0	24.9	0.7	0.4	0.5	5.6	29.2					35.7	33 - 38.2
Sulfamethoxazole*										69.3	0.4				0.1	0.1	1.3	28.9	30.3	27.7 - 32.7
Trimethoprim						69.8	5.5	0.2					24.5						24.5	22.1 - 26.8
Ciprofloxacin	67.4	15.0	0.5	1.2	10.4	3.3	0.7	0.1	0.1	0.6	0.7								17.1	15.1 - 19.1
Nalidixic acid									79.6	2.3	1.0	0.2	2.0	14.7					17.0	14.9 - 19
Chloramphenicol								0.1	8.6	70.8	11.6	1.3	1.8	5.8					9.0	7.4 - 10.5
Florfenicol								0.5	13.0	75.9	8.8	0.7		1.2					1.9	1.1 - 2.6

** Epidemiological cut off value for sulfamethoxazole changed from 256 mg/L in 2012 to 64 mg/L in 2013.*

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 breakpoint.

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Table Eco 02. Resistance (in %) of *E. coli* isolated from faecal samples of conventional (Conv.) and organic (Org.) broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2013.

E. coli N = 1328	Broilers		Pigs	Dairy	Veal calves	
	Conv. (301)	Org. (193)	(289)	(271)	White (160)	Rosé (157)
Ampicillin	56.5	20.7	23.5	0.7	38.1	11.5
Cefotaxime	2.7	2.6	1.7	0.0	0.6	0.0
Ceftazidime	2.7	2.6	1.4	0.0	0.0	0.0
Gentamicin	7.3	0.5	0.7	0.0	2.5	0.6
Kanamycin	8.0	1.6	1.4	1.1	13.1	3.2
Streptomycin	58.1	24.9	50.2	1.1	44.4	14.0
Tetracycline	41.2	27.5	52.6	2.6	72.5	23.6
Sulfamethoxazole	51.2	17.1	43.3	1.1	46.3	17.2
Trimethoprim	40.9	14.5	38.1	0.0	34.4	12.7
Ciprofloxacin	54.5	22.8	0.0	0.0	16.9	0.0
Nalidixic acid	54.2	21.8	0.0	0.0	16.9	0.6
Chloramphenicol	14.6	3.1	10.4	0.0	21.9	5.1
Florfenicol	1.3	0.0	1.7	0.0	6.9	3.8

Quinolones

Reduced susceptibility to quinolones was most commonly encountered in *E. coli* isolated from broiler chickens; 54% of all isolates showed non-wild type susceptibility[2] to nalidixic acid and ciprofloxacin. This indicates a slight increase compared to 50% recorded in 2012. This is surprising given the policy implemented in 2013 to reduce the use of quinolones in poultry and the decrease in resistance observed for most drugs in food-producing animals. In 2012 high level resistance (MIC >1 mg/L) to ciprofloxacin in broiler chickens was detected in 4.3%, which was similar as 4.5% 2012.
The percentage of *E. coli* with reduced susceptibility to ciprofloxacin was 22.8% in organic broilers, 16.9% in white veal calves compared to 0% in rosé veal, pigs, and dairy cattle. This likely reflects the use of quinolones in various animal husbandry systems, although the percentage in organic broilers was higher than anticipated.

Cefotaxime resistance

Resistance to third generation cephalosporins (cefotaxime and ceftazidime), indicative of ESBL producing *E. coli*, was detected in most animal host species except dairy cattle, rosé veal calves and veal and lamb meat included in this survey. Reduced susceptibility levels for cefotaxime ranged from 0.6% in samples from white veal calves to 2.7% in broiler chickens. The data demonstrate a continuous decrease of cefotaxime resistance in broilers which started in 2011 (Figure Eco 01). Among *E. coli* isolated from meat, resistance against third generation cephalosporins in poultry meat sharply decreased from 22.5% in 2011 to 8.0% in 2012. In 2013 the values remained stable at 10.7% (Figure Eco 03). The percentage of

2 a micro-organism is defined as wild type (WT) for a species by the absence of acquired and mutational resistance mechanisms to the drug in question. Wild type micro-organisms may or may not respond clinically to antimicrobial treatment (http://www.eucast.org).

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cephalosporin resistant *E. coli* in poultry meat is considered to partially reflect the percentage of cephalosporin resistance in broilers. However, an undefined part of the meat tested was of not-domestically produced poultry meat, which will affect the levels recorded.

Broilers

In commensal *E. coli* isolated from caecal samples from broiler chickens resistance to all antimicrobials tested was common as summarized in Table Eco02. For all antibiotics except the quinolones and streptomycin a reduction in resistance percentage varying from 0.4% to 13.4% was recorded. However, still very high levels were observed for ampicillin (56.5%), sulfamethoxazole (51.2%), streptomycin (58.1%), trimethoprim (40.9%), the quinolones nalidixic acid (54.2%) and ciprofloxacin (54.5%) and tetracycline (41.2%). The resistance levels in isolates from organic animals were substantially lower for all antibiotics tested, except the 3rd generation cephalosporins (2.6%), which may suggest that there is a common source for these ESBL-producers, originating from the environment of the poultry production pyramid.

Slaughter pigs

In swine very high levels of resistance in *E. coli* isolates in 2012 were recorded for tetracycline (52.6%), streptomycin (50.2%), sulfamethoxazole (43.3%), trimethoprim (38.1%) and ampicillin (23.5%). The tendency to decrease resistance in 2012 has somewhat stabilised in 2013 for most antibiotics tested (Figure Eco 01).
Reduced susceptibility to the 3rd generation cephalosporins was found at low levels in 2013, indicating that ESBLs are still present at low numbers.

Veal calves

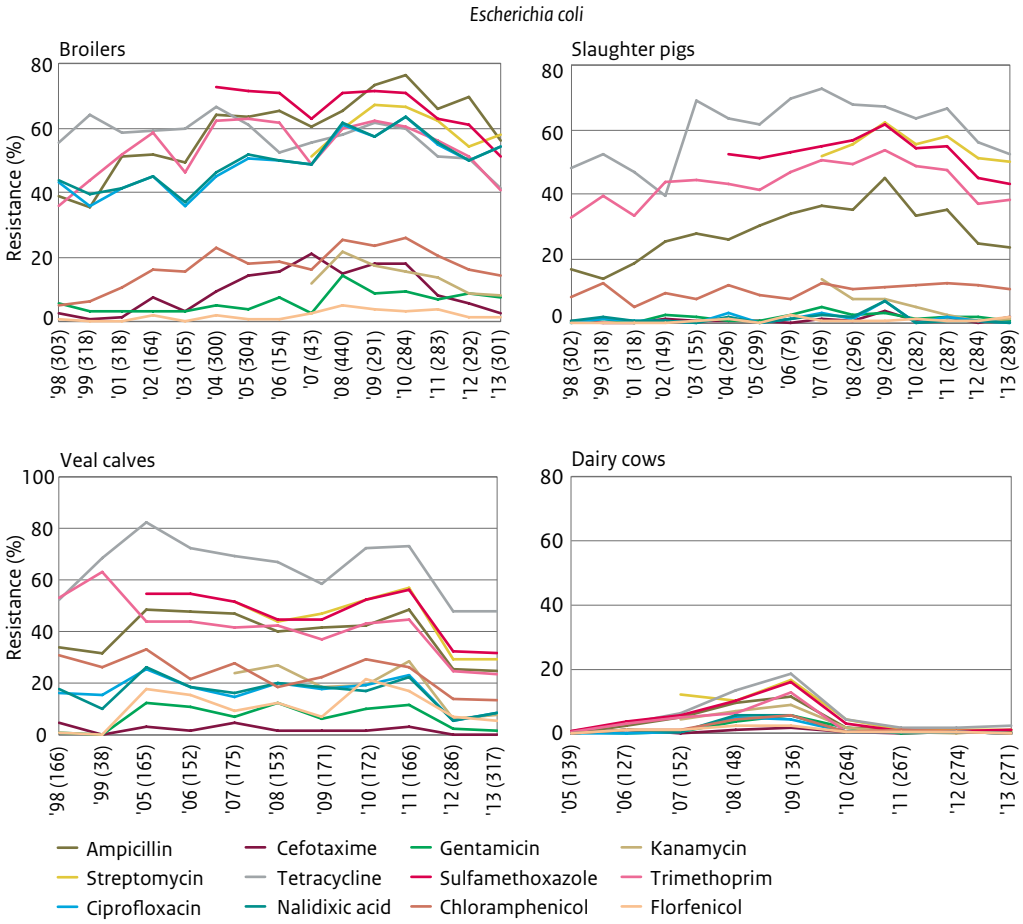
In 2013 as was also done in 2012, we report resistance data on two veal calf husbandry types separately: white veal and rosé veal calves. White veal calves are fattened on a milk diet with a required minimal uptake of roughage, while rosé veal calves are also fed corn silage, straw or pelleted feed. 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 Eco02).
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 2011 varying from 0% to 28%. In 2013 the decrease stabilised. A low resistance rate was recorded for 3rd generation cephalosporins (0.6%) in white veal calves. In rosé animals this type of resistance was even absent. Ciprofloxacin resistance was recorded in 16.9% of *E. coli* from white veal calves, while in rosé veal calves this was 0%.

Dairy cattle

In general, resistance in *E. coli* isolated from dairy cattle is very low compared to resistance levels seen in pigs, broilers and veal calves. The levels of resistance were below 1.2 % for all antibiotics tested except tetracycline (2.6%). In 2013 no resistance to cefotaxime and ciprofloxacin was observed in *E. coli* isolates from dairy cattle.

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Figure Eco 01. Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2013.



Multidrug resistance

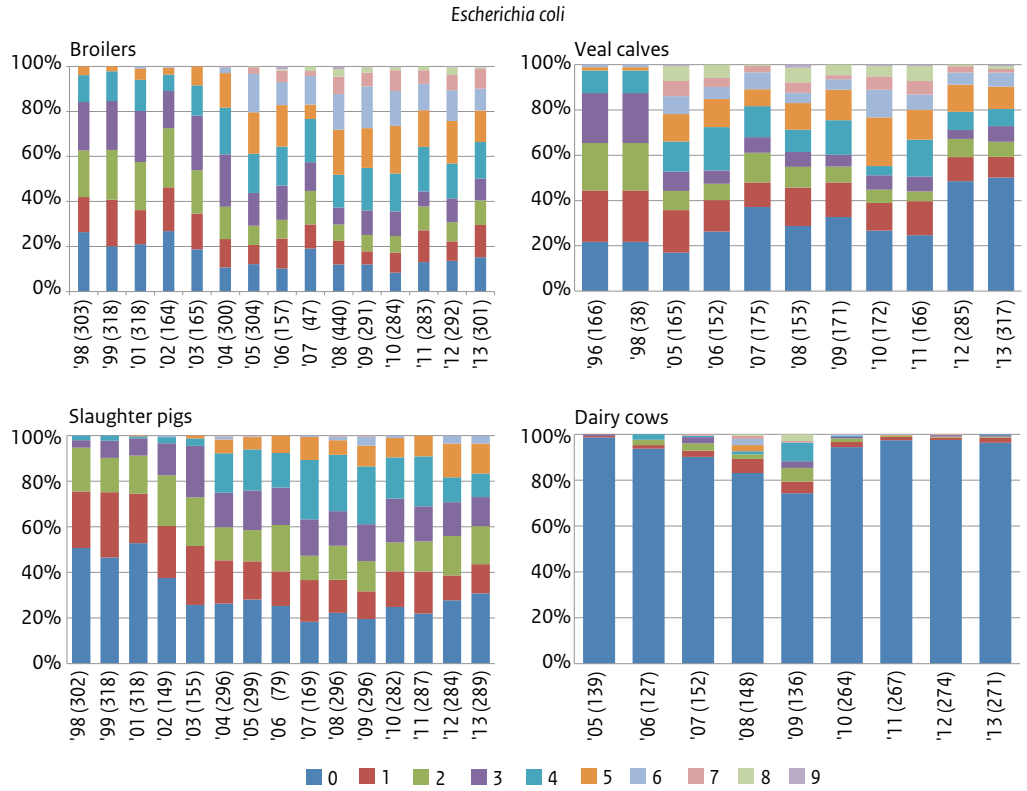
Data on multidrug resistance are shown in Figure Eco 02. The highest level of multidrug resistance was still present among *E. coli* originating from broilers. However, the situation seems to improve slightly. In 2012, more than 70% of the commensal *E. coli* strains from broiler chickens were resistant to two or more classes of antimicrobials included in the survey, while in 2013 this was almost 60%.

Among *E. coli* from veal calves and pigs, multidrug resistance was also common; in veal calves 41.0% (as in 2012) and in slaughter pigs 56.4%. However, the situation has stabilized or even slightly improved since 2010.

For *E. coli* from dairy cattle multidrug resistance was rare, with 1% resistant to two or more antibiotics. After an apparent increase in percentage multidrug resistant isolates up to 2009, the level has stabilized at a low level in the last three years.

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Figure Eco 02. Resistance (%) to 0-9 antimicrobial classes among *E. coli* strains from broiler chickens, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2013.



Overall, the slight increase of the number of totally susceptible *E. coli* isolates in most animal species included in the survey might reflect a more prudent use of antibiotics.

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

Table Eco 03 shows resistance percentages of *E. coli* strains isolated from raw meat products (including poultry, pork, veal, beef, lamb and turkey) sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (VWA), and the trends in resistance are presented in Fig Eco 03. Although the results are more variable than in isolates from faeces, probably due to the annual inclusion of imported meat products, the resistance rates show a slight tendency to decrease over the last 4 years. Cefotaxime resistance is still relatively high at 10.7% in isolates from poultry products, while isolates from pork and beef are incidentally resistant to 3rd generation cephalosporins.
In 2013, resistance percentages of *E. coli* isolated from poultry meat are still high, and have increased compared to 2012. This is possibly due to inclusion of meat from non-domestic sources (Table Eco 02). Resistance rates of *E. coli* from beef and pork samples are stable over the years. Interpretation of data

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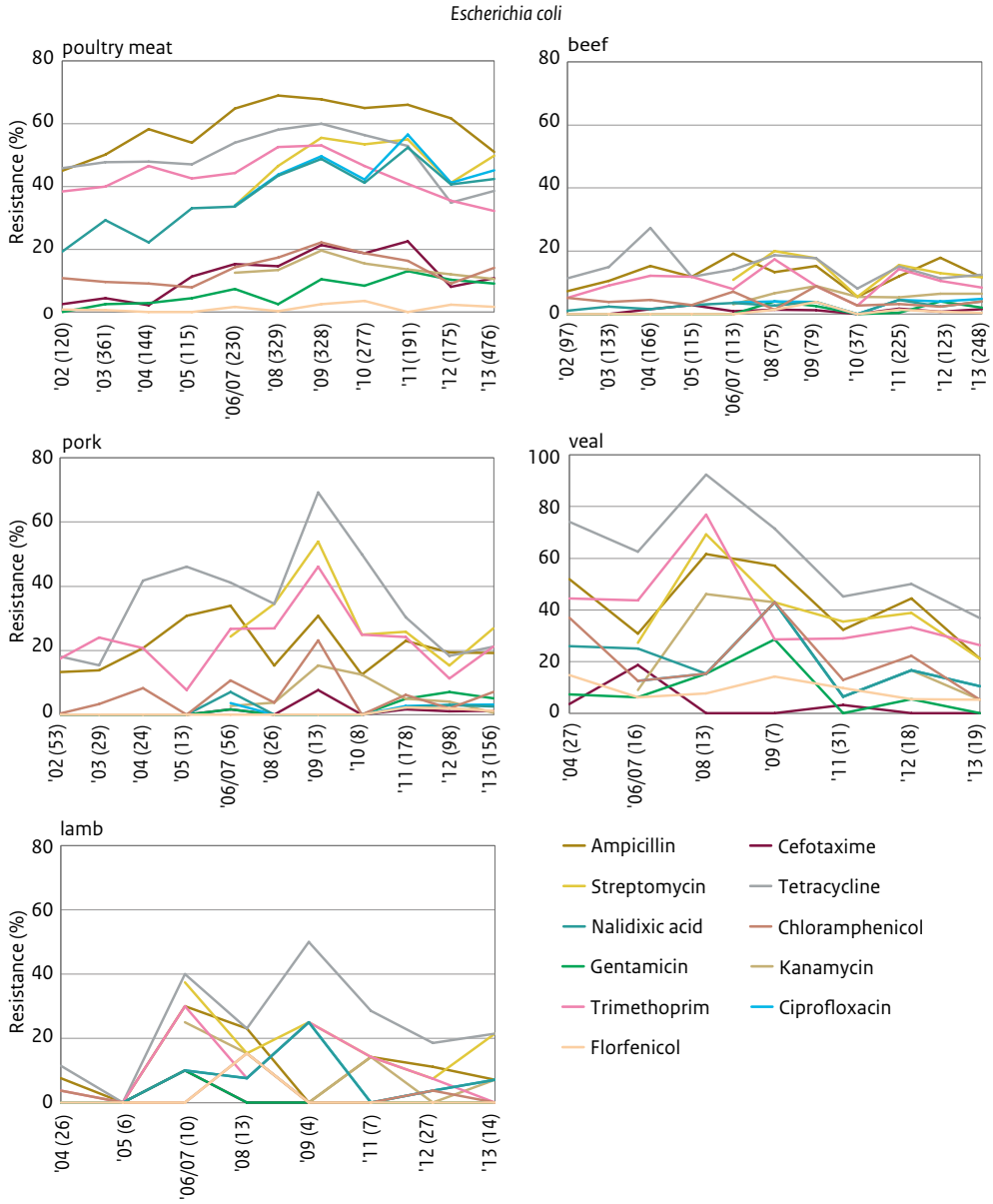
from veal and lamb is complicated by the sometimes low number of isolates from meat products that are tested. This is reflected in the variability in resistance rates over the years as shown in Figure Eco 03.

Table Eco 03. Resistance (in %) of *E. coli* isolated from raw meat products at retail in the Netherlands in 2013.

Meat products	Poultry N = 476	Pork N = 156	Veal N = 19	Beef N = 248	Lamb N = 14	Turkey N = 54
Ampicillin	51.1	19.2	21.1	11.7	7.1	72.2
Cefotaxime	10.7	1.3	0.0	1.6	0.0	3.7
Ceftazidime	10.7	0.6	0.0	2.4	0.0	3.7
Gentamicin	9.0	5.1	0.0	2.0	0.0	11.1
Kanamycin	10.5	0.6	5.3	6.5	7.1	27.8
Streptomycin	49.8	26.9	21.1	11.7	21.4	51.9
Tetracycline	38.7	21.2	36.8	12.5	21.4	53.7
Sulfamethoxazole	46.6	23.1	26.3	12.5	21.4	50.0
Trimethoprim	32.1	21.2	26.3	8.5	0.0	33.3
Ciprofloxacin	45.2	3.2	10.5	4.8	7.1	37.0
Nalidixic acid	42.4	2.6	10.5	4.0	7.1	37.0
Chloramphenicol	14.1	7.1	5.3	4.0	0.0	20.4
Florfenicol	1.7	1.3	5.3	0.4	0.0	3.7

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Figure Eco 03. Trends in resistance (in %) of *E. coli* isolated from raw poultry meat products, beef, pork, veal and lamb in the Netherlands.



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3.2.3 *Enterococcus faecalis* and *E. faecium* in faeces of food-animals

Highlights

1. In 2013 for the first year only isolates from poultry 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. Highest resistance levels were observed for tetracycline (80.5% in *E. faecalis* and 53.7% in *E. faecium*), erythromycin (68.8% in *E. faecalis* and 47.3% in *E. faecium*), and streptomycin (42.5% in *E. faecalis* and 29.8% in *E. faecium*). In *E. faecium*, additional high levels of resistance were observed for quinu/dalfopristin (72.3%), salinomycin (38.5%) and to a lesser extent to ampicillin (21.5%).
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 animals in 2013.

This paragraph 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 2013 *Enterococcus faecalis* and *E. faecium* isolates were isolated from fecal samples of chickens 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 poultry meat are presented as well.

Resistance levels

In 2013 MIC values have been determined for 266 *E. faecalis* and 423 *E. faecium* strains isolated from fecal samples of broilers (both conventional and organic) as well as for 72 *E. faecalis* and 244 *E. faecium* isolates from poultry meat samples. Table Ento1 presents MIC-distributions and Table Ento2 the resistance percentages specified for the isolates from conventional and organic broiler chickens. Trends over the years are depicted in Figure Ento1.
Data for 2013 on *E. faecalis* and *E. faecium* from poultry meats are presented in Table Ent 03. Trends over the years for enterococci from poultry meat sources are presented in Figure Ento2.

Broilers

Highest resistance levels were observed for tetracycline (80.5% in *E. faecalis* and 53.7% in *E. faecium*), erythromycin (68.8% in *E. faecalis* and 47.3% in *E. faecium*), and streptomycin (42.5% in *E. faecalis* and 29.8% in *E. faecium*). In *E. faecium*, additional high levels of resistance were observed for quinu/dalfopristin (72.3%), salinomycin (38.5%) and to a lesser extent to ampicillin (21.5%).
Over the years, resistance to the tested antimicrobials appears to have remained relatively stable in *E. faecalis* with a tendency to decrease for salinomycin. In *E. faecium*, pronounced fluctuations were observed. Resistance to salinomycin decreased briefly and resistance to ampicillin increased substantially from 2006 onwards from less than 5% to 30.4% in 2013. Vancomycin resistance was not detected.

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Table Ent 01. MIC distributions (in %) for *E. faecalis* (N = 266) and *E. faecium* (N = 423) isolated from conventional and organic broilers in the Netherlands in 2013.

E. faecalis	MIC (%) distribution mg/L														R%	95% CI
(N = 266)	0.125	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin			66.5	33.5											0.0	0 - 0.01
Linezolid			39.1	60.9											0.0	0 - 0.01
Tetracycline		14.7	4.9				0.4	26.3	22.6	31.2					80.5	75.5 - 85.3
Erythromycin			17.7	10.2	3.4	2.3	5.3	1.9	1.5	0.4	57.5				68.8	63.1 - 74.4
Vancomycin			66.9	24.4	8.6										0.0	0 - 0.01
Ciprofloxacin		13.9	75.9	6.8	0.8		0.8	1.5	0.4						2.6	0.6 - 4.5
Quino/dalfopristin		1.1	0.4		1.1	23.3	70.7	3.0	0.4						0.4	0 - 1.1
Salinomycin		4.1	33.8	8.6	47.7	5.6									5.6	2.8 - 8.4
Streptomycin									6.0	48.1	3.4			42.5	42.5	36.4 - 48.5
Gentamicin					0.4	22.6	73.3	1.5			0.8	0.4	1.1		2.3	0.4 - 4.0
Chloramphenicol					12.4	85.7	0.4	0.4		0.8					1.1	0 -2.4
Florfenicol			0.4	38.3	60.9	0.4									0.0	0 - 0.01

E. faecium	MIC (%) distribution mg/L														R%	95% CI
(N = 423)	0.125	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin			33.3	27.2	18.0	17.5		0.2	0.2	1.7	2				21.5	17.5 - 25.5
Linezolid			6.1	87.9	5.9										0.0	0 - 0.01
Tetracycline		45.9	0.2		0.2	0.2	2.8	1.4	17.7	31.4					53.7	48.8 - 58.5
Erythromycin			42.1	8.5	2.1	0.5	1.2	0.7			44.9				47.3	42.4 - 52.1
Vancomycin		53.4	41.6	4.0	0.9										0.0	0 - 0.01
Ciprofloxacin		0.7	9.7	20.6	56.5	12.3		0.2							12.5	9.3 - 15.7
Quino/dalfopristin		7.3	20.3	17.5	40.7	13.5	0.5	0.2							72.3	67.9 - 76.6
Salinomycin		0.2	15.1	7.8	38.3	38.5									38.5	33.8 - 43.2
Streptomycin							0.2	3.5	58.4	8.0	0.9	0.9	2.8	25.1	29.8	25.3 - 34.2
Gentamicin					4.5	44.4	46.1	3.3					1.7		1.7	0.4 - 2.8
Chloramphenicol			0.2	11.1	86.3	2.4									0.0	0 - 0.01
Florfenicol				0.2	11.1	72.3	7.3	9.0							0.0	0 - 0.01

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

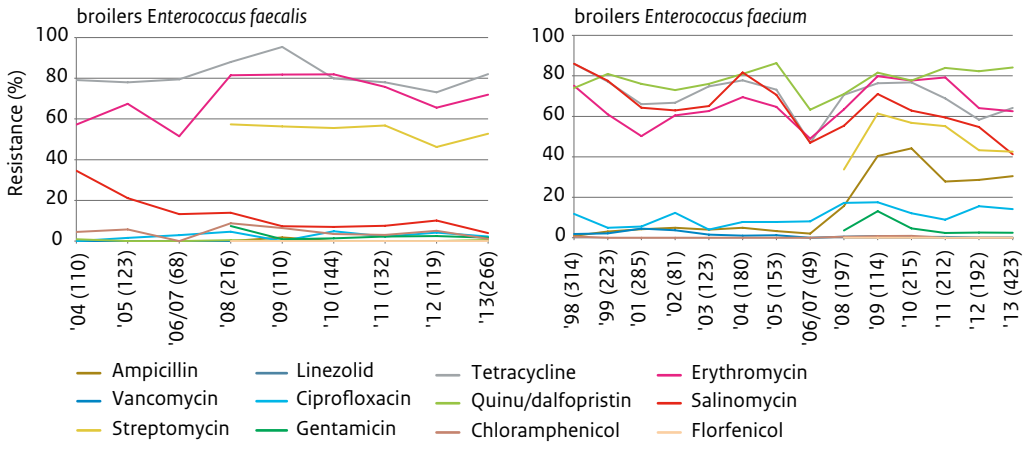
Table Ent 02. Resistance (%) of *Enterococcus faecalis* and *E. faecium* isolated from conventional and organic broilers in the Netherlands in 2013.

E. faecalis	Broiler chickens	
	Conventional N = 178	Organic N = 88
Ampicillin	0.0	0.0
Linezolid	0.0	0.0
Tetracyline	82.0	77.3
Erythromycin	71.9	62.5
Vancomycin	0.0	0.0
Ciprofloxacin	2.2	3.4
Quinu/dalfopristin	0.6	0.0
Salinomycin	3.9	9.1
Streptomycin	52.8	21.6
Gentamicin	1.7	3.4
Chloramphenicol	1.1	1.1
Florfenicol	0.0	0.0

E. faecium	Broiler chickens	
	Conventional N = 240	Organic N = 183
Ampicillin	30.4	9.8
Linezolid	0.0	0.0
Tetracyline	64.2	39.9
Erythromycin	62.5	27.3
Vancomycin	0.0	0.0
Ciprofloxacin	14.2	10.4
Quinu/dalfopristin	84.2	56.8
Salinomycin	41.3	35.0
Streptomycin	42.5	13.1
Gentamicin	2.5	0.5
Chloramphenicol	0.0	0.0
Florfenicol	0.0	0.0

In isolates from organic animals the resistance levels of *E. faecalis* were in the same order as those of conventional animals, except for streptomycin where the level was much lower in organic animals (52.8 versus 21.6%, respectively). For *E. faecium* all resistance levels where substantially lower in isolates from organic animals, the result of less selective pressure through minimal antibiotic use in the animals.

Figure Ent 01. Trends in resistance (%) of *Enterococcus faecium* and *E. faecalis* isolated from conventional broilers in the Netherlands from 1998-2013.



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3.2.4 *Enterococcus faecalis* and *E. faecium* in raw meat products of food-animals

Table Ent 03 shows resistance percentages of *E. faecalis* and *E. faecium* strains isolated from raw poultry meat products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (NVWA).

Table Ent 03. Resistance % of *Enterococcus faecalis* and *E. faecium* strains isolated from raw meat products from poultry in the Netherlands in 2013.

	Poultry meat	
	E. faecalis (N = 224)	*E. faecium* (N = 72)
Ampicillin	1,8	9,7
Linezolid	0,0	1,4
Tetracyline	75,0	38,9
Erythromycin	51,8	43,1
Vancomycin	0,0	0,0
Ciprofloxacin	3,1	23,6
Quinu/dalfopristin	2,2	72,2
Salinomycin	8,9	41,7
Streptomycin	37,5	22,2
Gentamicin	2,7	1,4
Chloramphenicol	0,4	0,0
Florfenicol	0,0	2,8

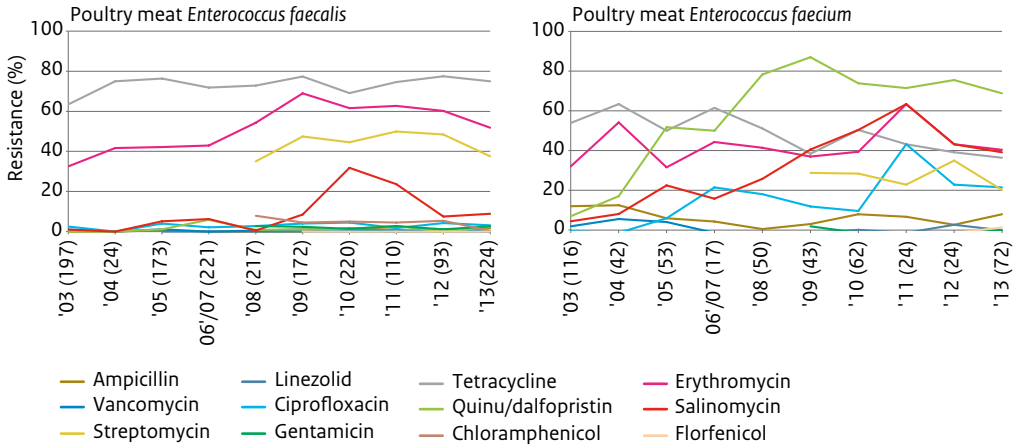
As in previous years, resistance in *E. faecalis* and *E. faecium* isolated from fresh poultry meat was not always comparable to resistance levels in isolates recovered from fecal samples from broiler chickens. For erythromycin in *E. faecalis* and ampicillin, tetracycline and streptomycin in *E. faecium* resulted this in lower resistance levels as found in isolates derived from feces. For ampicillin and quino/dalfopristin in *E. faecalis* and linezolid, ciprofloxacin and florfenicol in *E. faecium* resulted this in higher resistance levels compared to isolates recovered from feces. Moreover, the isolation rates differ between feces and meat. In meat samples *E. faecalis* is more frequently isolated than in feces. 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 feces and that data from feces cannot be one-in-one translated to data from meat and potential risks associated with the data. Variable resistance levels were observed among *E. faecalis* and *E. faecium* isolated from poultry meat (Table Ento3). Tetracycline resistance among *E. faecalis* was 75% in poultry meat and among *E. faecium* 38.9%. Erythromycin resistance levels were high in both species, 51.8 and 43.3%, respectively. Resistance to salinomycin and quinu/dalfopristin was highest in *E. faecium.*

Vancomycin resistance was not observed and reduced susceptibility to linezolid was detected in one *E. faecium* isolate.

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Trends over time are fairly stable for *E. faecalis* from different sources. The resistance percentages in *E. faecium* demonstrate more fluctuation over the years (Figure Ento2). Resistance to quinu/dalfopristin, ciprofloxacin and salinomycin showed a tendency to increase in *E. faecium* until 2011, after which they stabilized and slightly decreased.

Figure Ent 02. Trends in resistance percentages in *E. faecalis* and *E. faecium* isolated from raw meat products from poultry in the Netherlands from 2003-2013.



The overall differences between resistance levels in animals remain noteworthy and might suggest that certain selection pressures could favor the selection of certain biotypes in meat. Also meat from non-domestic sources may have biased the results.

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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 2013

Highlights

1. Prevalence of ESBL-producing *E. coli* from broilers using non-selective methods has decreased in 2013 (to 2.7%) compared to former years (18.3% in 2011 and 8% in 2012).
2. Selective isolation of ESBL-producing Enterobacteriaceae in faeces from batches of veal calves and slaughter pigs and individual dairy cows resulted in 46.1%, 57% and 7% ESBL-prevalence, respectively. In veal calves and slaughter pigs this suggests a slight decrease of ESBLs at farm level, although future sampling must reveal whether this is just a variation in results or indeed a decrease in prevalence.
3. This decreasing trend in ESBL-prevalence was not seen in targeted surveillance of meat which might be explained by e.g. the level of cross-contamination at meat processing and the inclusion of imported meat in the surveillance.
4. The prevalence of ESBL-producing *Salmonella* was in 2013 4%, which is more than two times as high as in previous years. This can mainly be attributed to an extra import project in which poultry meat from South America was extra sampled
5. In 2013, targeted screening for carbapenemase-producing strains in all faecal samples (>1000) from broilers, veal calves, slaughter pigs and dairy cows did not result in isolates with plasmid-mediated carbapenemase genes.

4.1 ESBL-producing bacteria

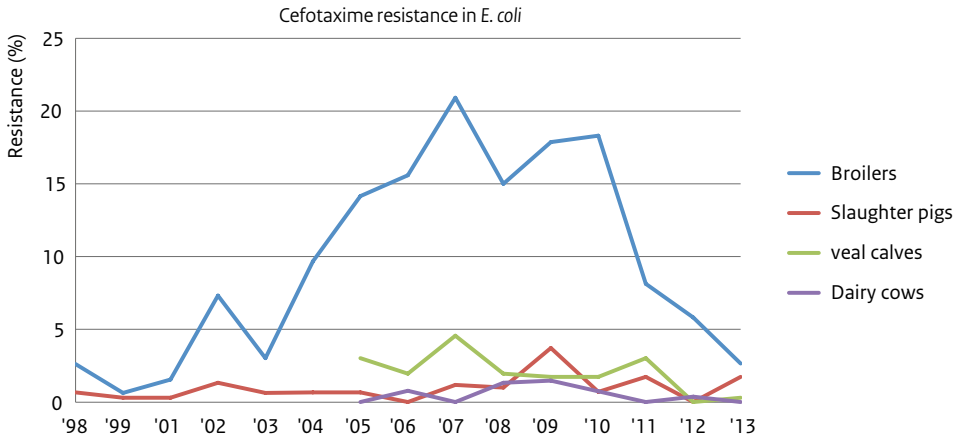
Surveillance of resistance to extended spectrum cephalosporins in the Netherlands is routinely done by random isolation of a minimum of 170 isolated *E. coli*, each representing one epidemiological unit, from faecal samples of food producing animals as prescribed by EFSA guidelines[1]. These isolates are tested for

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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susceptibility to cefotaxime and ceftazidime. Proportions of non-wild type isolates are determined based on EUCAST epidemiological cut-off values.
Since 1998 cefotaxime reduced susceptibility was observed at low levels in all animal species. Figure ESBL01 shows the percentage of cefotaxime non-wildtype phenotype in randomly picked *E. coli* isolates selected from non-selective media derived from broilers, slaughter pigs (1998 - 2013), veal calves and dairy cows (2005 - 2013). 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 decreased in 2010, and declined even further in 2011, 2012 to 2.7% in 2013. This is most likely the result of decreased usage of antibiotics in broilers and the fact that since spring 2010 no ceftiofur was used (off label use) at Dutch hatcheries.

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



From a total of 1371 *E. coli* isolates that were tested in 2013, eighteen displayed cefotaxime reduced susceptibility (see also 3.2.1). As also seen in Table ESBL01, thirteen were isolated from poultry (five from organic and eight from conventional broilers), one from veal calves (white), four from slaughter pigs and none from dairy cows. These isolates were screened for beta-lactamase gene families using the Check-Points CT101 miniaturised micro-array. Subsequently the genes were identified by dedicated PCR and sequence analysis. All isolates with a negative array result for ESBL or AmpC genes were examined for promoter mutants in the chromosomal *ampC*-genes. The results of this molecular typing are displayed in Table ESBL01. In the poultry isolates four types were almost equally present: $bla_{CTX\text{-}M\text{-}1}$ (n = 3), $bla_{TEM\text{-}52}$ (n = 4), $bla_{SHV\text{-}12}$(n = 3) and $bla_{CMY\text{-}2}$(n = 3). All four isolates from pigs contained $bla_{CTX\text{-}M\text{-}1}$. The isolate with cefotaxime reduced susceptibility from veal calves contained no plasmidic ESBL gene, but showed mutations in the promoter region of the chromosomal *ampC* gene (*ampC*-type-18).
In Table ESBL01 an overview is given of the different ESBL-genes found over the years since 2007. In all years most isolates were recovered from broiler faeces. In 2009 also a substantial amount of isolates were derived from faeces collected from slaughter pigs. In all years $bla_{CTX\text{-}M\text{-}1}$ was predominantly found. $bla_{CTX\text{-}M\text{-}9}$ and $bla_{TEM\text{-}20}$ (both found in *E. coli* from broilers) were only sporadically found and do not seem to play a role in the spread of ESBL enzymes in food-producing animals. On the other hand, next to $bla_{CTX\text{-}M\text{-}1}$,

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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 2007-2013.

Year	ESBLs isolated from					Total ESBL (n)	ESBL-genes detected										Total *E.coli* (n)	% ESBL of total *E. coli*
	Broiler	Veal calves	Slaughter pigs	Dairy cows	Turkey		CTX-M-1-group#	CTX-M-2	CTX-M-9	TEM-52(c)	TEM-20	SHV-12*	SHV-2	CMY-2	chromosomal *ampC*	no gene found		
2007	9	6	2			17	11	1		3			1	5	2	2	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
Total	242	23	27	7	6	305	120	19	1	35	2	37	9	41	15	26		

\# 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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bla$_{TEM-52}$, *bla*$_{SHV-12}$ and *bla*$_{CMY-2}$ were detected, almost every year and are still detected in 2013, indicating successful spread of these resistance genes among food-producing animals.

Active surveillance of ESBLs in 2013

Since 2011, prevalence studies of ESBL/AmpC-producing *E. coli* were initiated in Dutch food-producing animals (veal calves, dairy cows and pigs) in close collaboration between the Dutch Food and Consumer Product Safety Authority (NVWA) and the Central Veterinary Institute (CVI). At Dutch slaughterhouses a faecal sample was taken from ten (apparently healthy) animals per slaughter batch of animals. In 2013, 93 batches of slaughter pigs were sampled, 89 batches of veal calves and 93 individual dairy cows, each representing a different farm. Moreover, 1932 meat samples were analysed for ESBL/AmpC-producing *E. coli*.

Each faecal sample was analysed for the presence of ESBL/AmpC-producing *E. coli* using selective pre-enrichment in Luria Bertani broth with 1 mg/L cefotaxime, followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime by the NVWA. The pre-enrichment of the meat samples was followed by selective isolation on both MacConkey agar with 1 mg/L cefotaxime and Oxoid ESBL brilliance agar plates by the NVWA. From each plate colonies with the typical morphology of *Enterobacteriaceae* were selected and sent as pure cultures to CVI for identification of the bacterial species and confirmation of the ESBL/AmpC-genes present. One positive isolate per flock was screened for beta-lactamase gene families as described above.

Table ESBL02. Beta-lactamases detected in slaughter batches of veal calves (N = 89), pigs (N = 93) and individual dairy cows (N = 100) sampled at slaughter in the Netherlands in 2013.

N animal positive	Veal calves		Slaughter pigs		Dairy cows		
	N batches	%	N batches	%		N	%
0	48	53.9	40	43.0	neg.	93	93.0
1	5	5.6	14	15.1	pos.	7	7.0
2	9	10.1	13	14.0			
3	3	3.4	4	4.3			
4	6	6.7	10	10.8			
5	6	6.7	2	2.2			
6	3	3.4	4	4.3			
7	1	1.1	1	1.1			
8	4	4.5	1	1.1			
9	3	3.4	1	1.1			
10	1	1.1	3	3.2			
Total	89		93			100	
Batch prevalence		46.1%		57.0%			Not applicable

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ESBLs in faeces

Table ESBL02 shows the prevalence of ESBL-producing *E. coli* at slaughter batch level in 2013. In 46.1% of the veal calves batches examined and in 57% of slaughter pigs batches ESBL-producing *E. coli* were detected. The number of positive animals varied from 1 to 10 all animals per slaughter batch. In individually sampled dairy cows the prevalence of animals positive for ESBL-producing *E. coli* in their faeces was 7%. Table ESBL03 shows the prevalence found in the last three years. Since the start of this surveillance program in 2011, batch prevalence in pigs was highest in 2012 (75%) and decreased in 2013 to 57%. In veal calves the batch prevalence decreased in 2013 from 70% in 2011 and 2012 to 46% in 2013. In individual dairy cows the prevalence seems stable in 2012 and 2013 (between 7 and 8 %). Future sampling will reveal if the decreasing trends will continue.

Table ESBL03. ESBL *E. coli* farm prevalence (%) detected in slaughter pigs, veal calves and dairy cows in the Netherlands from 2011-2013.

	Pigs#	Veal calves#	Dairy cows*
2011	68	70	14
2012	75	70	8
2013	57	46	7

Per farm 10 animals were tested

* Individual animals, each representing a different farm were tested.

Table ESBL04 shows the ESBL/AmpC genes detected in the faeces of these animal species. A wide variation in beta-lactamase genes was identified. $Bla_{CTX\text{-}M\text{-}1}$, was the dominant variant in the animal species examined. In pigs the variation in genes was less than found in 2012. $bla_{TEM\text{-}52c}$ was frequently detected and two other variants $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CMY\text{-}79var}$ found in a *Citrobacter freundii* isolate were inciden-

Table ESBL04. Beta-lactamases identified in *E. coli* from veal calves, pigs and dairy cows in 2013. Data derived from the active surveillance of ESBL-producing *E. coli*.

		Veal calves	Slaughter pigs	Dairy cows	Total
CTX-M-1 group	CTX-M-1	17	26	1	44
	CTX-M-3	2			2
	CTX-M-15	3			3
	CTX-M-32	2		1	3
CTX-M-2 group	CTX-M-2		2		2
CTX-M-9 group	CTX-M-14	4			4
TEM	TEM-52c		5		5
CMY	CMY-2	3			3
	CMY-79-var		1		1
Chromosomal *ampC*	*ampC*-type-3	5	8	1	14
	ampC-type-34		5		5
Total		36	47	3	86

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tally found in pig isolates. In veal calves there was more variation in ESBL-types found. Next to *bla*$_{CTX-M-1,}$ *bla*$_{CTX-M-14,}$ which is considered a typical 'human' variant, was most predominant. Other types included the typical 'human' ESBLs *bla*$_{CTX-M-15, -32}$ and $_{-3}$. Promotor mutants of chromosomal *ampC*-genes were detected in all animal species.

ESBLs in raw meat products

Table ESBL05 shows the prevalence of ESBL suspected isolates in meat. The prevalences are compared to data from 2012. It is very important to distinguish between isolates that are ESBL-suspected and ESBL-confirmed. This 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 and not ESBL-positive. The vast majority of the species isolated that were not *E. coli* were negative for ESBLs/AmpCs. For this reason a genetic confirmation of ESBLs perceived to be present is essential. In 23% of the raw meat samples ESBL/AmpCs were confirmed to be present. Highest prevalence was observed in poultry meat (83%), although the prevalence was still lower than previously reported (84-100%) in the Netherlands by Cohen Stuart *et al* in 2012, it was somewhat higher than found in 2012 (73%). This may be due to sampling bias that varies between years. Thirty five percent of turkey meat was found positive (in 2012 this was 29%) while in beef and pork the prevalence of confirmed ESBLs was comparable to 2012 (respectively 5% in 2013 versus 6% in 2012 and 2% versus 1%). Surprisingly, in crocodile meat 4/10 (40%) of the isolates were confirmed ESBL producers. In kangaroo meat no ESBLs were detected.

Table ESBL05. ESBL-suspected and confirmed isolates from raw meat products in the Netherlands in 2013, prevalence (%) are compared to 2012.

Animal source	N total	ESBL suspected	tested	ESBL confirmed	% ESBL positive in 2013*	% ESBL positive in 2012
Beef	408	71	71	20	5%	6%
Pork	695	98	98	11	2%	1%
Chicken	728	636	118	112	83%	73%
Turkey	80	37	21	16	35%	29%
Crocodile	10	6	6	4	40%	-
Cangaroo	11	1	1	0	0%	-
Total	1932	849	315	163	23%	21%

* percentage is extrapolated to N total

Like in 2012, the ESBL/AmpC genes identified in the raw meat samples showed more variation than in isolates from faecal samples (Table ESBL06). Still *bla*$_{CTX-M-1}$, was by far the dominant variant both in meat and faecal samples. This strongly suggests that faecal contamination during slaughter or processing of the meat was the source of these genes. Other frequently found genes in isolates from meat were *bla*$_{CTX-M-2}$, *bla*$_{SHV-12}$ and *bla*$_{TEM-52}$, all typically associated with the food animals the meat originates from. The finding of poultry meat positive for *bla*$_{CTX-M-8}$ and *bla*$_{CTX-M-2}$ suggest that these meat samples were imported from South America, where these variants are known to dominate in poultry.

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Table ESBL06. Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2013.

	ESBL gene	Poultry	Beef	Pork	Turkey	Crocodile	Total
CTX-M-1 group	CTX-M-1	53	8	7	3	1	72
	CTX-M-3	2					2
	CTX-M-15	2	3		3		8
	CTX-M-55				1		1
	CTX-M-32	2	1	1			4
	CTX-M-32, TEM52cVar			1			1
CTX-M-2 group	CTX-M-2	10	1		3		14
CTX-M-8 group	CTX-M-8	3		1	1		5
CTX-M9 group	CTX-M-9	2					2
	CTX-M-14		3				3
TEM	TEM-52c	10					10
	TEM-52cVar	6			3		9
SHV	SHV-12	16	2	1	2	2	23
	SHV-12, CMY-2	1					1
	SHV-12, TEM-52c	1					1
	SHV-12, SHV-2a, TEM-52c	1					1
CMY-2	CMY-2	3	2			1	6
Total		112	20	11	16	4	163

ESBL-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-, phage or MLVA-typing were tested for susceptibility to cefotaxime and ceftazidime. The cefotaxime reduced susceptible *Salmonella* isolates were mainly from human and poultry sources. The prevalence of ESBL-producing *Salmonella* was in 2013 4%, which is more than two times as high as in previous years. This can mainly be attributed to an extra import project in which poultry meat from South America was over sampled. This was done according to article 24 of Counsil Directive 97/78/EC for re-enforced sampling of suspected batches. These samples were often positive for ESBL-producing S. Heidelberg isolates. Next to this serovar, a wide variation of 10 other serovars was identified to carry ESBLs. In these isolates the genes were identified as described above for *E. coli*. Table ESBL07 shows that in contrast to other years the poultry associated S. Paratyphi B Java variant which is often recognized as ESBL-producer in the past was only found once in poultry in 2013. As described, ESBL-producing S. Heidelberg was most prevalent carrying predominantly bla_{CMY-2}, which is frequently reported in South-America. Also the finding of $bla_{CTX-M-8}$ points in the direction of an import source as this gene is predominantly present in South America. In isolates from human sources a variety of ESBL-genes were found: bla_{CMY-2}, $bla_{CTX-M-65}$, $bla_{CTX-M-15}$, $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$. Table ESBL08 shows that these isolates were all highly multidrug resistant, which could affect the success of a therapy in infected humans.

In Table ESBL09 the ESBL-types found in *Salmonella* since 2007 are summarized. Every year genes belonging to bla_{CMY-2}, $bla_{CTX-M-2}$, bla_{TEM-52} and the $bla_{CTX-M-1}$-group, were found in several *Salmonella* isolates derived from different sources. The relatively high prevalence of bla_{CMY-2} positive isolates in 2013 can be attributed to the extra sampling of imported meat from South America.

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Table ESBL07. Beta-lactamases in *Salmonella* isolated in 2013.

Serovar	Humans	Poultry	Other	CTX-M-1 group	CTX-M-2 group	CTX-M-8 group	CTX-M-9 group		TEM		CMY		Total
				CTX-M-15	CTX-M-2	CTX-M-8	CTX-M-9	CTX-M-65	TEM-52c-Var	TEM-20	CMY-2	CMY-2-Var	
Agona	1	1	2			3					1		4
Anatum		2				2							2
Braenderup		4							4				4
Heidelberg		27	4		2						29		31
Infantis	1							1					1
Isangi	1			1									1
Kentucky	1											1	1
Minnesota		4									4		4
Paratyphi B var Java	1	1	1		1				1	1			3
Saintpaul		1									1		1
Typhimurium	3						3						5
Total	8	40	7	1	3	5	3	1	5	1	35	1	55

Table ESBL08. Resistance and multidrug resistance percentages of ESBL-producing *Salmonella* in the Netherlands in 2013.

	R%
Ampicillin	100
Cefotaxime	100
Ceftazidime	93
Gentamicin	11
Kanamycin	14
Streptomycin	32
Tetracycline	74
Sulfamethoxazole	75
Trimethoprim	16
Ciprofloxacin	81
Nalidixic acid	75
Chloramphenicol	12
Florfenicol	2

Multi drug resistance	N = 57
0	0%
1	0%
2	9%
3	9%
4	5%
5	49%
6	12%
7	5%
8	5%
9	5%

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Table ESBLog. ESBL-genes found in *Salmonella* isolates displaying reduced susceptibility to cefotaxime derived from human and chicken sources during 2007-2013.

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
Total	74	43	6	11	43	4	10	80	3	274		

\# contains CTX-M-1 (n = 59, in all years), CTX-M-55 (n = 6, 2008-2010, 2012), CTX-M-15 (n = 6, 2011-2013), CTX-M-3 (n = 3, 2010, 2012).

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

* contains CTX-M-9 (n = 6, 2008-2009, 2012-2013), CTX-M-14 (n = 4, 2009-2012) and CTX-M-65 (n = 1, 2013).

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

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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. 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. However, the dominant human ESBL (CTX-M-15) is only rarely found in animals or their products. This suggests that the attribution of ESBLs from food-animal sources is a relative small one. $Bla_{CTX\text{-}M\text{-}1}$ was the predominant ESBL gene identified in all animal species and sources tested. To estimate any possible attribution from these animal related sources to human health, more detailed identification and characterisation of both plasmids and isolates is needed. The results of this targeted surveillance of ESBLs in live animals suggest a slight decrease of ESBLs at farm level, although future sampling must reveal whether this is just a variation in results or indeed a decrease in prevalence. This decreasing trend in ESBL-prevalence was not seen in targeted surveillance of meat which might be explained e.g. the level of cross-contamination at meat-processing and by the inclusion of imported meat in the surveillance.

4.2 Carbapenemases

Carbapenemases are beta-lactamases with an extended spectrum that can also hydrolyse the last-generation of beta-lactam antibiotics called 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 extensive contact with Germany in terms of trade of live animals, which is a risk for introduction in the Netherlands. Therefore in 2012 and 2013 an extra screening was conducted with the aim to detect carbapenemase-producing *Enterobactericeae* in food-producing animals in the Netherlands.

From 2012 onwards this screening was done in isolates from faecal and meat samples of broilers, turkeys, slaughter pigs, veal calves and dairy cows, by disk diffusion tests using ertapenem, imipenem and meropenem. As carbapenemase producing *Enterobacteriaceae* are almost always also ESBL-producers, the screening included all *E. coli* and *Salmonella* isolates displaying reduced susceptibility to cefotaxime (N > 100/year). In 2012, all isolates tested were susceptible to these carbapenems and no further analysis was performed.

In 2013 a new and more 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 = 1126) were screened with this method. The samples were grown overnight in Trypton Soy Broth containing ertapenem (0.25 mg/L) and vancomycin (50 mg/L). 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, three steps to confirm the results were made:

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.

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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 2013, this sensitive screening resulted in three positive signals in the RT-PCR (two from pig samples and one from broilers). All signals indicated the presence of the OXA-48-gene. However, PCR and sequence analysis showed that the genes were OXA-48-like, which means that they were genetically not identical to the Genbank reference OXA-48 sequence AY23607. The genes detected differed 3-5 mutations to the reference OXA-48 gene and from the genes found in isolates from patients in the OXA-48 outbreak that occurred in 2012 in the Netherlands in the "Maasstad" hospital. The genes were identical to OXA-48 genes described to occur chromosomally in environmental *Shewanella* spp., which are considered to be not-pathogenic and not a source of transmission to humans.

Therefore the genes detected in pigs and broilers were considered not related to this outbreak and derived from environmental sources, and not a risk for public health. Finding these genes that are known to occur in the environment was considered the result of the high sensitivity of the method used.

Screening for carbapenemase producing isolates in faecal samples of food-producing animals (N > 1500) will continue in 2014 and in addition screening will also take place at clinical samples in pet animals at the veterinary faculty in Utrecht. Active screening in food products will be conducted based on risk evaluations.

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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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