NETHMAP 2012



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 2012



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

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National Institute for Public Health
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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.
NethMap can be ordered from the SWAB secretariat, c/o Secretariaat SWAB p/a Universitair Medisch Centrum St Radboud Medische Microbiologie, Huispost 574, route 574 Postbus 9101 6500 HB Nijmegen, Telefoon: (024) 36 19041/14356.
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1. Preface

This is NethMap 2012, the tenth SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in The Netherlands in 2011 and previous years. NethMap is a cooperative effort by members of The Netherlands Society for Infectious Diseases, The Netherlands Society of Hospital Pharmacists, The Netherlands Society for Medical Microbiology and the Centre for Infectious Disease Control Netherlands (CIb) at the National Institute for Public Health and the Environment (RIVM). In 1996, the Dutch Working Group on Antibiotic Policy was created, better known as SWAB (Stichting Werkgroep Antibiotica Beleid). Its mission is to manage, limit and prevent the emergence of resistance to antimicrobial agents among medically important species of micro-organisms in The Netherlands, thereby contributing to the quality of care in The Netherlands. For this effort SWAB received in 2008 an award from Prof Stuart Levy on behalf of the Alliance for the Prudent Use of Antibiotics (APUA) during the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in Washington DC.

Because of the multidisciplinary composition of SWAB, this working group can be considered the Dutch equivalent of the Intersectoral Coordinating Mechanisms (ICM's), as recommended by the European Union (2001), to control emerging antimicrobial resistance and promote rational antibiotic use.

SWAB has started several major initiatives to achieve its goals. Among these are training programmes on rational prescribing of antimicrobial drugs, development of evidence-based prescription guidelines, implementation of tailor-made hospital guides for antibiotic prophylaxis and therapy and an integrated nationwide surveillance system for antibiotic use and resistance. CIb has set up an Infectious Disease Surveillance Information System on Antibiotic Resistance (ISIS-AR) in collaboration with the medical microbiological laboratories, which was renewed in 2008. These surveillance data, together with surveillance data obtained in specific studies such as SERIN (Surveillance of Extramural Resistance in The Netherlands) and SIRIN (Surveillance of Intramural Resistance in The Netherlands), form the basis of resistance trends reported in NethMap. The initiatives correspond well with the recommendations by The Netherlands Council of Health Research (2001). In line with these recommendations, SWAB is fully funded by a structural grant from CIb, on behalf of the Ministry of Health, Welfare and Sports.

NethMap 2012 extends and updates the information of the annual reports since 2003. 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, published annually by the Veterinary Antibiotic Usage and Resistance Surveillance Working Group (VANTURES, see www.cvi.wur.nl). This year, for the first time, NethMap and MARAN are published together in one back-to-back report. 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. Because of the new format chosen, the size of NethMap was reduced significantly. The editors have tried to find a balance between sufficient detail for the interested professional, without losing too much of its content. Some chapters are now available electronically only (www.swab.nl, www.maran.wur.nl), such as the list of SWAB publications and detailed description of methods used for collecting data and part of the antibiotic usage data in animals. This trend will continue during the next years.

The interaction between the human and animal areas of antibiotic use and resistance is explored in a working group started in 2003 by both Ministries of health, Welfare and Sport and of Agriculture, Nature and Food Quality. Both SWAB and VANTURES are represented in this interdepartmental working group in which the evolution of antibiotic use and resistance in The Netherlands is discussed on the basis of surveillance data as provided by SWAB and MARAN.

NethMap thus provides extensive and detailed insight in the Dutch state of medically important antimicrobial resistance, and compares well with the data of the European Antimicrobial Resistance Surveillance System (EARSS, see http://www.rivm.nl/earss/). EARSS collects resistance data of a limited number of invasive bacterial species for the majority of European countries. In 2010, EARSS moved from CIb-RIVM to the European Centre for Disease Prevention and Control (ECDC) and has been renamed EARS-net.

We believe NethMap continues to contribute to our knowledge and awareness regarding the use of antibiotics and the resistance problems which may arise. We thank all who are contributing to the surveillance efforts of SWAB, and express our hope that they are willing to continue their important clinical and scientific support to SWAB.

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2. Summary

NethMap is the annual report of SWAB, in collaboration with the Centre for Infectious Disease Control Netherlands (CIb) on the use of and resistance to antimicrobial agents among common human pathogens isolated in the Netherlands, including resistance trends of antimycotic and antiviral drugs, the latter with focus on resistance in influenza virus.
NethMap's information on antimicrobial drug use and trends in antimicrobial resistance is based on systematically collected and analysed data over a period from 1996 until the present.

Use of antimicrobial agents

The use of antimicrobial agents in primary health care remained below 10 defined daily dosages (DDD) per 1000 inhabitants per day until 2005 and increased gradually since then to 11 DDD/1000 inhabitant days in 2008 that stabilised thereafter. The use in the Netherlands is still low in comparison with other European countries. The distribution of antibiotic usage over the different drug groups varies per patient population. Tetracyclines represented 23% of the antibiotics used in general practice, whereas these drugs are seldom prescribed in hospitals. Extramural nitrofurantoin use has been on the rise in recent years towards 1.31 DDD/1000 inhabitant-days in 2011, most probably because of the increased resistance to trimethoprim in *Escherichia coli* in urinary tract infection and the subsequent changes in treatment guidelines. Trimethoprim is nowadays a second choice antibiotic for treatment of urinary tract infections. Extra- and intramural fluoroquinolone use increased further during the last years with some minor changes within the class. NethMap 2012 reports a further substitution of amoxicillin by co-amoxiclav and an increase in macrolide use. The latter is caused by increased azithromycin use in primary health care and hospitals, whereas the use of other macrolides stabilized in primary health care or even decreased in hospitals.
The hospital use of the different subclasses of antibiotics, measured in DDD per 100 patient-days, remained relatively stable, except for combinations of penicillin, beta-lactamase resistant penicillins, cephalosporins, fluoroquinolones, glycopeptides, carbapenems and aminoglycosides, which showed a significant increase. However, in 2011 use of these classes of antibiotics stabilized. When use was expressed in DDD per 100 admissions no increase was observed. Different trends within the given groups of antibiotics are recognisable when usage per bed day, usage per admission and types of hospitals are compared. Penicillins, cephalosporins and quinolones still account for 72% of the antibiotics used in Dutch hospitals. The use of carbapenems and glycopeptides in university hospitals is higher in comparison to general hospitals whereas the latter showed a higher consumption of co-amoxiclav.
The use of systemic antimycotic drugs in university medical centres and general hospitals decreased; the use in university hospitals is almost four times higher than in general hospitals, which underlines the difference in patient populations between these two types of hospitals, the former harbouring a large group of severely immune compromised patients.
NethMap 2012 again shows an almost five times higher use of antiviral drugs in the university hospitals compared with general hospitals. The use of systemic antivirals in 2010 has been divided in use of antivirals for acute and for chronic infections. Overall use of antivirals for chronic infections was slightly higher than for acute infections. However, the use of antivirals for chronic and acute infections was higher in university hospitals compared to general hospitals.

Surveillance of resistance

NethMap 2012 presents data on antimicrobial resistance in the community and in hospitals. SWAB resistance surveillance data are derived from ISIS-AR (Infectious Disease Information System for Antibiotic Resistance) system, the inpatient SIRIN and the outpatient and community SERIN (Surveillance of Intra-/Extramural Resistance In the Netherlands) studies. In NethMap 2012 data derived from these three initiatives are compared and discussed taking into account the different methods used to collect and to study these data from different patient populations. Resistance data are presented for *Escherichia coli, Klebsiella* species, *Enterobacter* species, *Proteus mirabilis* and *Pseudomonas aeruginosa,* staphylococci, enterococci and respiratory pathogens and the results for patients visiting the general practitioner, patients in nursing homes, outpatient departments and hospital departments. In addition, data are presented in bacterial species associated with public health related infections e.g. meningococci, gonococci and *Mycobacterium tuberculosis*.
Resistance in the community was studied in *E. coli*, derived from uncomplicated urinary tract infections in general practice in men and women, collected through the NIVEL Sentinel Stations Network in 2009/2010, and compared with the results in men and women in 2004. Overall, the resistant rates for most antibiotics stabilized and decreased slightly for trimethoprim. Resistance rates were 32% for amoxicillin, 14% for co-amoxiclav, 19% for co-trimoxazole, 1% for nitrofurantoin and 5% for quinolones, respectively. The resistance rates in selected patients from general practice as reported from the ISIS-AR database are all higher. However, these include complicated urinary tract infections as well and patients that have been treated before. Resistance to amoxicillin

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was 43%, to co-amoxiclav 16%, to co-trimoxazole 24% and to quinolones 10%.
Resistance against ciprofloxacin of *N. gonorrhoeae* decreased further to 37% in 2011 (52 % in 2009). In 2011 the development of resistance against third generation cephalosporins decreased to 5% (was 9% in 2010), more often found in men who have sex with men (8%) than in heterosexual men and women (3%). Resistance in *M. tuberculosis* strains is slowly increasing with a level of multiresistance in 2011 at 1.5%.
Resistance in nursing homes was determined for *E. coli* and *Staphylococcus aureus*. Resistance levels in *E. coli* from urine in 308 residents without infection of six nursing homes appeared higher than in both GP populations, with 23% resistance to co-amoxiclav and 16% resistance to ciprofloxacin. Carriership and resistance of *Staphylococcus aureus* were studied in 2010 and compared with a similar study in 2007. Resistance levels did not change significantly over time and were similar to resistance patterns in hospitals, except for ciprofloxacin which was 23% in nursing homes vs 10% in hospitals.
Resistance in hospitals continues to increase steadily for many antimicrobials. Ciprofloxacin resistance among *E. coli* from Unselected Hospital Departments is now over 13%, and resistance to co-amoxiclav 21%. In general increasing resistance was found in all study populations for co-amoxiclav, cephalosporins, quinolones and co-trimoxazole. In intensive care units *E. coli* multiresistance was not yet reported before 1998 but increased to 18% in 2010 as reported by SIRIN. Similar trends were observed for the *Klebsiella spp, Enterobacter spp* and *Proteus mirabilis* strains studied. Carbapenems and the (toxic) colistin are then often the only remaining drugs to treat infections with such strains. Although carbapenem resistance is still low (< 1%), around 2% of *Enterobacteriaceae* strains now have an MIC just below the breakpoint for resistance (1-8 mg/l). Likewise, the MIC distributions over time identified significant "MIC creeps" (shifts within the susceptible populations) towards for cephalosporins *(K. pneumoniae)*, meropenem *(P. aeruginosa)*, quinolones *(K. pneumoniae, P. aeruginosa)* and aminoglycosides *(K. pneumoniae, E. cloacae)* which predict upcoming resistance in the following years. These strains are often not recognized in routine susceptibility testing but may already pose a problem for difficult to treat infections. Ceftazidime resistance increased further to 12% for *P. aeruginosa*.
The national surveillance of carbapenamase producing enterobacteriaceae was started during 2010. From patient samples taken in 2011, 169 strains from 142 patients were submitted by 40 hospitals to the National Institute for Public Health and the Environment (RIVM). Thirty five different CPE were detected. The majority (21) concerned OXA-48 producing *K. pneumoniae*. In 11 of the 35 cases, MICs for meropenem were within susceptible range (<= 2 mg/L) according to EUCAST criteria. As far as information was available (n=17), the origin of strains could either be traced back to the Rotterdam outbreak or to previous visits to endemic countries from North Africa or the Middle East, mainly including hospitalisation in these countries.
The resistance rates for *S. aureus* were not much different from previous years, although methicillin resistant *S. aureus* (MRSA) increased slightly and is now 1.8 % in Unselected Hospital Departments. Resistance to vancomycin, still the rescue drug for MRSA infection is rarely encountered in the Netherlands. Rifampicin resistance was lower than 1%, and mupirocin resistance 1%.Animal husbandry related MRSA isolates (CC398 strains) were at approximately the same level over the last year, 39% of the total number.
Data on pneumococci and *Haemophilus influenzae* were collected in hospitals. For the majority of these strains it can, by the nature of such public health related species, be suggested that these are community related rather than hospital acquired. Their resistance profiles may be considered a reflection of the situation in the general population. Therefore it is of interest that in *H. influenzae* an increase of resistance to amoxicillin to 16 % as well as to co-amoxiclav (4%) is observed in unselected departments. The increase is clearly not exclusively due to a rise in beta lactamase producing strains, therefore indicating an increasing prevalence of so called Beta Lactamase Negative Amoxicillin Resistant (BLNAR) strains. Doxycycline is still a reasonable alternative choice to combat infections with BLNAR *H. influenzae*.
In pneumococci resistance against macrolides is still at a critical 10% in unselected hospital departments and increasing in pulmonology services to 14%. Tetracycline resistance parallels this. Resistance to penicillin, the most important antibiotic prescribed for serious pneumococcal disease, increased to 2.4 %.
Studies in *Aspergillus spp.* indicate that resistance to azoles is further increasing. A large retrospective study in the Radboud UNMC showed that azole resistance emerged in 2000. Since then, resistance has slowly crept upwards and now has reached the critical value of 10%. It is expected that azole resistance will continue to rise in the near future. This will limit treatment options significantly and new guidelines for empiric treatment are necessary.
Finally, data from surveillance studies of influenza viruses in The Netherlands indicate treatment limitations due to emerging resistance against anti -influenza specific drugs such as oseltamivir.

We conclude that the data presented in NethMap 2012 increases concern with respect to the emergence of antibiotic resistance in the Netherlands. 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 defence. Diagnostic cultures and in particular susceptibility testing are increasingly necessary to guide antimicrobial treatment and antimicrobial stewardship.

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3. Use of antimicrobials

3.1 Primary health care

3.1.1 Use of antibiotics

Data on the use of antibiotics in primary health care were obtained from the Foundation for Pharmaceutical Statistics (SFK; http://www.sfk.nl) and expressed as the number of Defined Daily Doses (DDD) per 1000 inhabitants per day.

About 85% of antibiotic use in primary health care is prescribed by general practitioners (1) and the remaining by dentists and specialists.

From 1998-2004, the total antibiotic use was 10 DDD/1000 inhabitant-days. Over the past six years, use gradually increased to 11 DDD/1000 inhabitant-days. In 2011, the use of antibiotics remained stable as compared to 2010. The distribution of antibiotics per class in 2011 is presented in figure 3.1. Tetracyclines (mainly doxycycline) represented 23% of total antibiotic use in primary health care. Other frequently used antibiotics were penicillins with extended spectrum (mainly amoxicillin), combinations of penicillins with beta-lactamase inhibitors (essentially amoxicillin with clavulanic acid) and macrolides/lincosamides, each representing 17%, 16% and 13% of the total use, respectively.

From 2005 to 2011, the use of amoxicillin remained stable at about 1.9 DDD/1000 inhabitant-days. The use of amoxicillin/clavulanic acid increased steadily to 1.8 (table 3.1; figure 3.2).

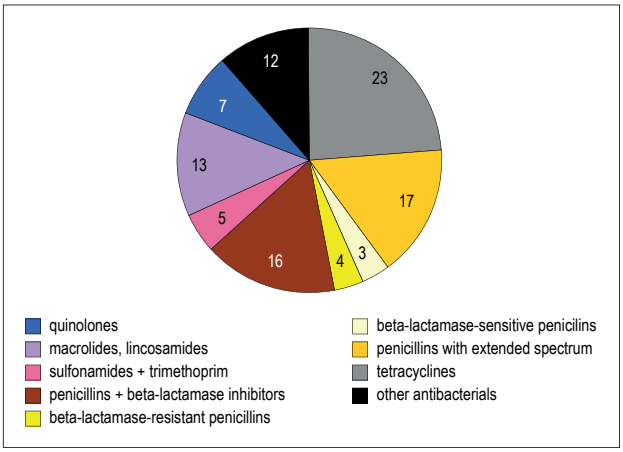

Figure 3.1. Distribution (%) of the use of antibiotics for systemic use (J01) in primary health care, 2011 (SFK).

After a small decrease from 2009 until 2010, the use of macrolides increased again to 1.34 DDD/1000 inhabitant-days in 2011 (table 3.1). In figure 3.2 the use of the different macrolides is depicted. Clarithromycin is still the most commonly used macrolide, but its use continues to decline from 2005-2011 in favour of the use of azithromycin. The use of azithromycin almost equals the use of clarithromycin now. The use of erythromycin declined to 0.09 DDD/1000 inhabitant-days in 2011. Use of fluoroquinolones remained about stable, except for norfloxacin which further declined to 0.17 DDD/1000

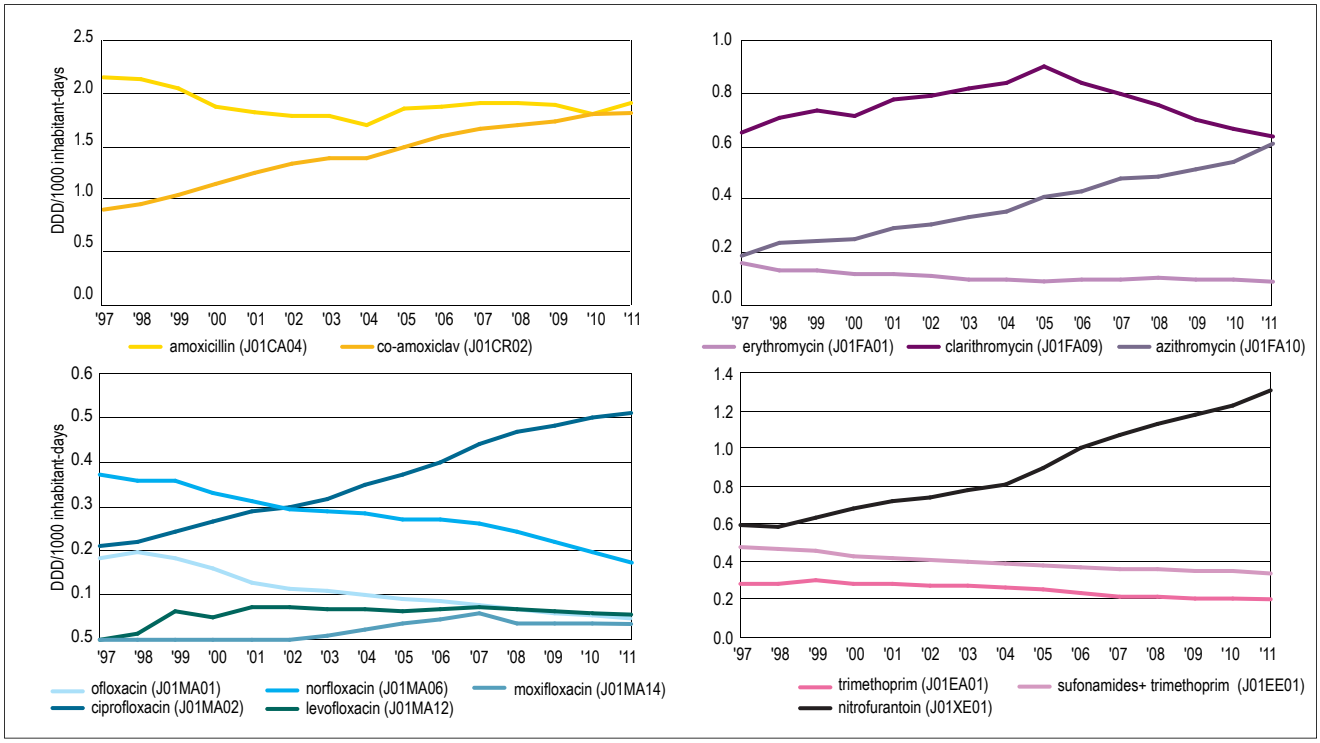

Figure 3.2. Use of antibiotics for systemic use in primary health care, 1997-2011 (SFK).

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Table 3.1. 13-years data on the use of antibiotics for systemic use (J01) in primary care (DDD/1000 inhabitant-days), 1999-2011 (Source: SFK).

ATC Group*	Therapeutic group	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
J01AA	Tetracyclines	2.49	2.48	2.40	2.34	2.24	2.24	2.41	2.37	2.57	2.66	2.67	2.67	2.60
J01CA	Penicillins with extended spectrum	2.05	1.88	1.83	1.78	1.78	1.71	1.86	1.87	1.91	1.91	1.89	1.81	1.91
J01CE	Beta-lactamase sensitive penicillins	0.52	0.52	0.49	0.46	0.44	0.43	0.44	0.50	0.46	0.42	0.39	0.37	0.35
J01CF	Beta-lactamase resistant penicillins	0.23	0.24	0.25	0.25	0.27	0.28	0.29	0.31	0.32	0.36	0.38	0.38	0.39
J01CR	Penicillins + beta-lactamase-inhibitors	1.04	1.15	1.25	1.34	1.40	1.39	1.50	1.59	1.66	1.71	1.74	1.80	1.82
J01D	Cephalosporins	0.10	0.08	0.07	0.07	0.06	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.04
J01EA	Trimethoprim and derivatives	0.30	0.28	0.28	0.27	0.27	0.26	0.25	0.23	0.22	0.21	0.21	0.20	0.20
J01EC	Intermediate-acting sulphonamides	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01EE	Sulphonamides + trimethoprim	0.46	0.43	0.42	0.40	0.40	0.39	0.38	0.37	0.36	0.36	0.35	0.35	0.34
J01FA	Macrolides	1.17	1.14	1.23	1.24	1.27	1.32	1.42	1.39	1.39	1.36	1.33	1.31	1.34
J01FF	Lincosamides	0.04	0.04	0.05	0.06	0.06	0.07	0.08	0.09	0.10	0.11	0.12	0.14	0.15
J01GB	Aminoglycosides	0.00	0.00	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03
J01MA	Fluoroquinolones	0.85	0.80	0.80	0.78	0.79	0.83	0.84	0.87	0.91	0.89	0.86	0.85	0.82
J01MB	Other quinolones	0.04	0.04	0.04	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01
J01XB	Polymyxins	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofuran derivatives	0.64	0.68	0.72	0.74	0.78	0.81	0.90	1.00	1.07	1.13	1.17	1.23	1.31
J01XX05	Methenamine	0.06	0.06	0.06	0.04	0.03	0.02	0.02	0.03	0.03	0.02	0.03	0.04	0.03
J01	Antibiotics for systemic use (total)	10.02	9.86	9.92	9.83	9.86	9.87	10.51	10.73	11.10	11.24	11.21	11.23	11.22

* From the 2011 edition of the Anatomical Therapeutic Chemical (ATC) classification system

inhabitant-days in favour of ciprofloxacin which slightly increased to 0.51 (table 3.1; figure 3.2).
The use of nitrofurantoin is still increasing to 1.31 DDD/1000 inhabitant-days in 2011 as compared to 1.23 DDD/1000 inhabitant-days in 2010. Over the past five years, the use of sulphonamides and trimethoprim (J01EA and J01EE combined) remained almost stable at 0.54 DDD/1000 inhabitant-days (table 3.1; figure 3.2).

Discussion
Despite the small increase in antibiotic use from 2005 to 2011 from 10 to 11.2 DDD/1000 inhabitant-days, use of antibiotics in the Netherlands is still low as compared to other European countries (2).
The largest increase was found for nitrofurantoin. The national guideline of the Dutch College of General Practitioners (3) was revised in 2005. According to this revision, antibiotic treatment can be considered without urine investigation for women recognising symptoms from a previous episode. The increases in incidence and antibiotic treatment for uncomplicated urinary tract infections need attention in the future.
Subtle shifts in the patterns of use within the various classes of antibiotics are observed. The increased use of ciprofloxacin seems to be offset by a decrease in ofloxacin and norfloxacin. Since its introduction in 2002 in the Netherlands, the use of moxifloxacin increased to 0.06 DDD/1000 inhabitant-days in 2007. After warnings about serious adverse events of moxifloxacin issued by the Dutch Medicines Evaluation Board in 2008, its use declined to 0.04 DDD/1000 inhabitant-days and remained stable until 2011. Within the class of macrolides, a shift is observed from erythromycin to the newer macrolides clarithromycin and azithromycin. Especially the use of azithromycin continues to increase. These trends may be relevant from the perspective of growing rates of resistance among common pathogens.

3.2 Hospitals

3.2.1 Use of antibiotics

Data on the use of antibiotics in Dutch hospitals were collected by the SWAB by means of a questionnaire distributed to all Dutch hospital pharmacists.
Data on antibiotic use are expressed in DDD per 100 patient-days, as well as in DDD per 100 admissions, because trends over time in these units of measurement do not always correlate (tables 3.2 and 3.3) (4).
For the first time in years, the total systemic use of antibiotics in our cohort of hospitals slightly decreased from 70.9 DDD per 100 patient-days in 2009 to 70.2 in 2010 (a decrease of 0.92%) (table 3.2). For comparison, in 2000 the total systemic use of antibiotics was 43.0 DDD per 100 patient-days. The total number of DDD per 100 admissions decreased by 1.6% from 321 DDD in 2009 to 316 DDD in 2010 (tables 2 and 3). A similar decrease was seen for almost all groups of antibiotics. The distribution of antibiotics per class in 2010 is depicted in figure 3.3. Over recent years, the use of the different subclasses

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Table 3.2. Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2000-2010 (Source: SWAB).

ATC group*	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	1,6	1,6	1,7	1,4	1,5	1,6	1,6	1,4	1,7	1,6	1,7
J01CA	Penicillins with extended spectrum	5,8	6,0	6,1	6,0	6,0	6,7	7,6	7,3	6,5	7,6	7,3
J01CE	Beta-lactamase sensitive penicillins	1,1	1,3	1,2	1,2	1,4	1,4	1,4	1,2	1,3	1,6	1,5
J01CF	Beta-lactamase resistant penicillins	4,3	4,3	4,4	5,4	5,7	5,8	5,9	5,7	6,4	6,6	6,8
J01CR	Penicillin combinations, incl. β-lactamase-inhibitors	8,9	9,9	12,2	12,1	12,8	13,9	15,1	14,5	16,2	16,5	16,0
J01DB -DE	Cephalosporins	5,6	6,1	6,3	6,5	7,0	7,4	8,4	8,4	8,8	10,1	10,2
J01DF	Monobactams	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
J01DH	Carbapenems	0,4	0,4	0,5	0,5	0,5	0,6	0,6	0,8	1,0	1,1	1,2
J01EA	Trimethoprim and derivatives	0,3	0,5	0,5	0,5	0,4	0,6	0,8	0,5	0,4	0,4	0,5
J01EC	Intermediate-acting sulfonamides	0,1	0,0	0,0	0,1	0,1	0,0	0,0	0,1	0,1	0,0	0,0
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2,3	2,3	2,4	2,3	2,1	2,3	2,1	2,3	2,4	2,0	2,0
J01FA	Macrolides	2,1	2,3	2,7	2,4	2,3	2,8	2,5	2,8	2,7	2,6	2,7
J01FF	Lincosamides	1,2	1,3	1,5	1,6	1,8	1,9	2,0	2,1	2,1	2,4	2,3
J01GB	Aminoglycosides	2,1	2,0	2,1	2,5	2,2	2,6	2,5	2,6	3,9	4,2	4,1
J01MA	Fluoroquinolones	4,7	5,5	5,7	6,4	6,5	7,3	8,0	7,6	8,8	9,3	9,0
J01MB	Other quinolones	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,0	0,1	0,1	0,0
J01XA	Glycopeptides	0,5	0,5	0,5	0,5	0,6	0,8	0,7	1,0	1,1	1,3	1,3
J01XB	Polymyxins	0,3	0,1	0,1	0,1	0,1	0,2	0,2	0,1	0,2	0,2	0,4
J01XC	Steroid antibacterials (fusidic acid)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0
J01XD	Imidazole derivatives	1,1	1,3	1,5	1,6	1,7	1,5	1,7	1,8	1,7	1,8	1,9
J01XE	Nitrofuran derivatives	0,5	0,5	0,5	0,7	0,9	1,0	1,0	1,1	1,2	1,1	1,2
J01XX05	Methenamine	0,0	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	0,0	0,0	0,0	0,1	0,1	0,1
J01	Antibiotics for systemic use (total)	43	46,5	50,2	51,9	53,8	58,3	62,2	61,6	66,8	70,9	70,2

* From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

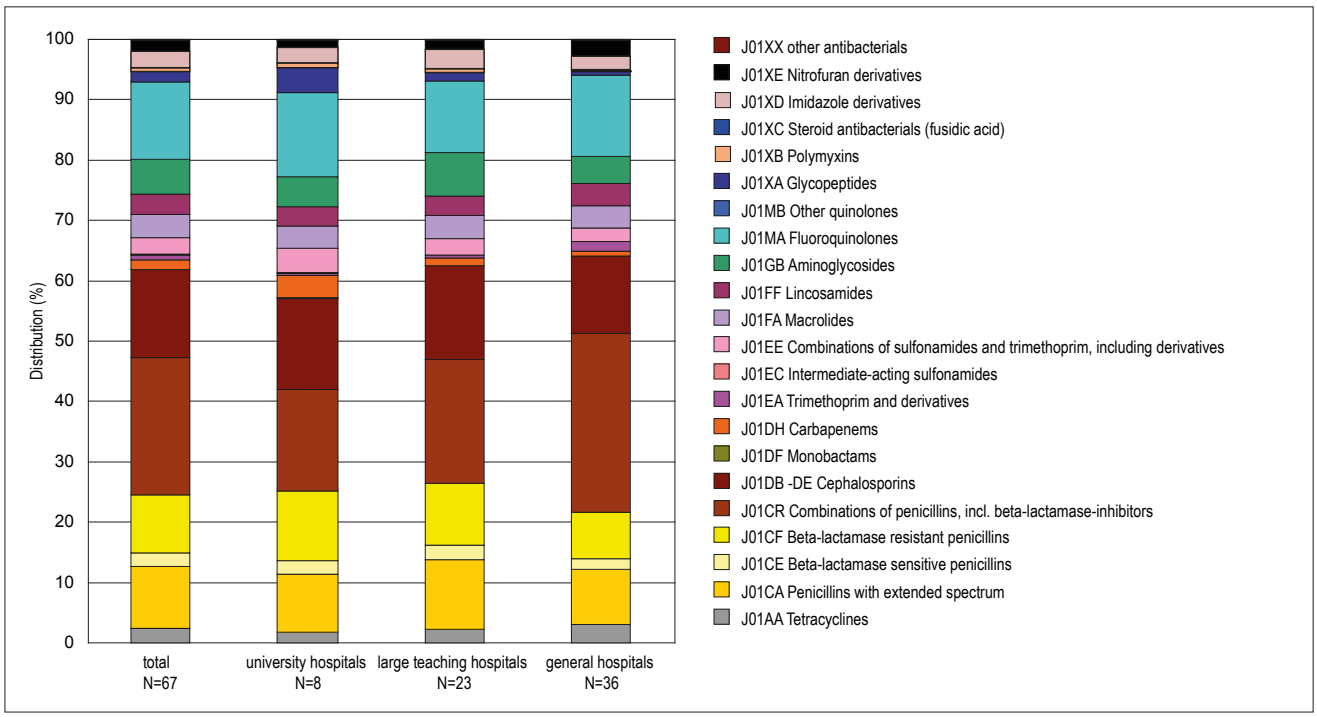

Figure 3.3. Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2010 (SWAB).

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Table 3.3. Use of antibiotics for systemic use (J01) in hospitals (DDD/100 admissions), 2000-2010 (Source: SWAB).

ATC-group*	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	12.1	11.3	11.2	8.8	8.4	8.8	8.7	7.8	8.5	7.4	7.5
J01CA	Penicillins with extended spectrum	44.3	41.5	41.5	38.6	34.3	36.4	41.0	39.9	33.7	34.3	32.6
J01CE	Beta-lactamase sensitive penicillins	8.1	9.2	8.2	7.8	7.8	7.5	7.7	6.8	6.6	7.3	6.9
J01CF	Beta-lactamase resistant penicillins	32.8	31.7	31.5	34.6	33.0	31.4	31.8	31.2	33.2	30.1	30.6
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	68.1	68.0	81.6	77.7	73.1	75.4	81.7	79.6	83.5	74.8	71.8
J01DB-DE	Cephalosporins	42.8	42.3	42.0	42.0	39.4	39.8	45.3	46.0	45.5	45.9	45.8
J01DF	Monobactams	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
J01DH	Carbapenems	3.3	2.4	3.2	3.3	2.8	3.2	3.0	4.6	5.1	5.1	5.4
J01EA	Trimethoprim and derivatives	2.5	3.6	3.3	3.1	2.3	3.0	4.2	2.7	2.0	1.7	2.4
J01EC	Intermediate-acting sulfonamides	0.5	0.1	0.2	0.8	0.3	0.3	0.1	0.4	0.3	0.2	0.2
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	17.3	15.6	16.0	14.4	12.1	12.2	11.5	12.7	12.2	9.3	9.1
J01FA	Macrolides	15.4	15.7	17.3	15.4	13.4	15.1	13.4	15.1	13.9	11.9	12.0
J01FF	Lincosamides	9.0	9.2	10.0	10.2	10.2	10.5	10.8	11.4	11.1	10.8	10.5
J01GB	Aminoglycosides	16.2	14.0	14.2	15.8	12.5	13.9	13.7	14.5	20.4	18.9	18.3
J01MA	Fluoroquinolones	35.9	38.0	38.2	41.0	37.2	39.7	43.3	41.9	45.6	42.2	40.6
J01MB	Other quinolones	0.4	0.5	0.5	0.6	0.8	0.5	0.3	0.2	0.3	0.5	0.0
J01XA	Glycopeptides	3.8	3.2	3.4	3.4	3.5	4.1	3.9	5.4	5.9	5.7	5.6
J01XB	Polymyxins	2.3	0.8	0.4	0.5	0.6	1.1	0.9	0.7	1.2	1.0	1.8
J01XC	Steroid antibacterials (fusidic acid)	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.4	0.4	0.1
J01XD	Imidazole derivatives	8.5	9.0	9.7	10.1	9.6	7.9	9.0	10.1	8.8	8.3	8.8
J01XE	Nitrofuran derivatives	2.8	3.3	3.6	4.7	4.9	5.6	5.2	6.1	6.2	5.0	5.3
J01XX05	Methenamine	0.3	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.1	0.1	0.2
J01XX08	Linezolid	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
J01	Antibiotics for systemic use (total)	327.1	320.2	336.6	333.2	306.8	316.9	335.9	335.9	344.7	321.3	315.9

* From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

of antibiotics, measured in DDD per 100 patient-days, remained relatively stable, except for the use of combinations of penicillin, beta-lactamase resistant penicillins, cephalosporins, fluoroquinolones, glycopeptides, carbapenems and aminoglycosides, which showed a significant increase. However, in 2011 use of these classes of antibiotics stabilized (table 3.2). When use was expressed in DDD per 100 admissions no increase was observed.

Figure 3.3 shows the relative distribution of antibiotic use per class, specified for the different types of hospitals. The use of penicillins includes the largest proportion, namely 45%. Second are the cephalosporins with 14%, followed by the quinolones with 13%. Notable is the high use of carbapenems (3.7%) and glycopeptides (4.2%) in university hospitals compared with the general hospitals (0.8 and 0.7%, respectively). General hospitals showed a higher consumption of amoxicillin/clavulanic acid.

All penicillins showed a slight decrease in use from 2009-2010, except for flucloxacillin which shows a minimal increase (figure 3.4).

Measured in DDD/100 admissions the total use of first generation cephalosporins remained stable compared from 2007-2010, the second generation cephalosporins showed a slight decrease from 2009-2010, whereas the third generation showed an increase in this period. The use of these three categories is rising when measured in DDD per 100 patient-days (figure 3.4).

Since 2006, a remarkable increase in the use of meropenem is seen (figure 3.4).

The patterns of macrolides use vary. Compared with last year the use of azithromycin increases while erythromycin decreases; this reflects the trend seen over the last decade (figure 3.5).

This year we splitted the use of gentamicin in parenteral and local use (implants of beads and cement), because of the important differences between these ways of usage. The parenteral use remains about constant over the past years, while local use shows a large increase. However, compared with 2009 the local and parenteral use showed a small decrease in 2010. Compared with the last year the use of tobramycin increased, though the trend over the years remains stable (figure 3.5).

After years of increase, the use of ciprofloxacin seems to stabilize. The use of the other quinolones is, compared with ciprofloxacin, relatively low and stable over the last

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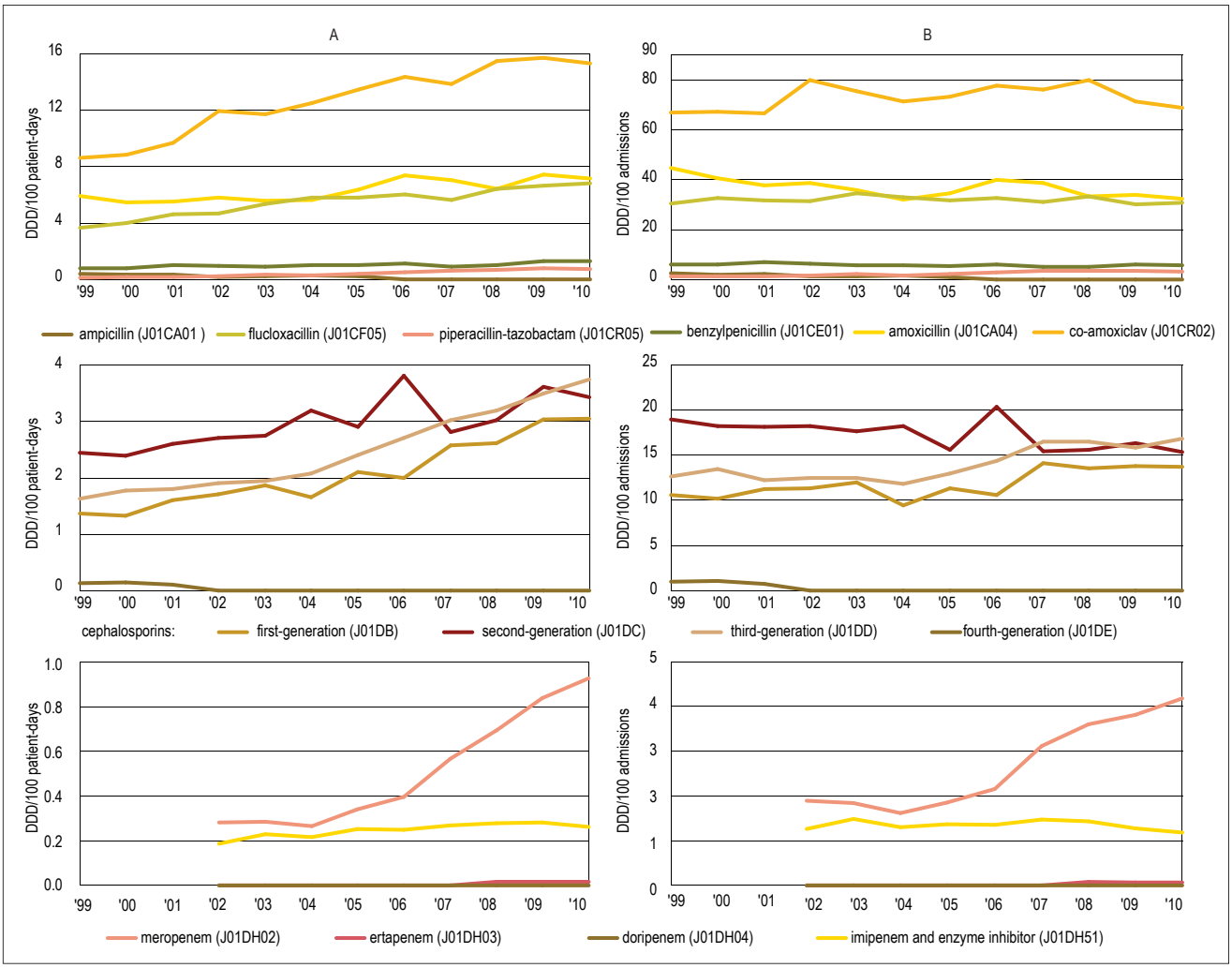

Figure 3.4. Use of beta-lactams in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2010 (SWAB).

years (figure 3.5). Vancomycin also stabilized this year after years of increasing use, while the use of teicoplanin remained stable and low (figure 3.5).

3.2.2 Use of systemic antimycotics

The total use of systemic antimycotics decreased from 3.65 to 3.49 DDD per 100 patient-days over the last year. Seventy-eight percent of the total use comprised triazole derivatives. Over the last four years their use remained stable when measured in DDD per 100 patient-days. Their DDD per 100 admissions is stable as well, except for a little decline in the use of fluconazole from 10.7 to 8.7 DDD per 100 admissions. There is a big difference in the total use in general hospitals (1.93 DDD per 100 patient-days) compared with university hospitals (8.66 DDD per 100 patient-days) (table 3.4)

3.2.3 Use of systemic antimycobacterials

The total use of systemic antimycobacterials showed a slight increase in use; 1.49 DDD per 100 patient-days in 2010 compared with 1.43 in 2009. The distribution of the different groups was more or less similar in university hospitals compared with the general hospitals, with the largest proportion (59%) represented by the antibiotics (mainly rifampicin) (table 3.5).

3.2.4 Use of systemic antivirals

A slight increase was shown in the total use of systemic antivirals, from 1.8 DDD per 100 patient-days in 2009 to 1.9 in 2010 (table 3.6). Over the past 4 years the pattern remained stable for the different groups. The largest proportion (41%) was represented by the nucleosides (without reverse transcriptase inhibitors), both in the general and the university hospitals (6).
The group of antivirals can be divided into 2 categories: antivirals mainly used for acute infections (J05AB, nucleosides without reverse transcriptase inhibitors; J05AD, phosphonic acid derivatives; J05AH, neuraminidase inhibitors) and antivirals used for chronic infections (J05AE, protease inhibitors; J05AF, nucleoside reverse transcriptase inhibitors; J05AG, non-nucleoside reverse transcriptase inhibitors; J05AR, antivirals for the treatment of HIV, combinations; J05AX, other antivirals). Overall the use of antivirals for chronic infections was

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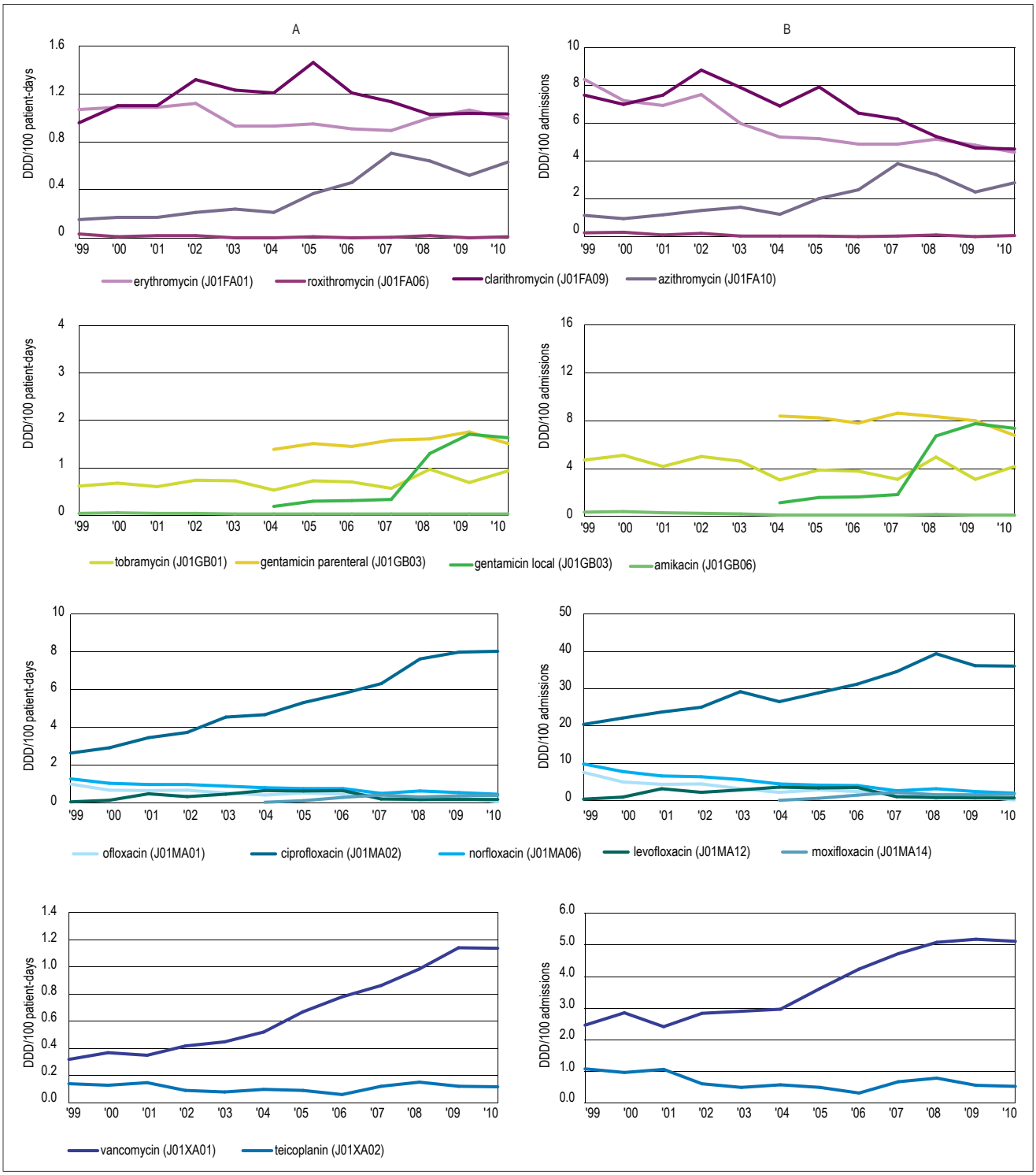

Figure 3.5. Use of macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2010 (SWAB).

slightly higher than for acute infections, which is shown in figure 3.6. This figure also shows a substantial higher use of antivirals in university hospitals compared with general hospitals. In the last year, there was an increase in use for chronic conditions in university hospitals, whereas use for acute conditions decreased. Contrarily in general hospitals use for chronic conditions slightly decreased, whereas use for acute conditions increased to the same level.

Discussion

In 2009, the total antibiotic use increased in reference to 2008 when expressed in DDD per 100 patient-days, but decreased when expressed in DDD per 100 admissions. In 2010 we see a slight decrease in both. Despite some small

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Table 3.4. Use of antimycotics for systemic use (J02) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2010 (Source: SWAB).

ATC group *	Therapeutic group	2007			2008			2009			2010		
		total 38	general 31	academic 7	total 51	general 43	academic 8	total 63	general 55	academic 8	total 67	general 59	academic 8
J02AA01	Antibiotics (amfotericin B)	1.50	0.12	4.44	0.41	0.13	1.12	0.42	0.14	1.35	0.46	0.10	1.65
J02AB02	Imidazole derivatives (ketoconazole)	0.04	0.01	0.12	0.05	0.03	0.11	0.05	0.04	0.08	0.06	0.03	0.15
J02AC	Triazole derivatives	2.74	1.59	5.18	3.06	1.75	6.36	2.89	1.74	6.72	2.74	1.66	6.31
J02AX	Other mycotics for systemic use	0.09	0.05	0.19	0.16	0.06	0.40	0.29	0.20	0.61	0.23	0.14	0.56
J02	Antimycotics for systemic use (total)	4.34	1.71	9.93	3.67	1.97	7.98	3.65	2.11	8.77	3.49	1.93	8.66

* From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 3.5. Use of antimycobacterials for systemic use (J04) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2010 (Source: SWAB).

ATC group *	Therapeutic group	2007			2008			2009			2010		
		total 37	general 30	academic 7	total 49	general 41	academic 8	total 63	general 55	academic 8	total 67	general 59	academic 8
J04AA	Aminosalicylic acid and derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J04AB	Antibiotics (mainly rifampicin)	0.83	0.52	1.44	0.92	0.75	1.34	0.86	0.74	1.27	0.89	0.73	1.41
J04AC	Hydrazides (mainly isoniazide)	0.28	0.22	0.39	0.21	0.18	0.29	0.21	0.14	0.40	0.21	0.16	0.34
J04AD	Thiocarbamide derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J04AK	Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol)	0.25	0.18	0.38	0.18	0.13	0.31	0.21	0.17	0.34	0.22	0.17	0.37
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J04BA	Drug for treatment of leprosy (dapson)	0.27	0.14	0.53	0.15	0.05	0.39	0.15	0.10	0.33	0.17	0.09	0.45
J04	Antimycobacterials for systemic use (total)	1.63	1.06	2.74	1.46	1.11	2.33	1.43	1.15	2.35	1.49	1.16	2.58

* From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

increases, there seems to be a stabilisation in use of all groups of antibiotics measured in DDD per 100 patient-days and in DDD per 100 admissions.
The unit in which antibiotic usage is expressed matters, especially for the hospital outcomes (4). This is of particular importance when hospital resource indicators change over a study period. In relation to antibiotic resistance development, the measure of antibiotic use should reflect the antibiotic selection pressure exerted. At the population level the selection pressure is thought to depend on the volume of antibiotics used in a particular geographical area, the number of individuals exposed and the proportion of the population treated with antibiotics (5). The denominator should therefore preferably include information on all these factors. However, there is a lack of studies determining the correlation between different measures of antibiotic use and the level of antibiotic resistance.

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Table 3.6. Use of antivirals for systemic use (J05) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2010 (Source: SWAB).

ATC group *	Therapeutic group	2007			2008			2009			2010		
		total 36	general 29	academic 7	total 44	general 36	academic 8	total 63	general 55	academic 8	total 67	general 59	academic 8
J05AB	Nucleosides excl. Reverse transcriptase inhibitors	0.78	0.27	1.72	0.78	0.29	2.00	0.79	0.35	2.22	0.77	0.39	2.02
J05AD	Phosphonic acid derivatives	0.02	0	0.06	0.03	0.01	0.11	0.03	0.00	0.13	0.03	0.01	0.10
J05AE	Protease inhibitors	0.35	0.06	0.7	0.34	0.11	0.92	0.31	0.18	0.75	0.31	0.17	0.78
J05AF	Nucleoside reverse transcriptase inhibitors	0.38	0.14	0.83	0.34	0.18	0.74	0.25	0.13	0.64	0.24	0.11	0.67
J05AG	Non-nucleoside reverse transcriptase inhibitors	0.11	0.05	0.2	0.11	0.06	0.25	0.14	0.11	0.23	0.10	0.07	0.22
J05AH	Neuraminidase inhibitors	0.01	0	0.02	0.03	0.02	0.05	n.a.#	n.a.#	n.a.#	0.11	0.07	0.21
J05AR	Antivirals for the treatment of HIV, combinations	0.16	0.07	0.33	0.22	0.10	0.52	0.24	0.15	0.55	0.29	0.14	0.76
J05AX	Other antivirals	0	0	0	0.02	0.00	0.06	0.03	0.02	0.06	0.05	0.02	0.15
J05	Antivirals for systemic use (total)	1.81	0.59	3.86	1.88	0.78	4.65	1.80	0.95	4.59	1.90	0.99	4.91

* from the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

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

Since NethMap 2004, data on antibiotic use in Dutch hospitals have been expressed in DDD per 100 patient-days and in DDD per 100 admissions. An increase in both outcomes is worrisome. With respect to development of resistance, decrease of the outcomes is favourable. When a constant use per patient is seen in combination with an increase in the number of admissions, selection pressure by antibiotics in hospitals increases over the years. An intensification of antibiotic therapy per 100 patient-days may in part be due to an increase in the number of admitted patients, and possibly to a shortening of the duration of antibiotic treatment. Such shortening might lead to less selection of resistant micro-organisms (6).
The total number of admissions has increased, whereas the total number of patient-days decreased. This reflects the decrease in the duration of hospital stay in the Netherlands (from an average of 7.6 days per admission in 2000, to 4.5 days per admission this year). The use per individual patient has remained constant. Therefore, the selection pressure on the hospital ward has increased.
The question that now arises is whether the development of resistance is more relevant in the patient or in the surrounding environment?
In university hospitals, the use of systemic antimycotics is more than four times higher than in general hospitals. This can be explained by the relatively larger patient population of haematology- and oncology-patients in university hospitals.
Although university hospitals use almost twice as much antimycobacterials, the distribution of the different groups is rather similar in both hospital settings. Higher usage in university hospitals might be explained by rifampicin being used as an adjuvant in certain infections with Staphylococci besides its use for tuberculosis.
In the Netherlands, tuberculosis is treated with a limited combination of antimycobacterials; there is not much room for variation (7) (except for MDR-TB). The use of dapson is explained by its role in the prophylaxis and treatment of *Pneumocystis carinii* and toxoplasmic encephalitis.
Use of systemic antivirals is much higher in university hospitals than in general hospitals as well, for both chronic and acute infections. The largest group of antivirals used are the nucleosides (reverse transcriptase inhibitors excluded), like (val)acyclovir and (val) ganciclovir. In the Netherlands, all university hospitals and a few general hospitals are specialised in the treatment of HIV patients.

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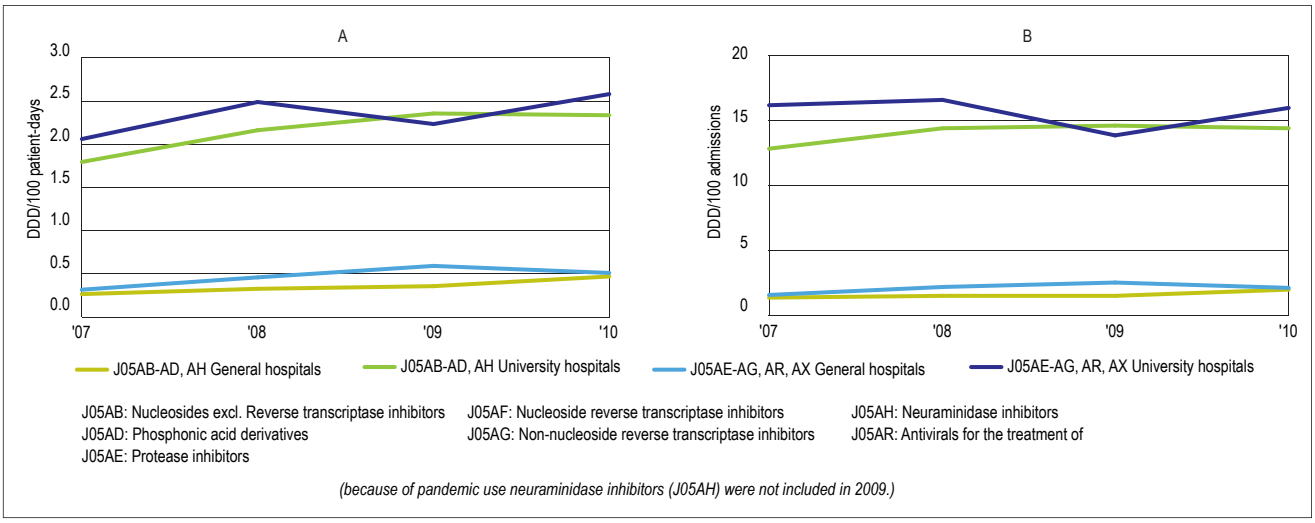

Figure 3.6. Distribution of the use of antivirals for chronic (J05 AE-AG, AR, AX) vs acute (J05 AB-AD, AH) infections in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2007-2010 (SWAB).admissions (B), 1999-2010 (SWAB).

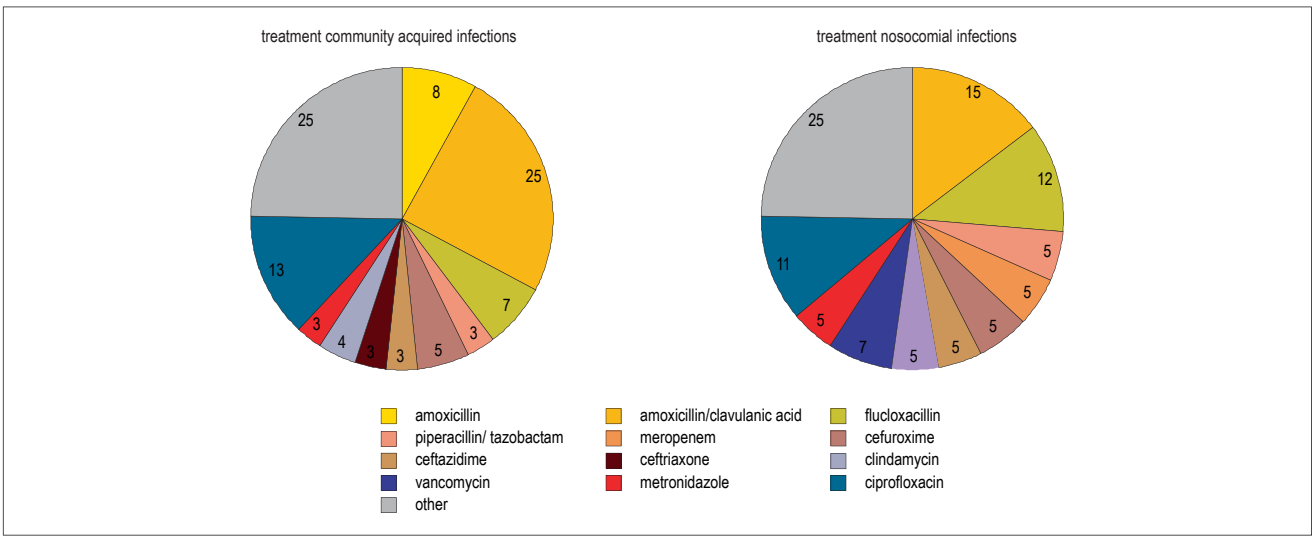

Figure 3.7. Distribution (%) of the use of antibiotics for systemic use (J01) for treatment of community acquired infections and of nosocomial infections, 2011 (PREZIES).

3.3 Results of the PREZIES point-prevalence study on antimicrobial use in hospitals

In 2011, for the first time, Dutch hospitals collected detailed data on antibiotic usage, (according to the methodology proposed by ECDC) combined with the PREZIES prevalence study on healthcare associated infections. Twenty nine hospitals participated: four university-, five large teaching- and 20 general hospitals. 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. Of 6360 patients, 2059 patients were on antibiotics with a total of 2683 prescriptions.

The antibiotics most often prescribed were amoxicillin/clavulanic acid (22.0%), ciprofloxacin (12.3%), flucloxacillin (6.3%), cefuroxime (6.1%) and amoxicillin (5.5%). The respective distribution for community-acquired and nosocomial infections are shown in figure 3.7.

For surgical as well as medical prophylaxis, a wide variety of antibiotics is used. For surgical prophylaxis, cefazolin is most often used (33.6%), followed by amoxicillin/clavulanic acid (22.5%) and cefuroxime (14.3%).

For medical prophylaxis, amoxicillin/clavulanic acid is used in 18.0% of cases, followed by ciprofloxacin (16.4%), trimethoprim/sulfamethoxazol (11.9%), colistin (8.4%) and tobramycin (7.3%).

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4. Resistance among common bacterial pathogens

4.1 Surveillance of resistance in the Community

The studies on resistance levels in the community include estimation of antimicrobial resistance in (1) the indigenous flora of various populations of healthy persons, (2) pathogens from patients visiting their general practitioner (GP) and (3) specific pathogens (meningococci, gonococci and mycobacteria).
Longitudinal multicentre Surveillance of Extramural Resistance in The Netherlands (SERIN) has been performed in cooperation with the Department for Medical Microbiology, University Hospital Maastricht, The Netherlands Institute for Health Services Research (NIVEL) and the regional Institutes for Public Health Services (GGDs).
Resistance levels among meningococci are recorded by the Netherlands Reference Laboratory for Bacterial Meningitis since 1993.
Surveillance of resistance of *Neisseria gonorrhoeae* among patients from outpatient-STI centres (Gonococcal Resistance to Antimicrobials Surveillance; GRAS) and the susceptibility testing and identification of first isolates of *Mycobacterium tuberculosis* of each patient with tuberculosis are carried out by the RIVM.

Table 4.1. Distribution (%) of isolated uropathogens in men per age category in 2009-2010.

	Age category (y)			
	18-50	51-70	> 70	Total
Patients (N)	60	166	164	390
Escherichia coli	55	53	48	51
Proteus mirabilis	3	2	7	5
Klebsiella pneumoniae	12	6	5	6
Other Gram-negatives*	8	9	14	11
Non-fermenters**	12	8	11	10
Enterococcus sp.	3	5	6	5
Other Gram-positives	7	16	9	11

* *Morganella sp., Citrobacter sp., Serratia sp., Providencia sp., Enterobacter sp.*and *Pasteurella sp.*
** *Pseudomonas sp.* and *Acinetobacter sp.*

4.1.1 General Practice

Escherichia coli
Antibiotic resistance among bacteria causing community acquired urinary tract infections (UTI) was determined for strains collected from male patients visiting GPs with symptoms indicative for an uncomplicated UTI in the absence of fever. The GPs (42) participated in the national NIVEL network. A total of 603 men (range 18-97 year) were included during 2009-2010, of whom 390 were positive; 83% of the isolated pathogens were Gram-negative bacteria with *E. coli* as the most common one in each age category (48-55%, table 4.1). Quantitative susceptibilities of all strains isolated towards indicator antibiotics were determined by microbroth dilution using the EUCAST breakpoints for resistance; resistance levels of all pathogens are presented in table 4.2.
The results for *E. coli* were compared with those obtained during a comparable study in 2004 and with data obtained from comparable studies among women in 2004 and 2009. Overall a slight increase of resistance over time was observed for all antibiotics tested among *E. coli* from men, except for nitrofurantoin (figure 4.1), but these differences were not statistically significant. Resistance levels in women were stable for amoxicillin, co-amoxiclav, quinolones and nitrofurantoin, whereas the resistance to trimethoprim and co-trimoxazole decreased. Gentamicin resistance was < 1% in men and women. Resistance levels for co-amoxiclav, trimethoprim, co-trimoxazole and quinolones among strains from men were higher than those found in strains from women (significant for trimethoprim and co-trimoxazole, $p < 0.05$) (figure 4.1). No differences in resistance levels were found between the different age categories. Only one ESBL producing *E. coli* (0.3%) was isolated.

Table 4.2. Resistance (%) among uropathogens from men with uncomplicated UTI in 2009-2010.

Species	Resistance (%)						
	amoxicillin	co-amoxiclav	trimethoprim	co-trimoxazole	norfloxacin	ciprofloxacin	nitrofurantoin
Escherichia coli (N=200)	35	16	24	23	6	6	0
Proteus mirabilis (N=18)	18	0	18	12	0	0	59
Klebsiella sp (N=25)	100	14	5	0	0	0	5
other Gramnegative facultative bacteria* (N=43)	82	53	16	11	16	8	13
Non-fermenters** (N=39)	62	38	64	62	19	16	47

* *Morganella sp., Citrobacter sp., Serratia sp., Providencia sp., Enterobacter sp.and Pasteurella sp.*
** *Pseudomonas sp.and Acinetobacter sp.*

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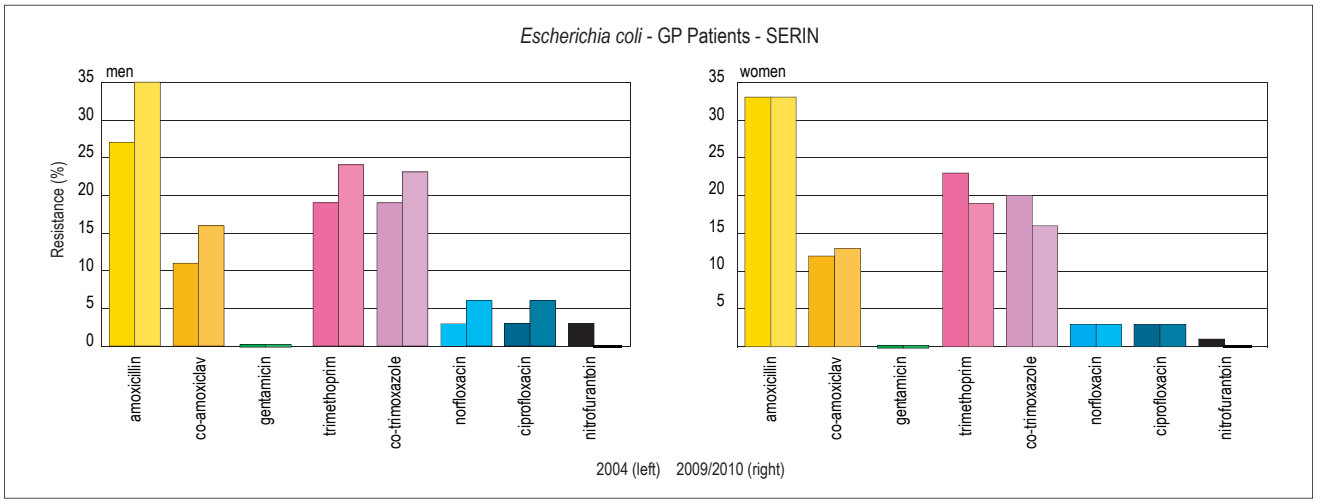

Figure 4.1. Resistance to antibiotics among *Escherichia coli* from male patients in 2004 (N=103) and in 2009/2010 (N=200) and female patients in 2004 (N=1724) and in 2009 (N=489) in the community.

Streptococcus haemolyticus

De prevalence of *S. haemolyticus* in the normal nasopharyngeal flora was investigated by taking nasal swabs from patients visiting the general practitioner with non-infectious- and non-throat complaints. A total of 41 GPs from the NIVEL network participating in this study sent swabs from 1925 patients from which 33 strains of *S. haemolyticus* serogroup A were isolated (1.7%). This study is still ongoing.

4.1.2 Nursing homes

Escherichia coli

Urine (freshly voided urine or pressed in incontinence materials) from 308 residents without infection of six nursing homes in the province of Limburg were cultured by taking dipslides in 2010-2011 and examined for the presence of *E. coli*. In total 208 strains were obtained; the resistance levels are presented in figure 4.2. Amoxicillin-, co-amoxiclav- and quinolone resistance levels are higher ($p<0.05$), than those found in GP patients (figure 4.1), the resistance levels of trimethoprim, co-trimoxazole and nitrofurantoin are comparable with those found in GP patients. Two *E. coli* strains (1%) were ESBL positive. Gentamicin resistance was 6%. This surveillance will be continued in 2012 by recruiting more nursing homes in other parts of the Netherlands and to determine also the prevalence of health care related infections and its relationship with antibiotic use.

Staphylococcus aureus

Nasal swabs from 308 residents having somatic disabilities without infections, living in six nursing homes in the province of Limburg were cultured for carriership of *S. aureus* in the commensal flora in 2010. A total of 99 strains of *S. aureus* were isolated (32%), of which one was an MRSA (1%). This is somewhat lower than the carriership found in 2007 in a comparable population of nursing home residents (N= 260, with 40% carriers and 2.6% MRSA). The resistance to penicillins, macrolides,

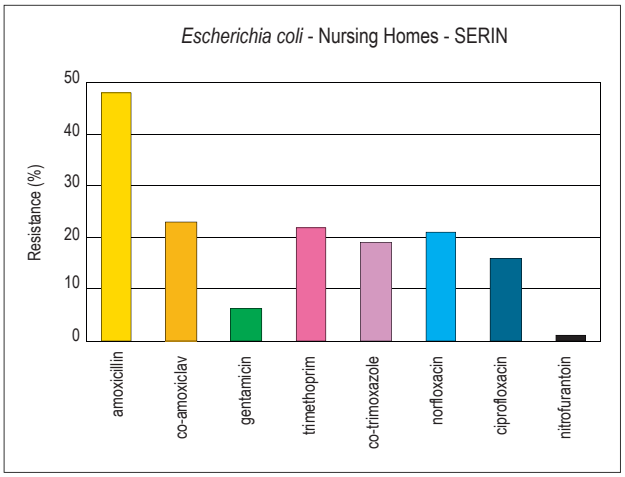

Figure 4.2. Resistance to antibiotics among *Escherichia coli* (N=208) from Nursing Home residents in 2010.

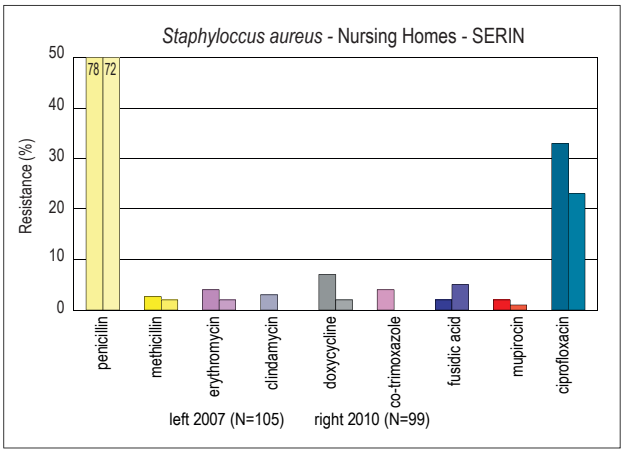

Figure 4.3. Resistance to antibiotics among *Staphylococcus aureus* from Nursing Home residents in 2007 (N=105) and 2010 (N=99).

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doxycycline, co-trimoxazole, fusidic acid, mupirocin and ciprofloxacin is presented in figure 4.3 and compared with the results found in 2007. No significant changes were recorded in resistance patterns. Resistance levels are comparable with those found in strains isolated from patients in Unselected Hospital Departments and Intensive Care Units (see later) except for ciprofloxacin, which was 23% in nursing home residents compared with 10% in hospital patients in 2010 ($p< 0.02$).
No resistance to clindamycin, gentamicin, co-trimoxazole, vancomycin or linezolid was recorded. Resistance to daptomycin was 1% (not shown).

GP and Nursing Homes - Conclusion

1. Overall resistance levels among *E. coli* in UTI strains in male GP patients is higher than in female GP patients; difference is significant for trimethoprim and co-trimoxazole ($p<0.05$).
2. Slight increase in resistance among *E. coli* in men since 2004; stable resistance in women.
3. Amoxicillin-, co-amoxiclav- and quinolone resistance levels among *E. coli* in nursing home residents are higher ($p<0.05$) than in GP patients.
4. Low prevalence of ESBL producing strains in GP patients (0.3%) and in nursing home residents (1%).
5. Gentamicin resistance in nursing home residents is 6% vs. < 1% in the community.
6. Carriership of *S. aureus* in commensal flora of nursing home residents is 32% with 1% MRSA.
7. Resistance levels among *S. aureus* in nursing homes are similar to those found for Unselected Hospital Departments and Intensive Care Units, except for ciprofloxacin which was significantly higher in nursing homes (23%, $p< 0.05$).

4.1.3 Community

Neisseria meningitidis

From 1994-2011 a total of 4657 strains from cerebrospinal fluid (CSF) and 2859 strains from blood were included in the surveillance project of The Netherlands Reference Laboratory for Bacterial Meningitis of the Academic Medical Centre, Amsterdam. The number of strains moderately susceptible to penicillin (MIC 0.125-0.25 mg/l) increased since 2000 from less than 2% to 22% in 2011 and from 1% to 35% for strains isolated from CSF and blood, respectively (figure 4.4). Penicillin resistance (MIC >0.5 mg/l) was occasionally found in strains both from CSF and blood in some years, the last time in 2006 (4.4). The MIC distributions of penicillin showed a slow movement of the peak to the right from 0.03 to 0.06 mg/l from 2008 onwards and broadening of the range, including more strains with MICs 0.125-0.25 mg/l in 2011.
Nineteen of the 27 strains isolated in 2011 which were moderately susceptible belonged to serogroup B, six to serogroup Y, one to serogroup C and one was not groupable.
Resistance to rifampicin and ceftriaxone was not found.

***Neisseria meningitidis* - Conclusion**

1. Penicillin resistance not found since 2006.
2. 22% of strains from blood and 35% from CSF were moderately susceptible to penicillin in 2011.
3. Resistance to ceftriaxone and rifampicin not found.

Neisseria gonorrhoeae

The national project GRAS started in 2006 and collects epidemiologic data on gonorrhea and resistance patterns of isolated strains from Sexually Transmitted Infections (STI) centres. The participating STI centres represent 83% of the total population of STI centre attendees, but exclude GP patients. Diagnosis of gonorrhea is made by culture or PCR on patients' materials, with an obvious decrease in number and percentages of cultures over time (figure 4.5) (data from the yearly questionnaire sent by the RIVM to all laboratories).
Susceptibility testing for 5789 isolates was performed by E-test for penicillin, tetracycline, ciprofloxacin and cefotaxime (2006-2011); in 2011 ceftriaxone, azithromycin and spectinomycin were added to the panel for which 1283 isolates were tested. Resistance levels were calculated using the EUCAST breakpoints for resistance.
Overall penicillin resistance decreased significantly until 2010 ($p< 0.05$) and increased thereafter to 13% in 2011 (figure 4.6). It is difficult to estimate this increase since only 20% of strains was tested for penicillin 2011.
Tetracycline resistance increased to 60% in 2009 and decreased to 47% in 2011 (figure 4.6).
Ciprofloxacin resistance increased to 52% in 2009 and decreased to 37% in 2011.
Cefotaxime resistance (MIC > 0.12 mg/l) increased from 1% in 2006 up to 9% in 2010 and decreased to 5% in 2011 (figure 4.6). The MIC distribution of cefotaxime (figure 4.7) showed a unimodal shape with broadening over the range since 2009 and a growing subpopulation with MIC 0.12-0.25 mg/l. Cefotaxime resistant strains were more common in MSM (8%), than in heterosexual men and women (3%).
Ceftriaxone resistance was not found, the MIC distribution was also unimodal (figure 4.7). Resistance to azithromycin was 7%; one isolate (0.1%) was resistant to spectinomycin.
The changing antibiotic resistance pattern among gonococci underlines the need for a continuous surveillance to detect trends which might necessitate modification of treatment guidelines.

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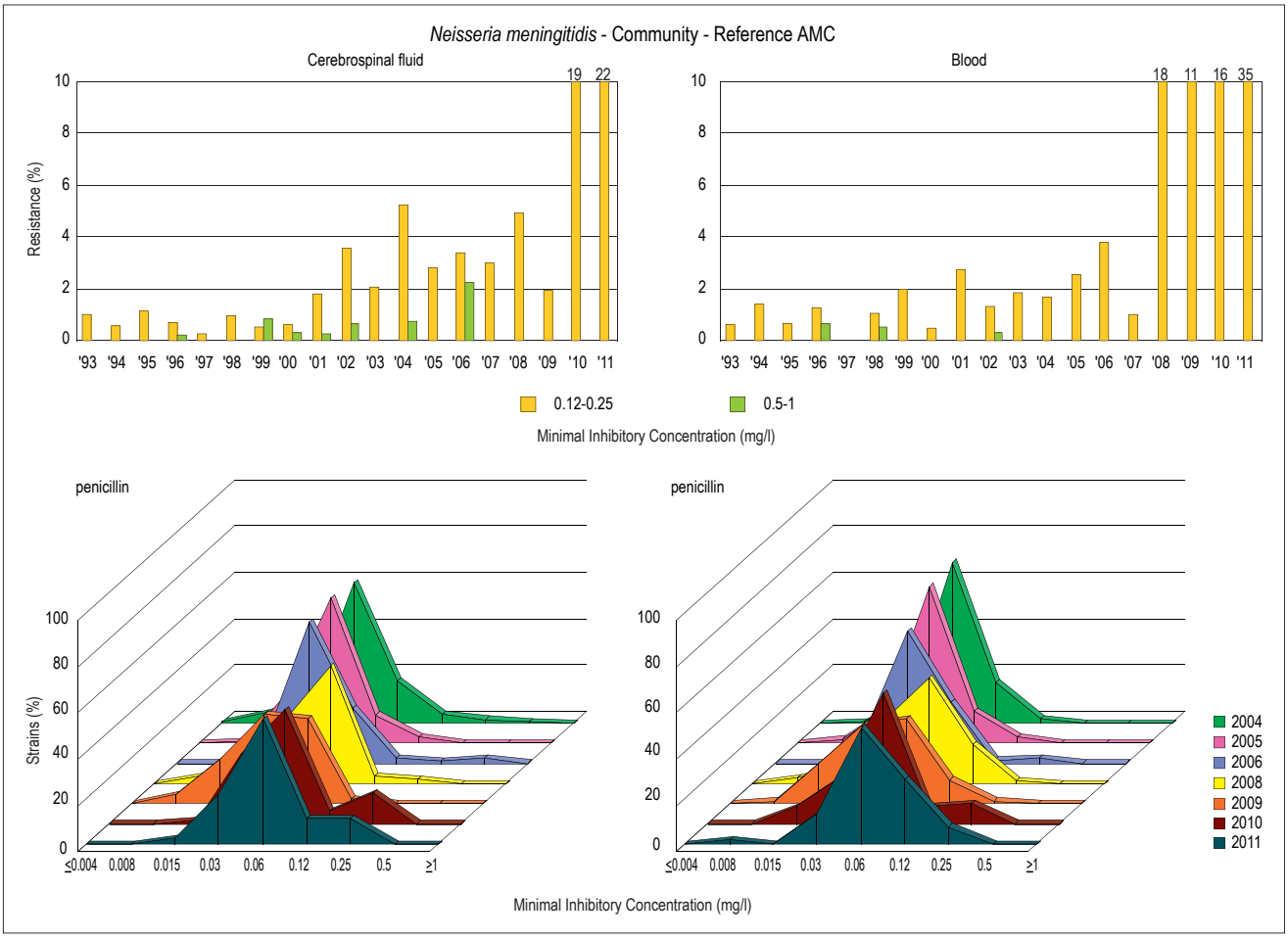

Figure 4.4. Trends in penicillin resistance and MIC distributions of penicillin for *Neisseria meningitidis* from CSF (N= 4.657) and blood (N=2.859). MIC data for 2007 were not available.

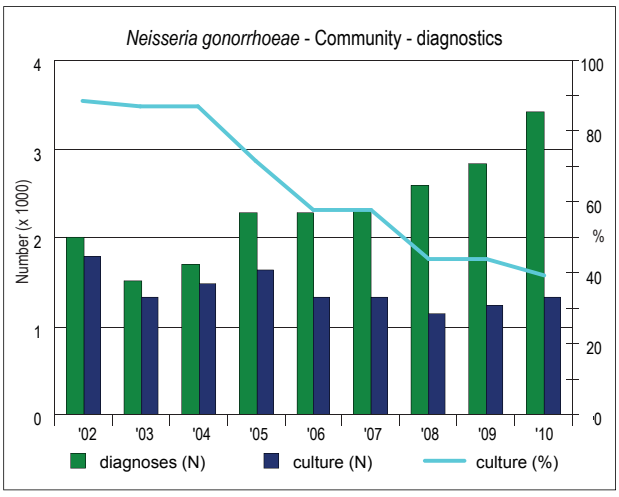

Figure 4.5. Diagnoses of gonorrhoea in The Netherlands since 2002 (yearly RIVM questionnaire).

***Neisseria gonorrhoeae* - Conclusion**

1. Resistance to tetracycline (47%) and ciprofloxacin (37%) decreased.
2. Overall cefotaxime resistance was 5%, highest resistance (8%) among isolates in men having sex with men. Ceftriaxone resistance was not found.
3. Azithromycin resistance was 7% in 2011.

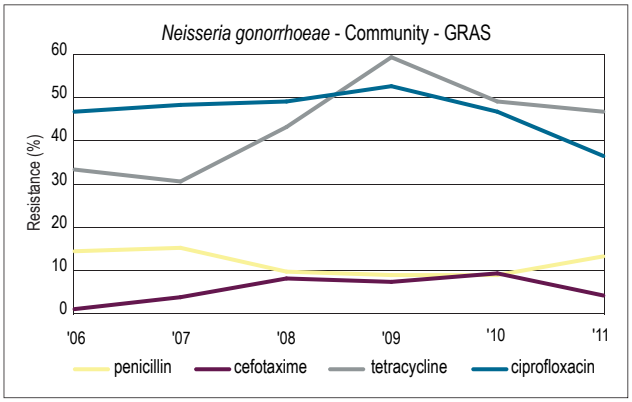

Figure 4.6. Trends in antibiotic resistance among *Neisseria gonorrhoeae* (N=5.789).

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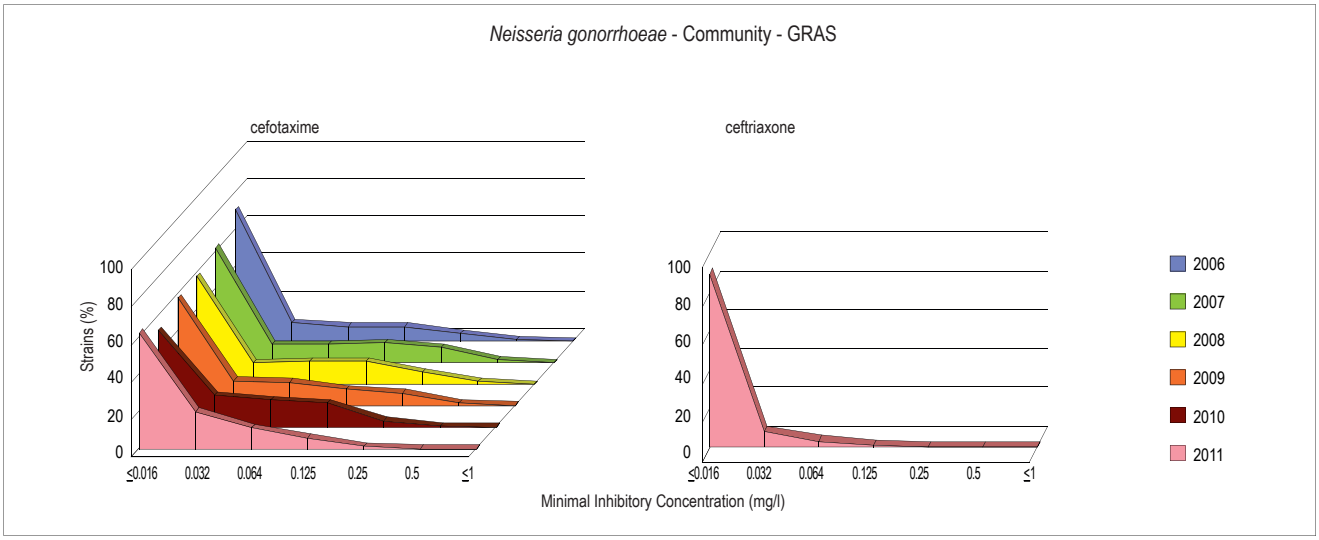

Figure 4.7. MIC distributions of cefotaxime and ceftriaxone for *Neisseria gonorrhoeae.*

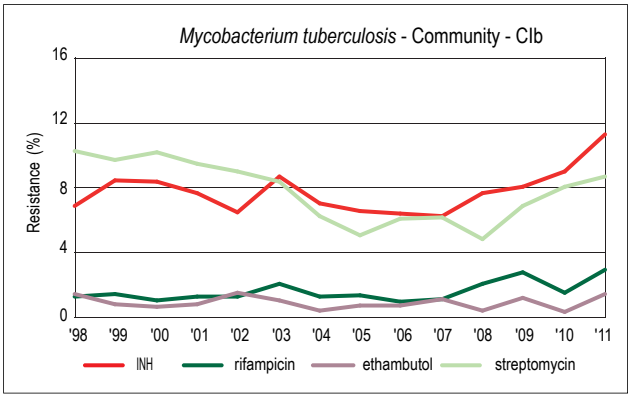

Figure 4.8. Trends in antibiotic resistance among *Mycobacterium tuberculosis* (N= 12,250).

Mycobacterium tuberculosis

A total of 12,250 strains of *M. tuberculosis* complex were obtained during 1998-2011. In the period of 2001 till 2006, the number of strains isolated yearly gradually decreased, from 1080 in 2001 to 727 in 2006 (33% decline), since then this number is stable. In 2011, 734 strains were sent to the RIVM; susceptibility was determined for 546 strains. INH resistance increased since 2008 to 11.3% in 2011 (figure 4.8).
Rifampicin resistance increased to 3% in 2011.
Ethambutol is also routinely used as a first line drug in TB treatment. Resistance to ethambutol remained low, fluctuating between 0.2% and 1.6% and was 1.5% in 2011.

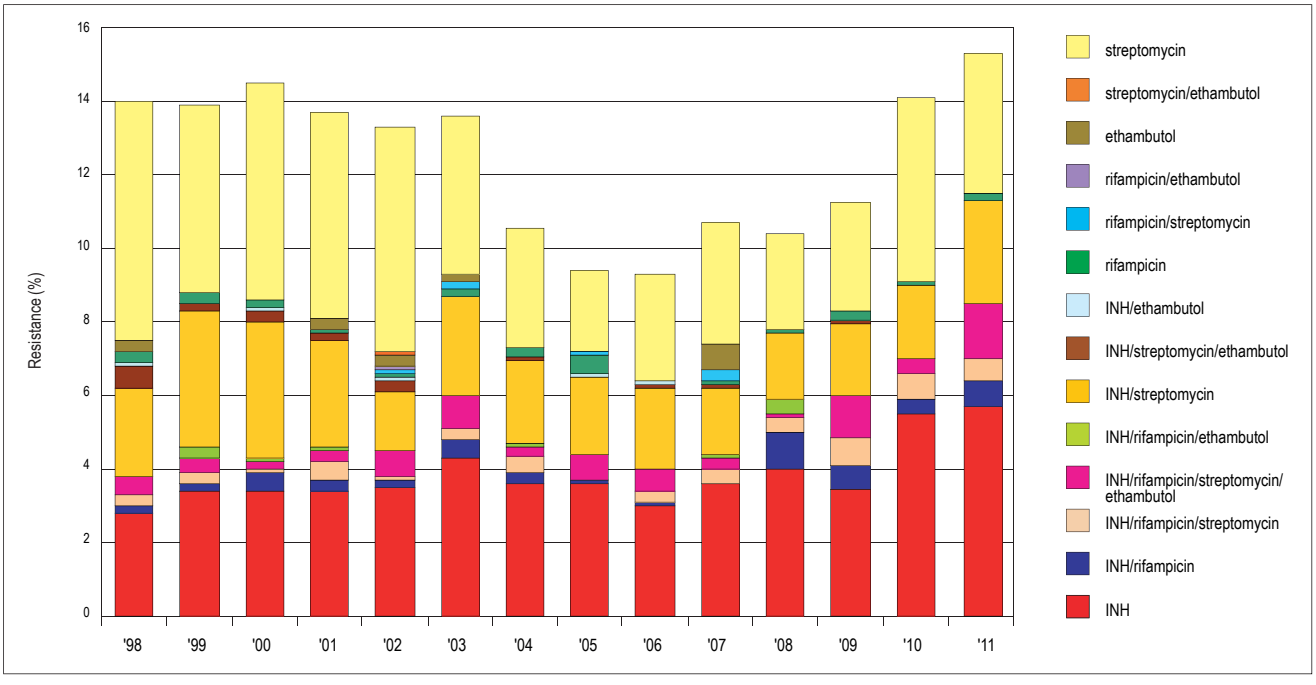

Figure 4.9. Trends in combined resistance among *Mycobacterium tuberculosis* (N= 12,250).

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Streptomycin is not used as a first line drug in the western world anymore; resistance to streptomycin decreased from 10.2% in 2000 to 4.9% in 2008, but has raised since then to 8.7% in 2011.
Combined resistance to more than one drug increased from 3.5% in 2010 to 5.6% in 2011 (figure 4.9); co-resistance to rifampicin and INH was recorded in 2.8% of the isolates, whereas resistance to all four antimicrobial agents re-increased from 0.4% in 2010 to 1.5% in 2011 (figure 4.9).

***Mycobacterium tuberculosis* - Conclusion**
1. Increasing resistance to INH (11%), rifampicin (3%) and streptomycin (8%).
2. Varying and low resistance to ethambutol (1.5% in 2011).
3. Combined resistance to INH and rifampicin was 3.5%; multiresistance to the four drugs tested increased to 1.5% in 2011.

4.2 Surveillance of resistance in specific patient populations

ISIS-AR
Between 2008 and 2011, 22 laboratories continuously reported susceptibility data to the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) including MICs, breakpoint MICs (≤ or >), S I R interpretations and disk zone diameters. When MICs or breakpoint MICs were available the EUCAST breakpoints for resistance (R) were applied to the available MICs to determine the annual proportion of resistant isolates for *E. coli, P. mirabilis, K. pneumoniae, E. cloacae, P. aeruginosa, Acinetobacter spp., S. aureus*, and coagulase-negative staphylococci including *S. epidermidis*. These results may differ from previous interpretations (NethMap 2009-2011) where only S I R data as reported by the individual laboratories were evaluated. In 2009-2011, the S I R interpretations of the laboratories were primarily based on CLSI breakpoints of which the I+R proportion for most antimicrobial agents corresponds to the proportion of resistant (R) isolates when using EUCAST breakpoints. In NethMap 2009-2010 I+R values, as reported by the laboratories, were therefore used to determine resistance. Now the reported S I R interpretations were only used when no MICs were available or when < 80% of the MICs was available for a specific antimicrobial agent within a given species, such as for enterococci, *H. influenzae, S. pneumoniae* and *M. catarrhalis*. Not all antimicrobial agents were tested for all species by all participating laboratories; therefore the range of the minimum and maximum numbers of isolates tested within each species is given. A more detailed overview of the materials and methods used can be found at www.swab.nl.

4.2.1 General Practice - ISIS-AR
Data from urinary isolates from GP patients were included in this evaluation. General practitioners use to send urine samples for culture and susceptibility testing in case of complicated UTI or when there is no response to antimicrobial therapy (selected GP patients). The presented resistance levels are therefore not representative for all patients with UTI presenting at the GP.

Escherichia coli
Isolates tested (N): 4089-54,685
Laboratories participating (N): 13-20.
Trends. Amoxicillin resistance decreased slightly from 42% in 2008 to 40% in 2011, co-amoxiclav resistance was around 15% during the whole period (figure 4.10); both levels were higher than the resistance level in GP patients with uncomplicated UTI (figure 4.1)). Cefuroxime resistance remained stable at 6%, resistance to ceftriaxone/cefotaxime increased slightly (from 2% in 2008 to 3% in 2011, figure 4.10). Trimethoprim resistance decreased to 28%, but co-trimoxazole resistance increased from 24% to 26%. Normally these two drugs follow the same trend. Not all participating laboratories tested both trimethoprim and co-trimoxazole and when restricting the analysis to those laboratories which tested both agents, a significant increase in resistance over time for both drugs was observed. Differences in methodology used by the participating laboratories may also contribute to these discrepancies and need further analyses. Gentamicin resistance was 4% which is higher than the level found in GP patients with uncomplicated UTI in the community (figure 4.1)
Quinolone resistance was stable at 14-15% for norfloxacin and 9% for ciprofloxacin.
The resistance levels of amoxicilline, co-amoxiclav, trimethoprim, co-trimoxazole and the quinolones are all significantly higher than the levels found in GP patients with uncomplicated UTI (figure 4.1), indicating that the selected GP patients were exposed to these drugs before. Nitrofurantoin resistance was 2% during the whole period, fosfomycin resistance was less than 1%, but was only reported by 13 laboratories.

Klebsiella pneumoniae
Isolates tested (N): 1213-5359
Laboratories participating (N): 13-20.
Trends. Co-amoxiclav resistance remained stable at 9% (figure 4.10), cefuroxime resistance was around 12%, the resistance level of ceftriaxone/cefotaxime (3% in 2011) and ceftazidime (2%) resembled those for *E. coli*. Trimethoprim resistance decreased with fluctuations to 26% in 2011 with a simultaneous increase of co-trimoxazole resistance from 15% in 2008 to 18% in 2011 ($p < 0.05$). Ciprofloxacin resistance level remained low (4% in 2011), whereas norfloxacin resistance appeared to be 20%.
Data on fosfomycin resistance were only reported by 13 laboratories for a limited number of strains from 2009-2011 (1213-4547); a slight increase in resistance was observed

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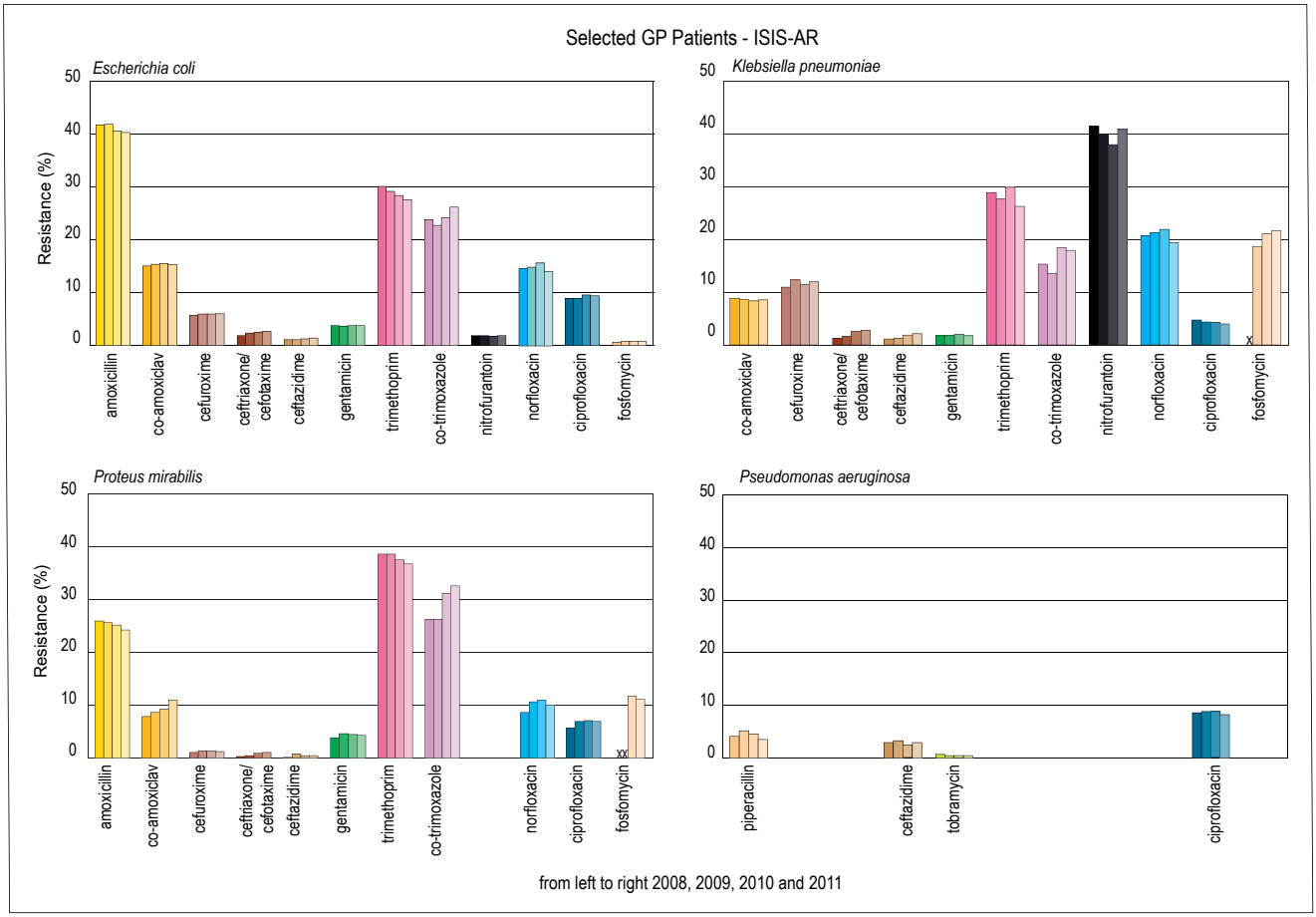

Figure 4.10. Trends in antibiotic resistance (2008-2011) among urinary strains of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* from Selected Patients of General Practice, reported to ISIS-AR.

Table 4.3. Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa from Selected Patients of General Practice in 2011.

Antibiotic	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
amoxicilin	40		24	
co-amoxiclav	15	9	11	
piperacillin				3
cefuroxime	6	12	1	
cefotaxime/ceftriaxone	3	3	1	
ceftazidime	1	2	1	3
gentamicin	4	2	4	
tobramycin				1
trimethoprim	28	26	37	
co-trimoxazole	26	18	33	
norfloxacin	14	20	10	
ciprofloxacin	9	4	7	8
nitrofurantoin	2	41		
fosfomycin	1	22	11	

increasing since 2008
decreasing since 2008
stable since 2008

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from 19% in 2009 to 22% in 2011. Data on nitrofurantoin resistance varied highly between the different laboratories which may be associated with the fact that the EUCAST breakpoints fall within the wild type distribution; overall 41% resistance was reported in 2011 (figure 4.10).

Proteus mirabilis
Isolates tested (N): 3591-5360
Laboratories participating (N): 13-20.
Trends. Amoxicillin resistance decreased to 24% in 2011 (figure 4.10) with a simultaneous increase in resistance to co-amoxiclav (11%, p< 0.05). Cephalosporin resistance remained low over time (1%). Also here, a decrease in trimethoprim resistance was observed from 39% in 2008 to 37% in 2011, with a simultaneous increase in resistance to co-trimoxazole from 26% to 33%. Norfloxacin resistance was around 10%, ciprofloxacin resistance 7%. Data on fosfomycin resistance were available from 13 laboratories; the resistance was stable at 11% over time.

Pseudomonas aeruginosa
Isolates tested (N): 1353-1999
Laboratories participating (N): 8-20.
Trends. Stable resistance levels over time were found for piperacillin (4%), ceftazidime (3%), tobramycin (0.5%) and ciprofloxacin (8%) (figure 4.10).

Selected GP Patients - Conclusion (see also table 4.3)
1. All resistance levels in selected GP patients were significantly higher than in GP patients from the community with an uncomplicated UTI.
2. Co-amoxiclav resistance was 9-15% among *Enterobacteriaceae*. Empirical therapy with co-amoxiclav should be reconsidered in selected GP patients.
3. Cephalosporin resistance remained low, except for cefuroxime in *K. pneumoniae* (12% resistance).
4. Trimethoprim- and co-trimoxazole resistance levels are too high for empirical therapy in selected GP patients.
5. Quinolone resistance is stable, but high, except for ciprofloxacin in *K. pneumoniae.*

4.2.2 Outpatient Departments - ISIS-AR

Data from urinary isolates from patients visiting Outpatients Departments reported to ISIS-AR were included in this evaluation.

Escherichia coli
Isolates tested (N): 1034-16,372
Laboratories participating (N): 9-21.
Trends. Resistance levels for amoxicillin (45%) and co-amoxiclav (19%) were stable over time (figure 4.11) and higher than in selected GP patients. Cefuroxime resistance was around 10%, that of ceftriaxone/cefotaxime increased slightly (4% in 2011). The resistance levels of trimethoprim (31% in 2011) and co-trimoxazole (29%) showed the same trends as those among selected GP patients, but at a higher level (figure 4.11). Norfloxacin resistance fluctuated around 23% and ciprofloxacin showed a slight increase to 17% in 2011. Both levels were significantly higher than those found in selected GP patients and are therefore no option for empirical therapy. Nitrofurantoin resistance was stable at 3% and fosfomycin resistance remained 1% during the whole study period (figure 4.11).

Klebsiella pneumoniae
Isolates tested (N): 1689-2512
Laboratories participating (N): 9-21.
Trends. Co-amoxiclav resistance (10%) and cefuroxime resistance (12%) remained stable over time and were comparable with the levels found in selected GP patients. Resistance to ceftriaxone/cefotaxime increased from 2% in 2008 to 5% in 2011 (p<0.05); that to ceftazidime from 1% to 4% (p<0.05). Trimethoprim resistance level was stable at 26%, that of co-trimoxazole increased to 18% (figure 4.11), both levels being similar to those found in selected GP patients. Norfloxacin resistance was high (20%), resistance to ciprofloxacin remained around 7%. Nitrofurantoin resistance levels reported by the different laboratories showed the same large variations as observed for selected GP patients, and. decreased since 2008 to 36% in 2011. Data on fosfomycin resistance were reported by nine laboratories; resistance increased over the years from 14% to 25% (p< 0.05).

Proteus mirabilis
Isolates tested (N): 1302-1912
Laboratories participating (N): 9-21.
Trends. Amoxicillin resistance was stable and remained at 26%, but co-amoxiclav resistance increased from 7.5% to 13% (figure 4.11), p< 0.05); both levels were somewhat higher than those found in selected GP patients. Resistance levels to cefuroxime, ceftriaxone/cefotaxime and ceftazidime were 2% or less. Trimethoprim and co-trimoxazole resistance levels were similar to those found in selected GP patients that to co-trimoxazole showed an increase and was 33% in 2011 (p<0.05). Gentamicin resistance was stable at 6%. Norfloxacin resistance was 20%, similar to the level in selected GP patients, ciprofloxacin resistance increased slightly in 2011 to 11% (figure 4.11, not significant). Data on fosfomycin resistance were obtained from 887 isolates from nine laboratories; resistance fluctuated from 10-16% (figure 4.11).

Pseudomonas aeruginosa
Isolates tested (N): 863-1112
Laboratories participating (N): 11-21.
Trends. Stable resistance levels were observed for piperacillin (6%), ceftazidime (4%), tobramycin (1%) and ciprofloxacin (10%).

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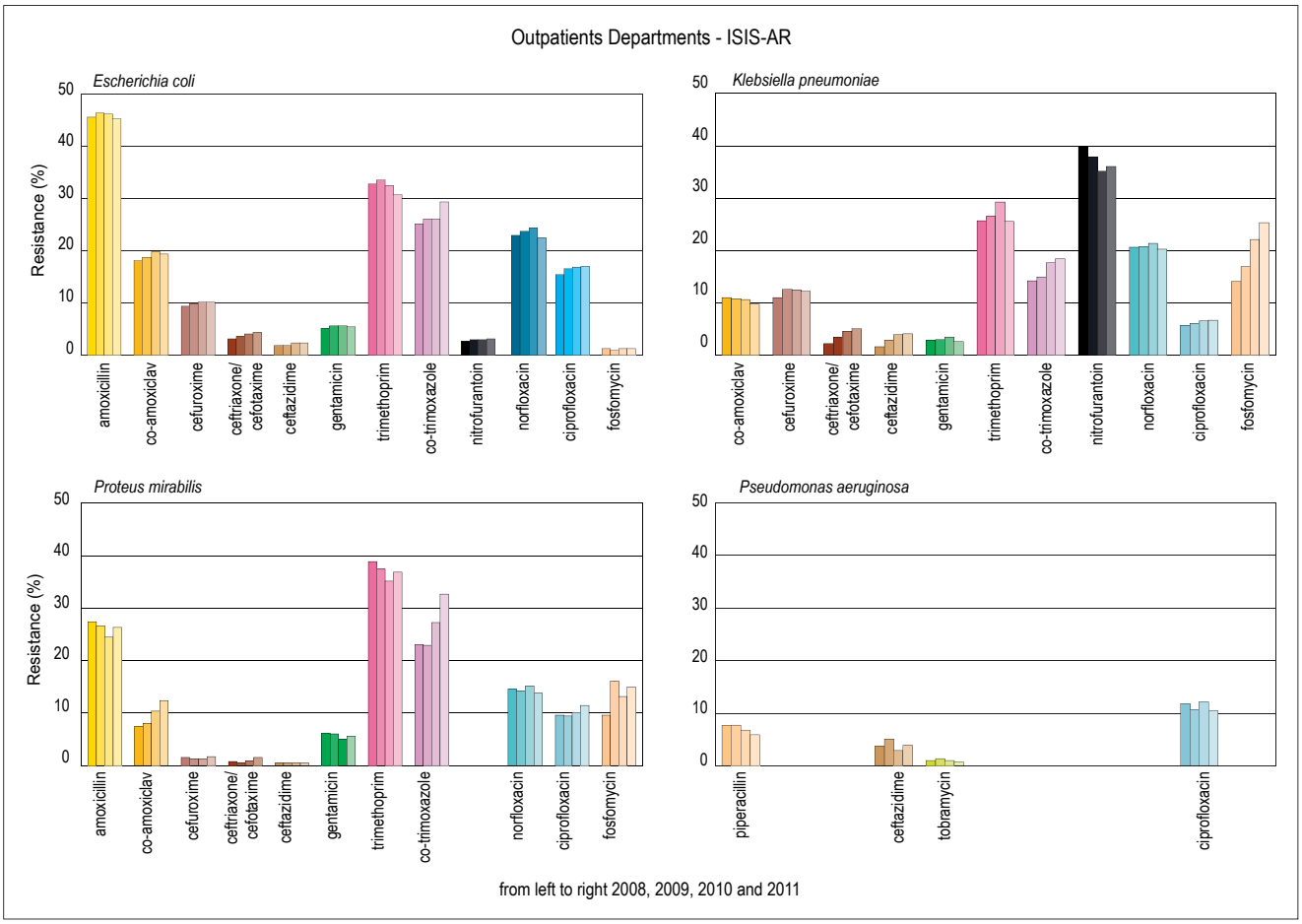

Figure 4.11. Trends in antibiotic resistance (2008-2011) among urinary strains of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* from patients of Outpatients Departments reported to ISIS-AR.

Table 4.4. Resistance levels among Enterobacteriaceae and P. aeruginosa in Outpatients Departments in 2011.

Antibiotic	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
amoxicilin	45		26	
co-amoxiclav	19	10	13	
piperacillin				6
cefuroxime	10	12	2	
ceftriaxone/cefotaxime	4	5	2	
ceftazidime	2	4	1	4
gentamicin	6	3	6	
tobramycin				1
trimethoprim	31	26	37	
co-trimoxazole	29	18	33	
norfloxacin	23	20	14	
ciprofloxacin	17	7	11	10
nitrofurantoin	3	36		
fosfomycin	1	25	15	

increasing since 2008
decreasing since 2008
stable since 2008

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Outpatient Departments - Conclusion (see also table 4.4)

1. Resistance levels among urinary isolates from patients visiting Outpatient Departments were similar or 1-3% higher than those in selected GP patients.
2. The resistance levels of amoxicillin, co-amoxiclav, trimethoprim, co-trimoxazole and the quinolones are too high for empirical therapy of infections by *Enterobacteriaceae*.
3. Fosfomycin resistance low in *E. coli*, but increasing in *K. pneumoniae* and *P. mirabilis* to levels which may make the drug unusable for empirical therapy.
4. Empirical therapy of urinary infections by *E. coli* (most frequently) is only possible with nitrofurantoin or fosfomycin in case of uncomplicated UTI or with a 3rd generation cephalosporins or gentamicin for complicated UTI.
5. Empirical therapy of urinary infections by pathogens other than *E. coli* is only possible with cephalosporins or gentamicin.

4.2.3 Unselected Hospital Departments - ISIS-AR

Data from clinical isolates (urine, wound, blood, respiratory tract) of patients hospitalized in Unselected Hospital Departments excluding Intensive Care Units, reported to ISIS-AR were evaluated. Resistance to carbapenems among *Enterobacteriaceae* was only evaluated in 2011, since isolates from previous years were not confirmed by a second phenotypic antimicrobial susceptibility test. For further details see chapter 6.

Escherichia coli
Isolates tested (N): 12,336-18,427
Laboratories participating (N): 18-21.
Trends. Amoxicillin resistance (figure 4.12) was similar to the level found in Outpatient Departments; co-amoxiclav resistance increased from 19% in 2009 to 21% in 2011, piperacillin-tazobactam resistance from 2% to 5%. A slight increase was observed for all cephalosporins tested (figure 4.12). One of 16.932 isolates in 2011 was resistant to the carbapenems. Gentamicin resistance was stable at 5% over time, similar to the level found in Outpatient Departments and selected GP patients. Co-trimoxazole resistance was increasing from 21% in 2008 to 28% in 2011. Resistance to ciprofloxacin increased from 11% to 13%. Nitrofurantoin resistance remained stable at 2% (figure 4.12), similar to the level found for *E. coli* in other patient groups.

Klebsiella pneumoniae
Isolates tested (N): 2644-3373
Laboratories participating (N): 20-21.
Trends. Resistance to co-amoxiclav increased slowly to 12%, to cefuroxime to 13% and the resistance levels of ceftriaxone/cefotaxime and ceftazidime increased to 7% and 6% respectively (figure 4.12). One of 2.931 isolates tested was resistant to imipenem/meropenem in 2011. Co-trimoxazole resistance increased to levels similar to those found in selected GP patients and Outpatient Departments. Ciprofloxacin resistance remained stable around 7%, whereas nitrofurantoin resistance decreased to 28%.

Enterobacter cloacae
Isolates tested (N): 1624-1953
Laboratories participating (N): 18-21.
Trends. Data on susceptibility to the carbapenems, gentamicin, co-trimoxazole, ciprofloxacin and nitrofurantoin were available for interpretation. One of 1.796 isolates tested was confirmed to be resistant to imipenem/meropenem in 2011. Resistance to gentamicin and ciprofloxacin was 5% or less over time (figure 4.12), whereas that to co-trimoxazole increased significantly from 5% in 2008 to 8% in 2011 ($p<0.05$). Nitrofurantoin resistance fluctuated around 19-20%.

Proteus mirabilis
Isolates tested (N): 2633-3539
Laboratories participating (N): 18-21.
Trends. Resistance levels to amoxicillin (25%) and co-amoxiclav (increasing from 8% to 12%, $p< 0.05$) were similar to the levels found in the former patient groups. Piperacillin-tazobactam resistance was low (3% in 2011); resistance to cephalosporins remained low (1-2%). Carbapenem resistance was not recorded. Significantly increasing were the resistance levels to co-trimoxazole (from 19% to 30%) and to ciprofloxacin (from 5% to 8%).

Pseudomonas aeruginosa
Isolates tested (N): 2976-3928
Laboratories participating (N): 18-21.
Trends. Piperacillin resistance increased slowly from 8% in 2008 to 10% in 2011 (figure 4.12); piperacillin-tazobactam resistance, reported for 1.395 isolates from 14 laboratories in 2011, was also 10%. Carbapenem resistance was stable at 2%, ceftazidime resistance remained 5%. Resistance to gentamicin increased significantly between 2008 (4%) and 2011 (8%), that to tobramycin remained 2% over time. Ciprofloxacin resistance was stable (8%) and similar to the level in selected GP patients and lower than in Outpatients Departments.

Acinetobacter species
Isolates tested (N): 276-433
Laboratories participating (N): 20-21.
Trends. Data on susceptibility to the carbapenems, ceftazidime, gentamicin, co-trimoxazole and ciprofloxacin were available for interpretation. Resistance levels fluctuated considerably over time (figure 4.12). Carbapenem resistance was 1-4%, gentamicin

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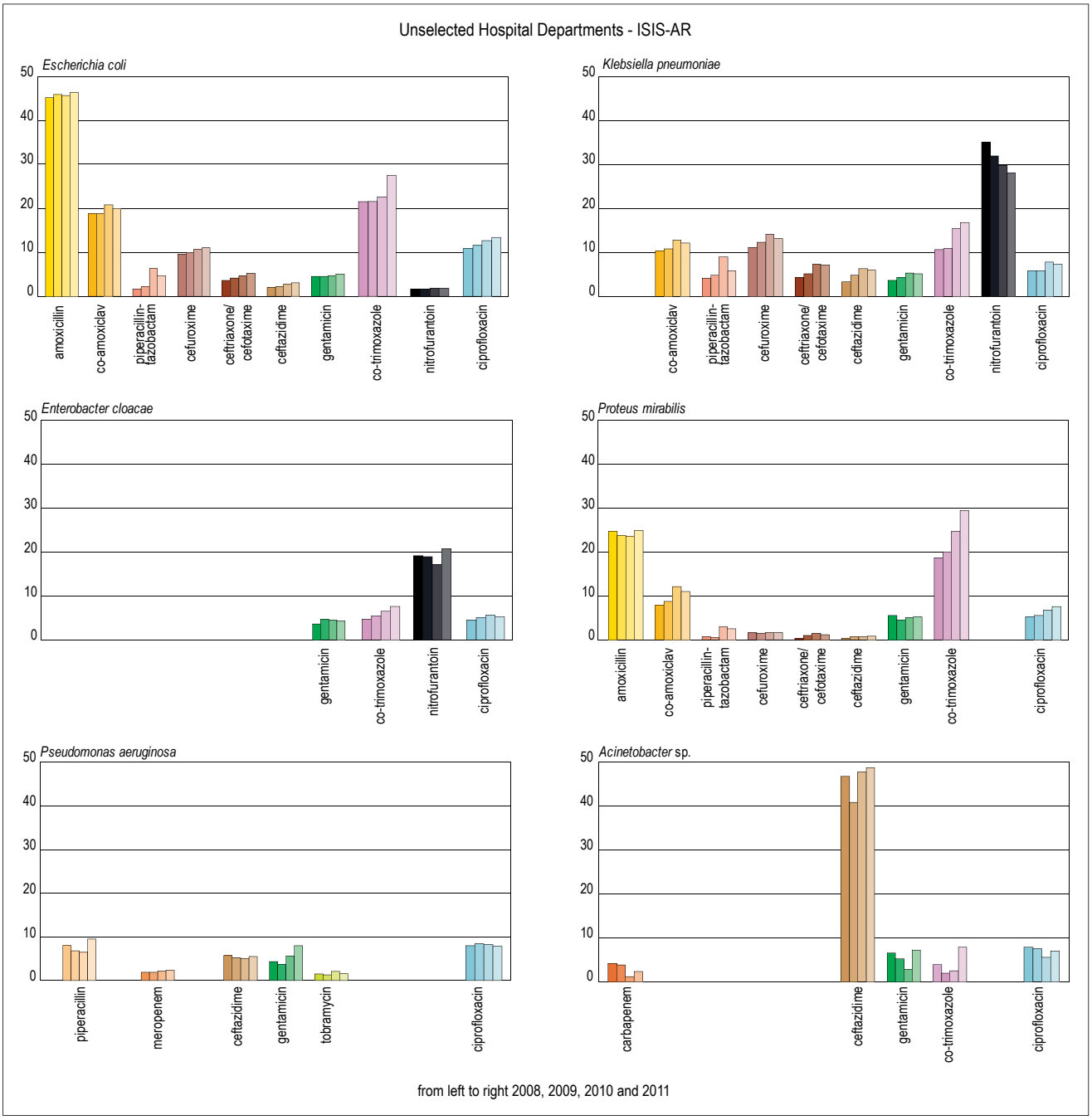

Figure 4.12. Trends in antibiotic resistance (2008-2011) among clinical strains of *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Pseudomonas aeruginosa* and *Acinetobacter spp.* from patients of Unselected Hospital Departments reported to ISIS-AR.

resistance ranged from 3-7%, co-trimoxazole 2-8% and ciprofloxacin resistance was 6-8%.

Enterococcus species
Isolates tested (N): *E. faecalis* 1535-3663; *E. faecium* 350-1490
Laboratories participating (N): *E. faecalis* 12-19; *E. faecium* 12-21.
Trends. Data on susceptibility to vancomycin and teicoplanin were evaluated by taking I+R interpretations of the participating laboratories. Vancomycin resistance among *E. faecalis* ranged from 0.2-0.6%, teicoplanin resistance was 0.1-0.4%; no significant trend could be observed for both glycopeptides. Vancomycin resistance among *E. faecium* isolates fluctuated between 0.1-0.8% from 2008-2010 and was 1.5% in 2011. This increase was associated with an outbreak of VRE in several hospitals in the northern part of the Netherlands. Most of these strains were teicoplanin susceptible, as only 0.3% resistance to teicoplanin was observed in 2011.

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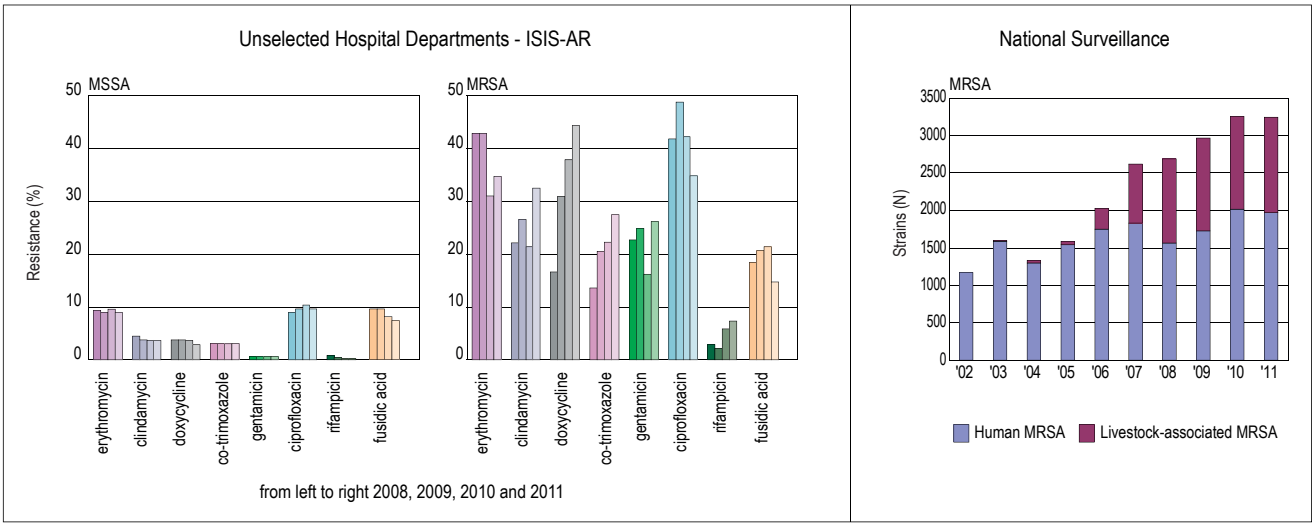

Figure 4.13. Trends in antibiotic resistance (1998-2011) among clinical strains of *Staphylococcus aureus* from patients of Unselected Hospital Departments, reported to ISIS-AR. and the numbers and origin of MRSA strains sent to the RIVM for typing.

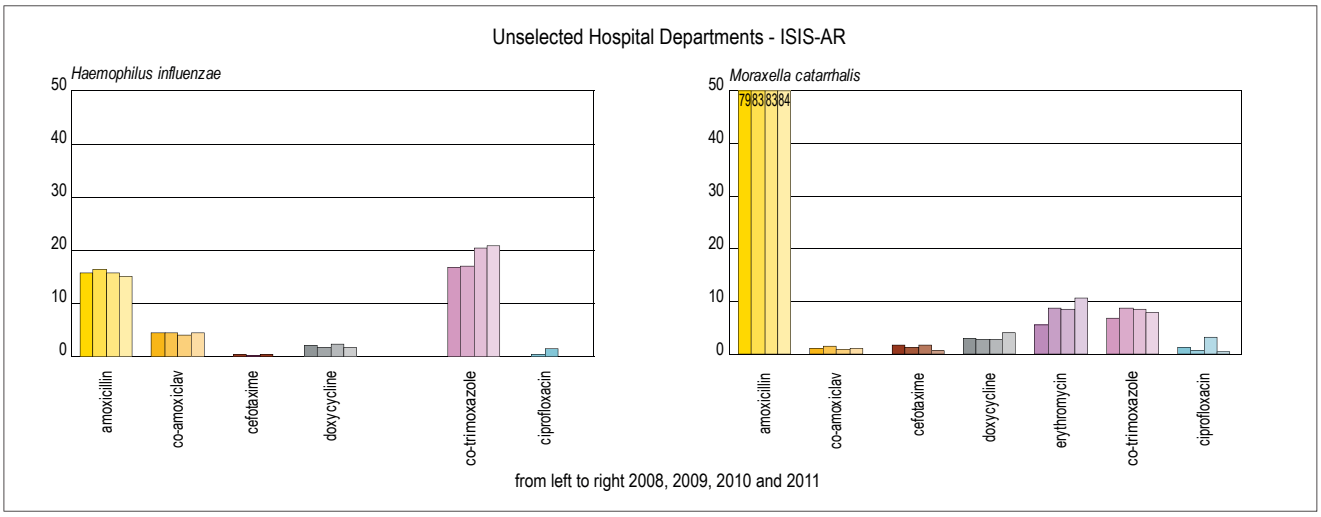

Figure 4.14. Trends in antibiotic resistance (1998-2011) among clinical strains of *Haemophilus influenzae* and *Moraxella catarrhalis* from patients of Unselected Hospital Departments, reported to ISIS-AR.

Staphylococcus aureus
Isolates tested (N): 8140-8824
Laboratories participating (N): 18-22.
Methicillin resistance increased slowly to 1.8% in 2011. In 2011 a total number of 3,246 MRSA isolates were forwarded to the National Institute for Public Health and the Environment (RIVM) for typing, 16 isolates less than in 2010. A total of 1271 strains (39%) appeared to be livestock-related CC398 strains (figure 4.13).

MSSA - Number of isolates available for interpretation: 4646-6313
Trends. No significant changes were observed for resistance levels of erythromycin (9%), clindamycin (4%), doxycycline (3-4%), gentamicin (< 1%), co-trimoxazole (3%) or ciprofloxacin (9%) (figure 4.13).

Less than 0.5% of the strains were resistant to rifampicin; resistance to fusidic acid decreased from 10% in 2008 to 8% in 2011 ($p < 0.05$).

MRSA - Number of isolates available for interpretation: 55-125
Trends. Resistance levels to all antibiotics tested were significantly higher among MRSA compared to MSSA (figure 4.13). Resistance rates for clindamycin, doxycycline, co-trimoxazole and rifampicin increased over time, those of erythromycin, ciprofloxa cin and fusidic acid tended to decrease. None of the isolates was resistant to linezolid.

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Table 4.5. Resistance levels among Enterobacteriaceae and Non-fermenters in Unselected Hospital Departments in 2011.

Antibiotic	*E.coli*	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*Acinetobacter sp.*
amoxicilin	46			25		
co-amoxiclav	21	12		12		
piperacillin					10	
piperacillin-tazobactam	5	6		3		
carbapenem			0.1		2	3
cefuroxime	11	13		2		
ceftriaxone/cefotaxime	5	7		1		
ceftazidime	3	6		1	5	49
gentamicin	5	5	4	5	8	7
tobramycin					2	
co-trimoxazole	28	17	8	30		8
ciprofloxacin	13	7	5	8	8	7
nitrofurantoin	2	28	20			

increasing since 2008
decreasing since 2008
stable since 2008

Table 4.6. Resistance levels among Staphylococcus aureus and respiratory pathogens in Unselected Hospital Departments in 2011.

Antibiotic	*MSSA*	*MRSA*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
penicillin			2		
amoxicillin				15	84
co-amoxiclav				4	1
ceftriaxone				0.1	1
erythromycin	9	35	9		11
clindamycin	4	32			
doxycycline	3	44	10	2	4
co-trimoxazole	3	27		21	8
gentamicin	1	26			
ciprofloxacin	9	35		0	0.5
rifampicin	0.1	8			
vancomycin					
linezolid					
fusidic acid	8	15			

increasing since 2008
decreasing since 2008
stable since 2008

Coagulase negative staphylococci including Staphylococcus epidermidis
Isolates tested (N): 3450-4089
Laboratories participating (N): 16-21.
Trends. Resistance data of linezolid and vancomycin were taken for evaluation. Linezolid resistance appeared stable around 0.7%; nine strains (0.2%) were vancomycin resistant.

Streptococcus pneumoniae
Isolates tested (N): 1640-2226
Laboratories participating (N): 20-22.
Trends. Data on susceptibility to benzylpenicillin, erythromycin and doxycycline were evaluated by using the I+R interpretations of the participating laboratories. Resistance to (benzyl) penicillin (2-3%) and erythromycin (9%) were relatively stable over time, resistance to doxycycline/tetracycline increased significantly from 7% in 2008 to 10% in 2011.

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Haemophilus influenzae
Isolates tested (N): 859-3105
Laboratories participating (N): 10-22.
Trends. Data on susceptibility were evaluated by using I+R interpretations of the participating laboratories. Amoxicillin resistance was 15-16% and stable over time (figure 4.14), 4% of all strains were also co-amoxiclav resistant, indicating that resistance was not exclusively caused by production of beta-lactamase. Cefotaxime resistance remained low (0.4% or less), doxycycline resistance level was 2% during the whole study period. Co-trimoxazole resistance increased significantly from 17% in 2008 to 21% in 2011. Ciprofloxacin resistance was rare.

Moraxella catarrhalis
Isolates tested (N): 220-1124
Laboratories participating (N): 7-22.
Trends. Data on susceptibility were evaluated by using the I+R interpretations of the participating laboratories. Amoxicillin/ampicillin resistance increased from 79% to 84% ($p<0.05$) figure 4.14); resistance to co-amoxiclav was observed in 1% of the strains tested. Resistance to cefotaxime was 1-2%. Erythromycin resistance increased from 6% to 11% ($p<0.05$) between 2008 and 2011. Resistance to doxycycline was 3-4%, that to ciprofloxacin 1-3%, whereas around 8% of all strains appeared to be resistant to co-trimoxazole (figure 4.14)

Unselected Hospital Departments - Conclusion (see also tables 4.5 and 4.6)

1. Co-amoxiclav resistance is increasing in *E. coli* (21%) and *P. mirabilis* (12%), and its use for empirical therapy should be reconsidered. Piperacillin-tazobactam is 3-6%, but increasing.
2. Cefuroxime resistance is increasing in *E. coli* and *K. pneumoniae* and its use for empirical therapy should be reconsidered. Cefotaxime- and ceftriaxone resistance is low, but increasing in *E. coli* and *K. pneumoniae.*
3. Aminoglycoside resistance is stable at 4-5% for *Enterobacteriaceae*. Gentamicin resistance is increasing in *P. aeruginosa* (8%); tobramycin resistance was 2%, reflecting its higher intrinsic activity towards *P. aeruginosa*.
4. Trimethoprim- and co-trimoxazole resistance is too high for empirical therapy, except for Acinetobacter sp. infections.
5. Quinolone resistance is increasing in *E. coli* and *P. mirabilis* and a matter of concern.
6. Prevalence of MRSA is low (1.8%), but seems to increase; resistance to other antibiotics among MRSA is significantly higher than among MSSA.
7. Standard antibiotics for respiratory tract infections by pneumococci remain first choice (penicillin/amoxicillin, macrolides, and doxycycline); high resistance to amoxicillin and co-trimoxazole among *H. influenzae* necessitates other treatment schedules.

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SIRIN

Resistance in selected hospital departments was recorded by studying susceptibility patterns in 14 large referral centres participating in the longitudinal SWAB study for Surveillance of Intramural Resistance in the Netherlands (SIRIN). Unique unrelated consecutive isolates isolated from various clinical materials of patients admitted to Intensive Care Units, Urology Services and Pulmonology Services were yearly collected for quantitative susceptibility testing in one central laboratory. MICs were determined by broth micro-dilution assays and breakpoints for resistance according to the recommendations of EUCAST (January 2012) were used. A total of 34.150 strains were collected from 1996-2010.

4.2.4 Intensive Care Units - SIRIN

Escherichia coli

Isolates tested (N): 2844

Trends. Amoxicillin resistance increased from 46% in 1998 to 60% in 2010 (figure 4.15); the bimodal MIC distribution showed a flattening and decrease of the susceptible subpopulation and a growing resistant subpopulation with MIC > 32 mg/l (figure 4.16).

Co-amoxiclav resistance increased from 22% in 1998 to 44% in 2010 (figure 4.15); the unimodal MIC distribution showed a growing number of strains with MIC 16-32 mg/l (figure 4.16).

Piperacillin resistance increased in all Units to 43% in 2010 (figure 4.15). The MIC distribution became clearly bimodal after 2001 with a growing subpopulation of resistant strains (MIC > 16 mg/l). Resistance to piperacillin-tazobactam was 2-5% during the whole study period with a unimodal MIC distribution; 90% of the strains were inhibited by 2 mg/l or less.

Imipenem- and meropenem resistance was occasionally found in 2000 and 2005; MIC_{90} was 0.25 mg/l for imipenem and 0.125 mg/l for meropenem. Around 1-2% of strains had MIC 1-8 mg/l (table 4.7), which categorizes the strains as susceptible, but indicate the emergence of non Wild Type (WT) strains. Strains in the higher MIC range may form a problem in the treatment of infections at the currently recommended dosage schemes.

Cefuroxime resistance levels rose from 9% to 20%

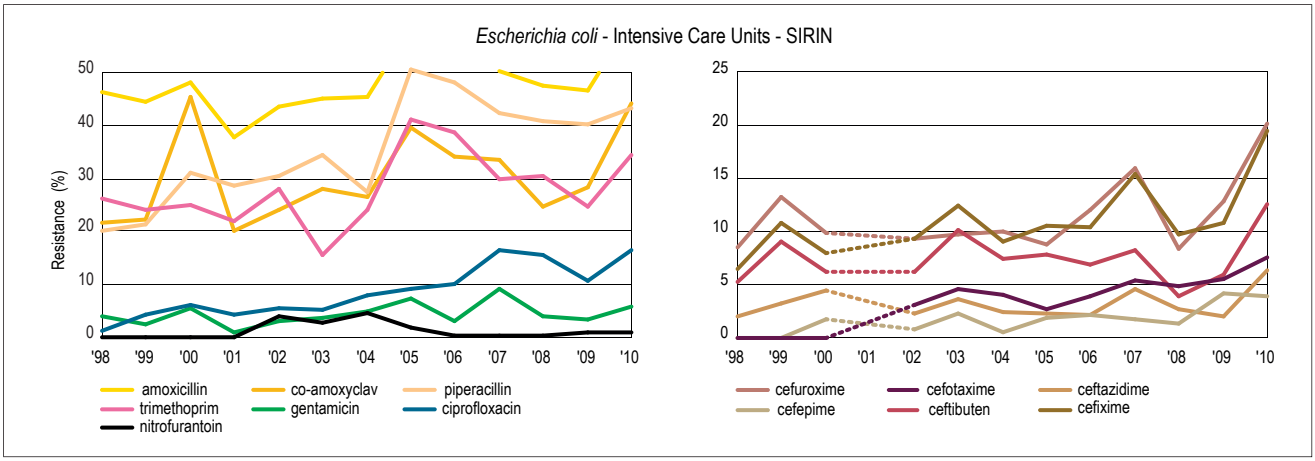

Figure 4.15. Trends in antibiotic resistance among clinical strains of *Escherichia coli* from Intensive Care Units.

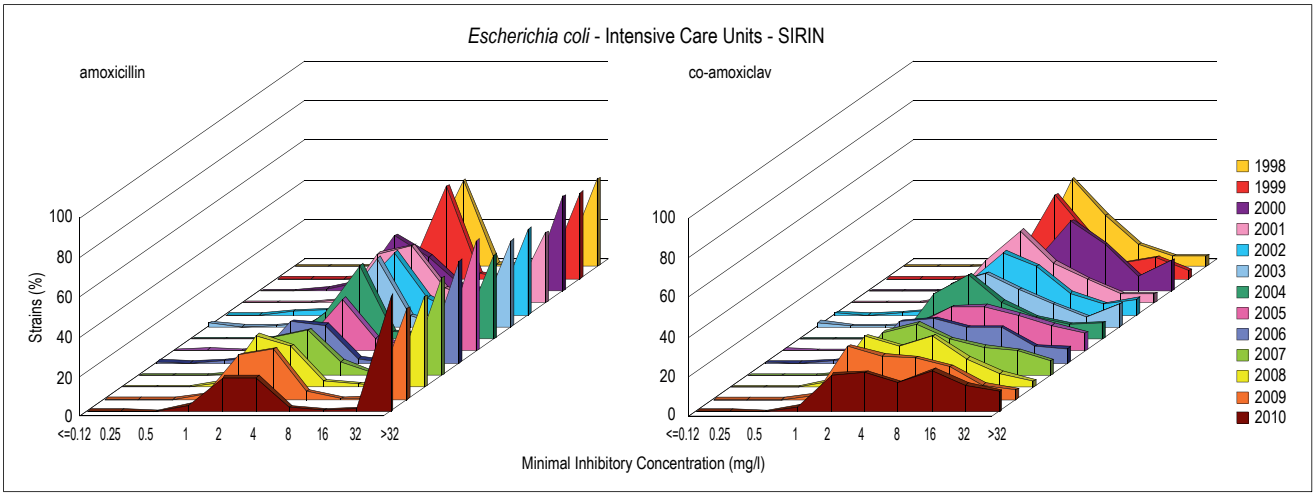

Figure 4.16. MIC distributions of amoxicillin and co-amoxiclav for *Escherichia coli* from Intensive Care Units.

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Table 4.7. MIC distribution (% of strains) of meropenem for *Escherichia coli* from Intensive Care Units.

Minimal Inhibitory Concentration (mg/l)										
Year	<=0.12	0.25	0.5	1	2	4	8	16	32	>32
'98	100.0	-	-	-	-	-	-	-	-	-
'99	100.0	-	-	-	-	-	-	-	-	-
'00	97.3	-	-	-	0.9	0.9	-	0.9	-	-
'01	100.0	-	-	-	-	-	-	-	-	-
'02	99.2	-	-	-	0.8	-	-	-	-	-
'03	99.1	0.5	-	-	-	-	0.5	-	-	-
'04	98.5	0.5	0.5	-	0.5	-	-	-	-	-
'05	98.2	-	-	0.5	0.9	-	-	-	-	0.5
'06	100.0	-	-	-	-	-	-	-	-	-
'07	100.0	-	-	-	-	-	-	-	-	-
'08	100.0	-	-	-	-	-	-	-	-	-
'09	100.0	-	-	-	-	-	-	-	-	-
'10	99.4	-	-	-	-	0.3	-	-	-	0.3

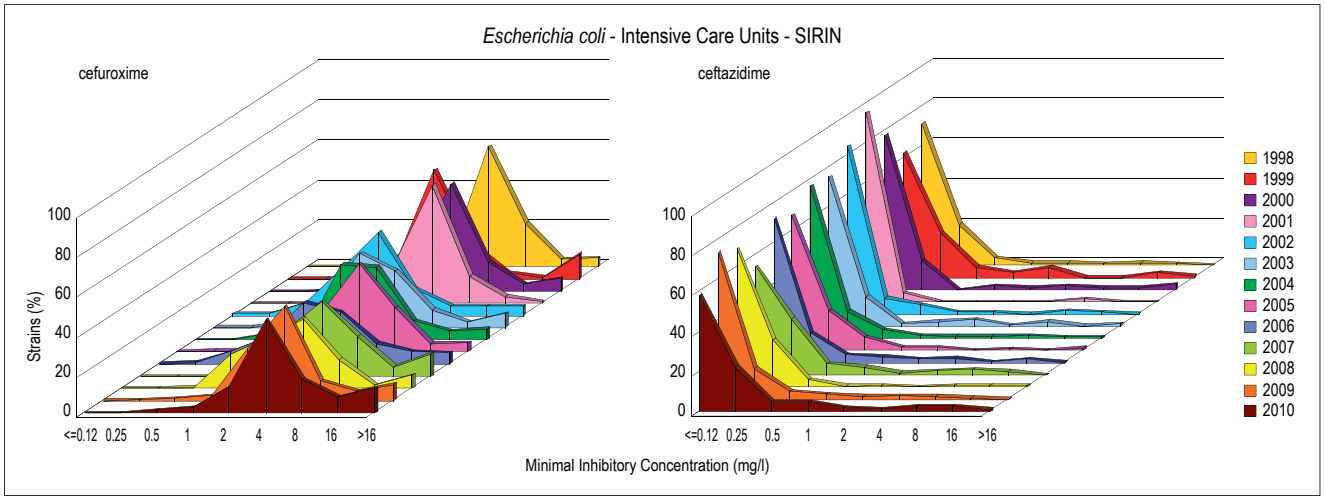

Figure 4.17. MIC distributions of cephalosporins for *Escherichia coli* from Intensive Care Units.

(figure 4.15).The MIC distribution changed to bimodal during the years with lowering of the peak at 4 mg/l and an increase of the resistant subpopulation with an MIC > 16 mg/l (figure 4.17). Cefotaxime- and ceftazidime resistance increased slowly to 7.7% and 6% in 2010, respectively. The MIC distribution of cefotaxime became bimodal, that of ceftazidime remained unimodal over a broad range (figure 4.17). No highly resistant strains were found. Resistance to cefixime increased from 6% in 1998 to 19% in 2010 like cefuroxime but its intrinsic activity was higher with 75% of strains susceptible to 0.1-0.5 mg/l cefixime compared to 1-8 mg/l of cefuroxime. Gentamicin resistance ranged from 1-9% with 6% resistance in 2010 (figure 4.15). The fluctuations were caused by an unusual high resistance level in some centres (up to 15%); resistance was not found in all centres (figure 4.18). Therefore the increasing trend found does not reflect a real national trend and underlines the importance of local surveillance of resistance. The bimodal MIC distribution showed a large susceptible subpopulation with MIC 0.25-1 mg/l and a small subpopulation with MIC > 32 mg/l (figure 4.19). Amikacin resistance remained less than 1% over the years. The MIC distribution was unimodal over a broad range (0.5-8 mg/l). Incidentally strains with MIC > 32 mg/l were found (figure 4.19). Tobramycin resistance was higher (4-9%) with 8% in 2010 (not shown).

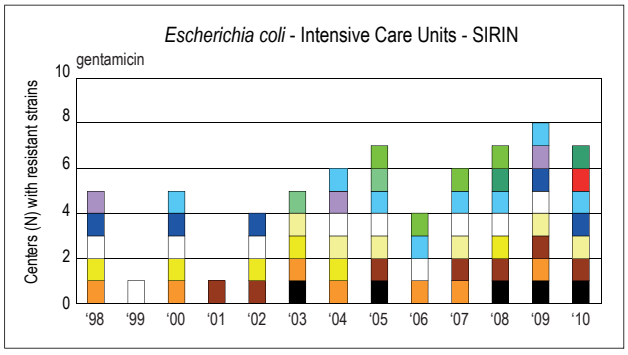

Figure 4.18. Number of centres with gentamicin-resistant *Escherichia coli* on Intensive Care Units. Each color represents one specific centre.

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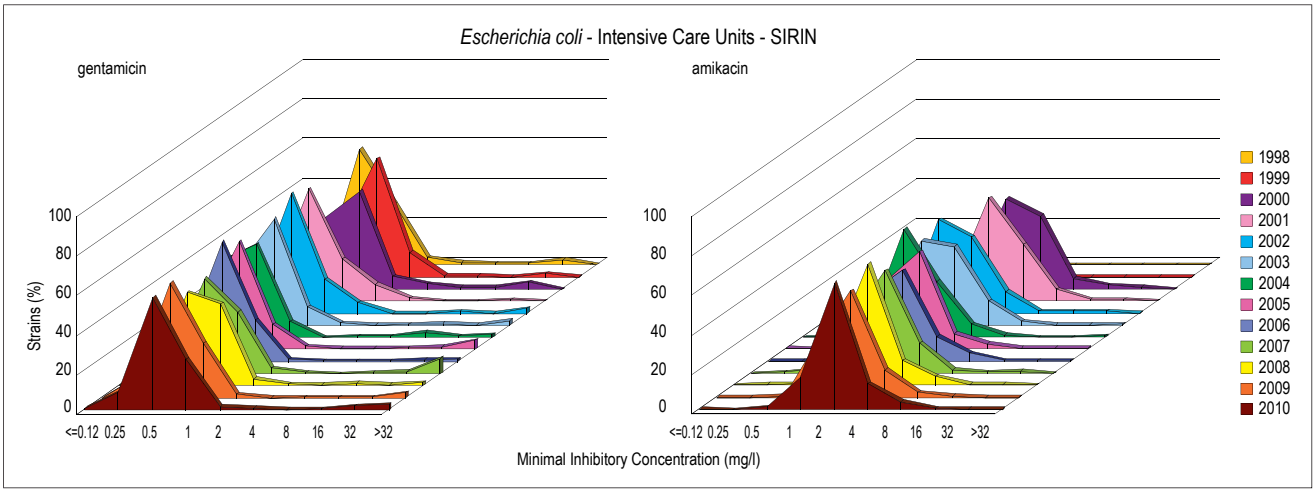

Figure 4.19. MIC distributions of aminoglycosides for *Escherichia coli* from Intensive Care Units..

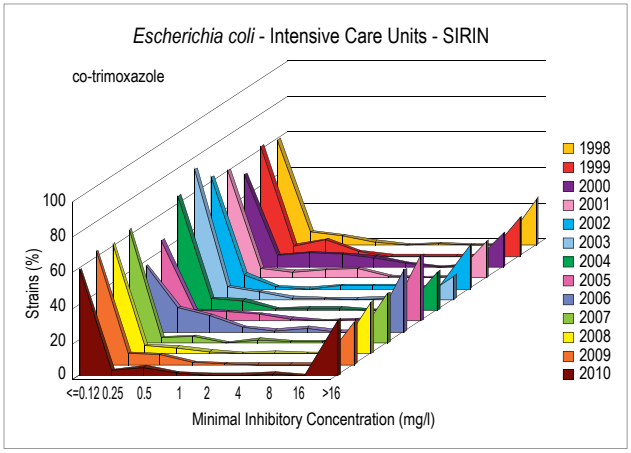

Figure 4.20. MIC distributions of co-trimoxazole for *Escherichia coli* from Intensive Care Units.

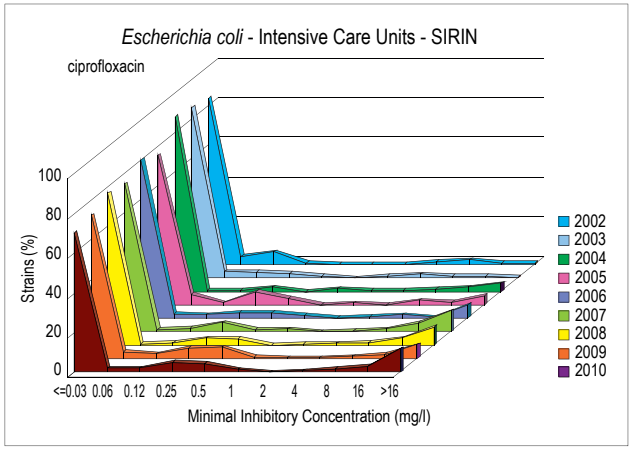

Figure 4.21. MIC distributions of ciprofloxacin for *Escherichia coli* from Intensive Care Units.

The MIC distribution of tobramycin resembled that of amikacin (not shown).
Trimethoprim resistance increased with fluctuations from 26% in 1998 to 34% in 2010 (figure 4.15). Co-trimoxazole resistance followed that trend at a somewhat lower level (32% resistance in 2010). The MIC distributions for co-trimoxazole (figure 4.20) were bimodal with one susceptible (MIC < 0.25 mg/l) and one highly resistant subpopulation (MIC > 16 mg/l). Nitrofurantoin resistance was not found until 2001, increased to 4.5% in 2004 and decreased 1% in 2006 and remained less than 1 mg/l thereafter (figure 4.15). Overall quinolone resistance (ciprofloxacin, norfloxacin, levofloxacin, moxifloxacin) increased from 1% in 1998 to 17% in 2010 (figure 4.15). Quinolone resistance was found in 11 of 13 centres and the level of resistance varied between the centres from 5-53%. This underlines the importance of local surveillance. The MIC distributions were bimodal with a large susceptible subpopulation over a small range (< 0.12 -0.25 mg/l) and a small subpopulation of strains with MIC >8 mg/l (figure 4.21, only ciprofloxacin is shown). The intrinsic activity of ciprofloxacin remained superior: 70% of the isolates were susceptible to <0.03 mg/l ciprofloxacin compared to 46% for levofloxacin, 32% for moxifloxacin and 4% for norfloxacin in 2010.

Multiresistance of *Escherichia coli* in Intensive Care Units

Resistance to three or more classes of antibiotics (multiresistance) was recorded for various combinations at increasing levels from 4% in 1998 to 17.7% in 2010 (figure 4.22). A total of 10.8% was resistant to three classes, 4.2% to four and 2.7% to five classes of antibiotics. Resistance to the combination co-amoxiclav/co-trimoxazole with another drug (cefuroxime / gentamicin / ciprofloxacin) was prevalent (12.6%). Numbers and origin of Intensive Care Units with multiresistant strains varied over time, but increased; 10 of 14 Units had multiresistant strains in 2010 (figure 4.22). So multiresistance was a local problem, but becomes more and more a problem in most Intensive Care Units.

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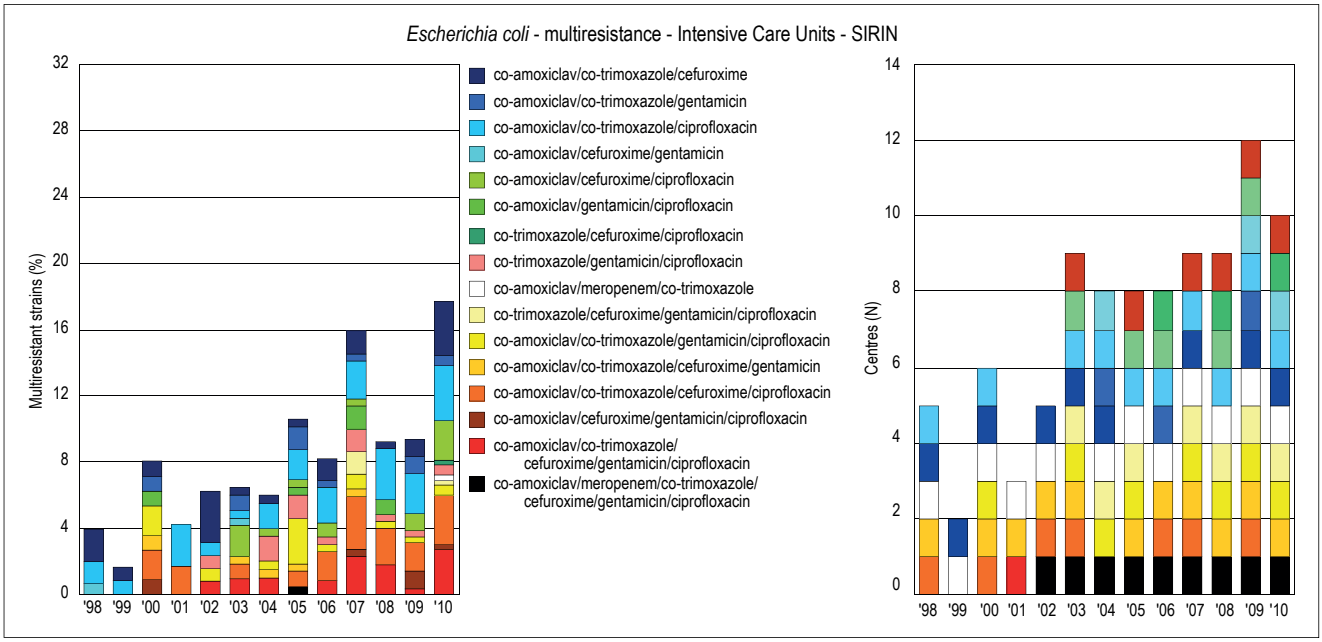

Figure 4.22. Trends in multiresistance among *Escherichia coli* from Intensive Care Units and the number of centres with multiresistance. Each color represents one specific centre.

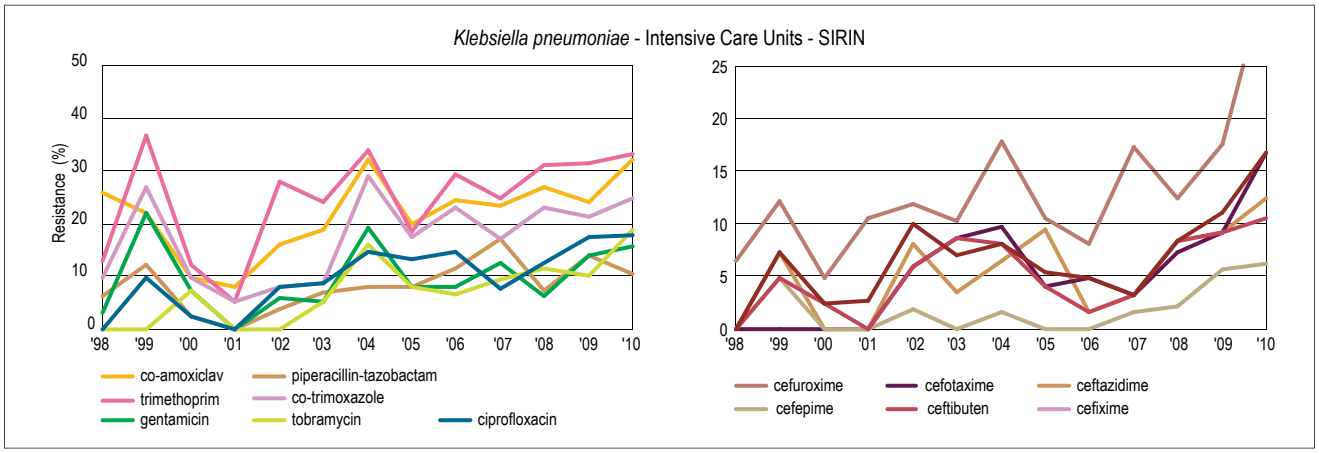

Figure 4.23. Trends in antibiotic resistance among clinical strains of *Klebsiella pneumoniae* from Intensive Care Units.

Klebsiella pneumoniae
Isolates tested (N): 884

Trends. Resistance to all antibiotics tested increased slowly during the last five years of the study period (figure 4.23). Co-amoxiclav and piperacillin-tazobactam resistance reached levels of 32% and 10% respectively in 2010. The MIC distribution of piperacillin-tazobactam was clearly bimodal with one susceptible and one resistant subpopulation, the bimodality for co-amoxiclav was less prominent - the MICs of the strains were distributed over a broad range (figure 4.24).
Carbapenem resistance was rare; MIC_{90} of meropenem was < 0.12 mg/l, but occasionally strains with an MIC 1-4 mg/l were found in 2007 and 2010 in two centres.
Resistance to all cephalosporins tested increased very rapidly from 2008 on, with levels of resistance of 12.5% for ceftazidime, 17% for cefotaxime, 33% for cefuroxime and 6-17% for the fourth generation cephalosporins (figure 4.23). The MIC distribution of cefuroxime (figure 4.25) showed a significant shift to the right in the susceptible subpopulation: the MIC_{50} was 1 mg/l in 2008 compared to 2-4 mg/l in 2010. This "MIC creep" may predict further upcoming resistance in the near future. The MIC distributions of cefotaxime, ceftazidime and cefixime were similar, but here also a change was observed with lowering of the peak at MIC 0.012 mg/l (80% of strains until 2009 and 60% in 2010) and appearance of high resistant strains with MIC > 16 mg/l (figure 4.25).
Trimethoprim resistance increased with fluctuations over the years from 13% in 1998 to 33% in 2010.
The resistance to co-trimoxazole followed this trend

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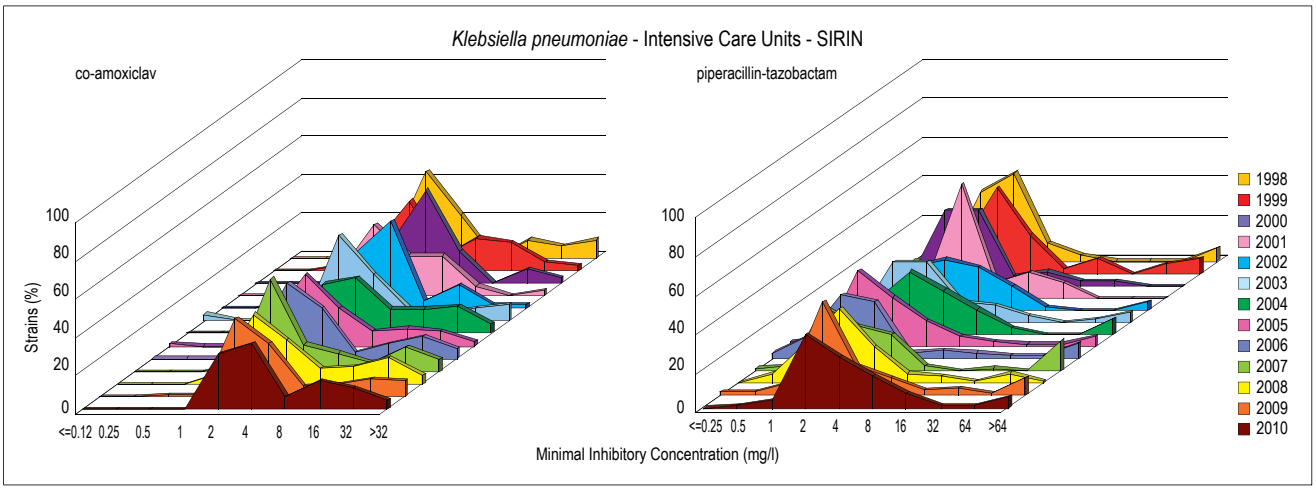

Figure 4.24. MIC distributions of co-amoxiclav and piperacillin-tazobactam for *Klebsiella pneumoniae* from Intensive Care Units.

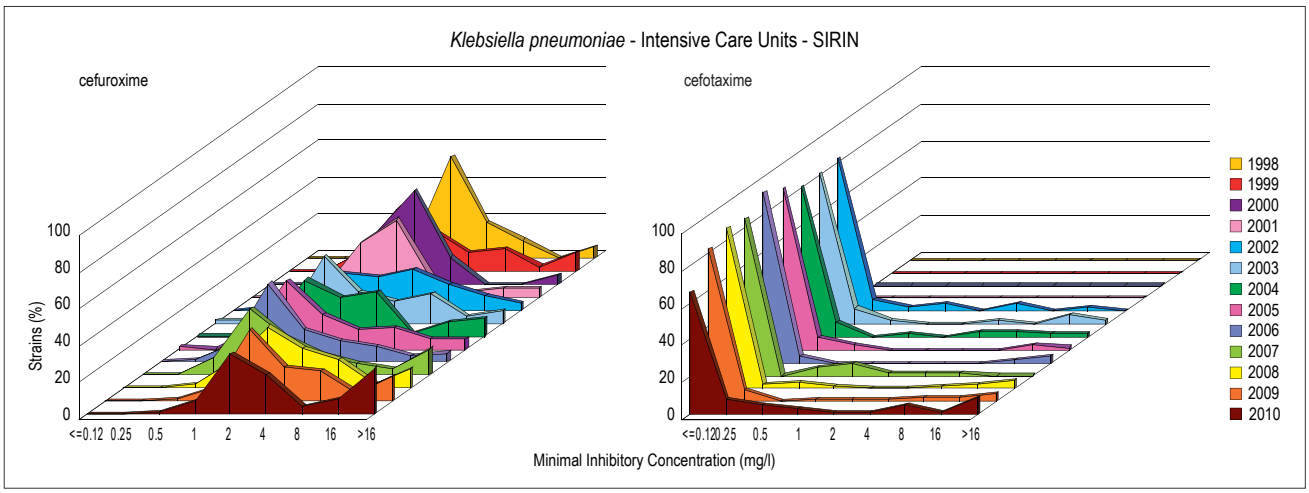

Figure 4.25. MIC distributions of cephalosporins for *Klebsiella pneumoniae* from Intensiv e Care Units.

at a lower level and reached 25% in 2010. The MIC distribution was bimodal (figure 4.26).
Quinolone resistance showed an increasing trend from less than 1% for the four quinolones in 1998 to 22% (norfloxacin), 18% (ciprofloxacin, figure 4.23), 15% for levofloxacin and 16% for moxifloxacin (not shown). The MIC distributions of all quinolones tested showed the same pattern: change from unimodal over broad range with a peak at MIC < 0.03 mg/l to a bimodal shape with one subpopulation of susceptible strains and one with highly resistant strains (MIC > 32 mg/l. The appearance of so many strains in the intermediate area during the past years warned us for this change to bimodality and high resistance (figure 4.27).
Gentamicin-resistant strains were observed continuously in two Intensive Care Units from 1999 onward and intermittent in nine other Units resulting in large fluctuations in gentamicin resistance rates (0-23%) over the years of surveillance. Yet the increase that started in 2009 seems to continue resulting in a mean resistance rate of 14% in 2009 and 15.5% in 2010 (figure 4.23). These figures are not representative for the individual Intensive Care Units and again underline the need for local surveillance. Tobramycin resistance followed the pattern of gentamicin increasing to 19% in 2010. The MIC distributions of gentamicin (figure 4.28) and tobramycin (not shown) showed significant changes compared to the years before: they became clearly bimodal (one subpopulation with MIC 0.12-1 mg/l and one with MIC > 32 mg/l) and showed a shift to the right with lowering of the peak at MIC 0.5 mg/l (80% of strains in 2008 were susceptible to 0.5 mg/l, 67% in 2009 and 53% in 2010). Amikacin resistance was found sporadically in 2003 and 2004, but also here the MIC distributions showed a significant change in 2010 with lowering of the peak at 0.5 mg/l (70% of strains in 2007 vs. 53% in 2010) and broadening of the range in the area 4-16 mg/l (24% of all strains). Such changes are predictable for emerging resistance.

Multiresistance of *Klebsiella pneumoniae* in Intensive

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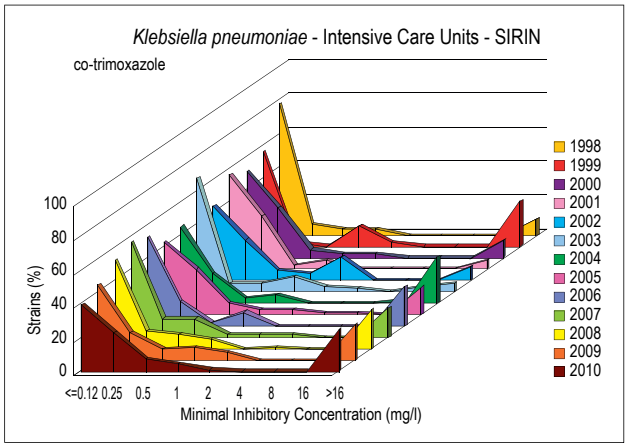

Figure 4.26. MIC distributions of co-trimoxazole for *Klebsiella pneumoniae* from Intensive Care Units.

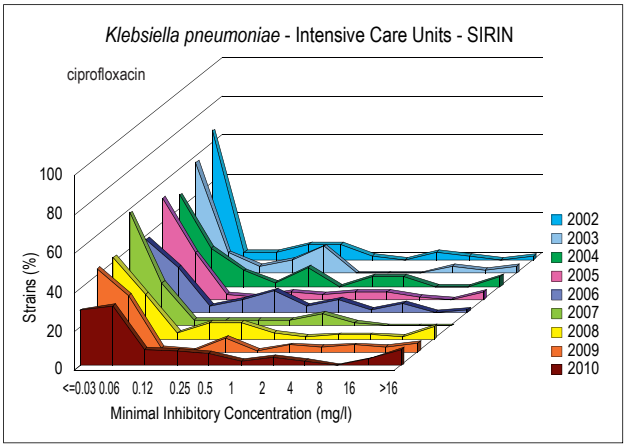

Figure 4.27. MIC distributions of ciprofloxacin for *Klebsiella pneumoniae* from Intensive Care Units.

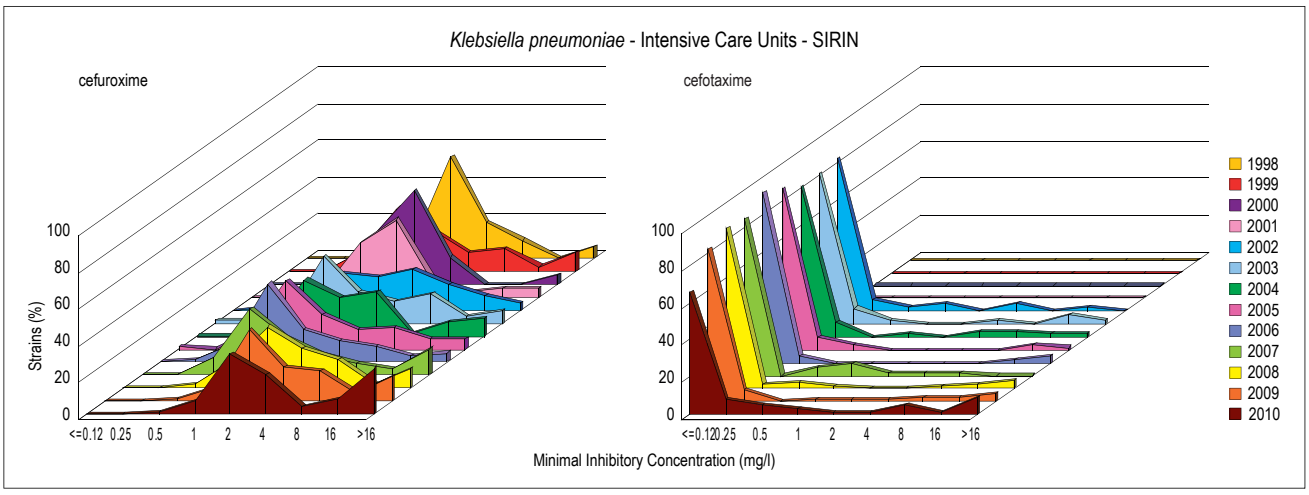

Figure 4.28. MIC distributions of aminoglycosides for *Klebsiella pneumoniae* from Intensive Care Units.

Care Units

Multiresistance (resistance to three or more classes of antibiotics) was recorded yearly except in 2001 at varying percentages; 22% of all strains were multiresistant in 2010 (figure 4.29). Multiresistance was not common in all centres and not found every year in a given centre (figure 4.29); it was often related to an outbreak or circulation of resistant clones in a centre and disappeared after appropriate measures were taken. Therefore these figures are not representative for the country as a whole, but the overall increasing trend is a matter of concern. Resistance to four classes of antibiotics was common (17.8%) among which the combination with gentamicin was prevalent (15.7%).

Enterobacter cloacae

Isolates tested (N): 722

Trends. The resistance trends for *E. cloacae* strains were evaluated from 2003 onward; resistance to piperacillin-tazobactam fluctuated around 15% (figure 4.30). Ceftazidime resistance fluctuated around 25% without a significant increase, that of cefotaxime increased to 33% in 2010. Resistance to cefixime and ceftibuten were also increasing to high levels (50-70%, not shown); the resistance level of cefepime fluctuated between 0% and 13%. The fluctuations were partly related to existence and circulation of resistant clones in some Intensive Care Units. Therefore the overall resistance percentage does not reflect the general situation in Intensive Care Units and does not indicate a trend. Because of the high resistance rates and the presence of an inducible chromosomal beta-lactamase in every *Enterobacter* spp, cephalosporins and penicillins are not recommended as empiric therapy in Intensive Cares with circulating *E. cloacae* strains. Imipenem- and meropenem resistance was rare (3% in 2003, 0% thereafter). The intrinsic activity of meropenem (MIC_{90} < 0.06 mg/l) was significantly higher than that of imipenem (MIC_{90}= 0.25 mg/l) with less strains in the area with MIC 1-8 mg/l.

Co-trimoxazole resistance increased with annual

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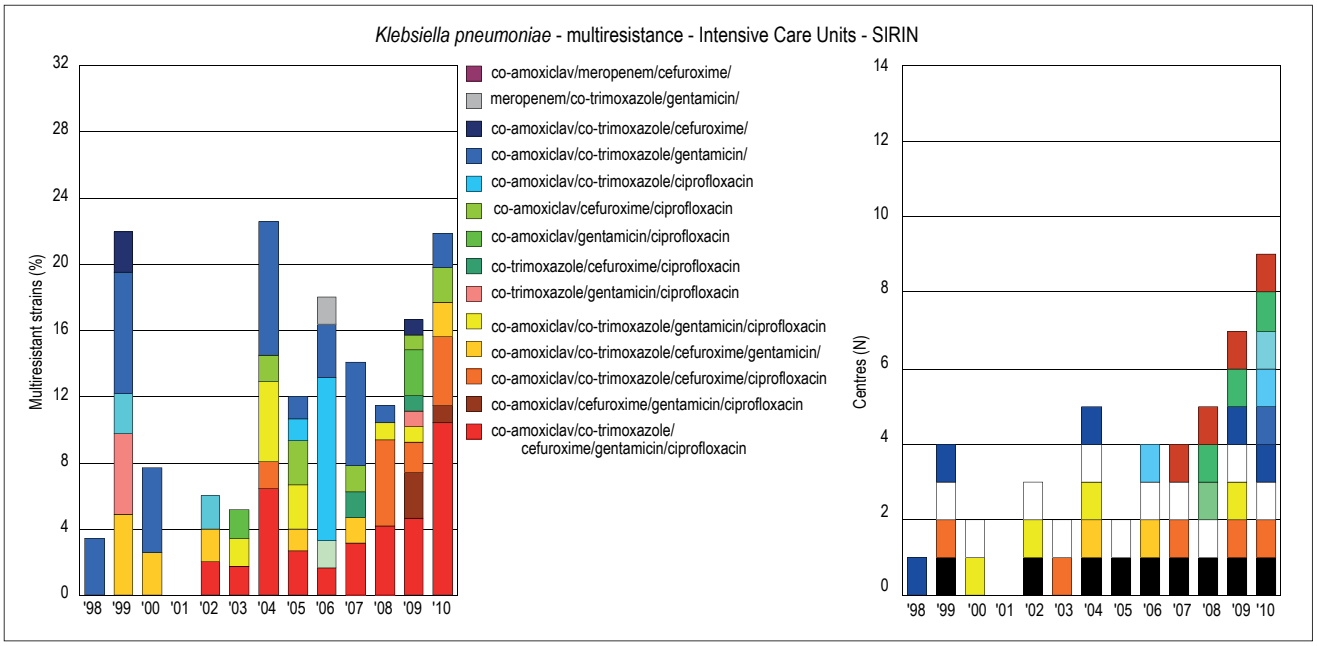

Figure 4.29. Trends in multiresistance among *Klebsiella pneumoniae* and number of centres with multiresistant *Klebsiella pneumoniae* from Intensive Care Units.

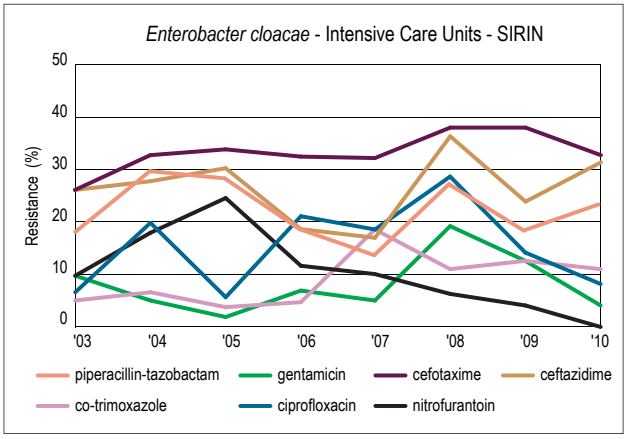

Figure 4.30. Trends in antibiotic resistance (2003-2010) among clinical strains of *Enterobacter cloacae* from Intensive Care Units.

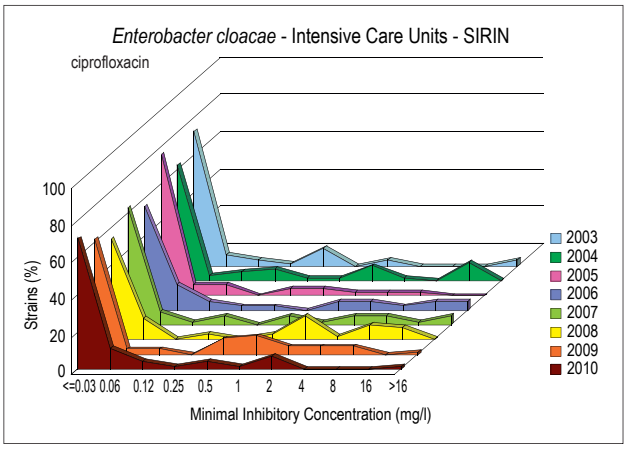

Figure 4.31. MIC distributions of ciprofloxacin for *Enterobacter cloacae* from Intensive Care Units.

fluctuations from 5% in 2003 to 11% in 2010. Ciprofloxacin resistance increased from 7% in 2003 to 28% in 2008 with a sharp decrease to 14% in 2009 and 8% in 2010. These fluctuations were due to the existence of strains around the breakpoint of 1 mg/l (figure 4.31). The MIC distributions of the quinolones showed a consistent unimodal shape over a broad range with a large subpopulation with MIC < 0.12 mg/l (70-80%) and small subpopulations with MIC 1-16 mg/l. Co-resistance with gentamicin and tobramycin occurred in 50% or more of ciprofloxacin-resistant strains.
Gentamicin resistance was recorded in 10 of 14 centres during the study period, but only in 1-5 centres each year (figure 4.32); the overall resistance was 5-10% with two peaks in 2008 (19%) and 2009 (13%) respectively. There

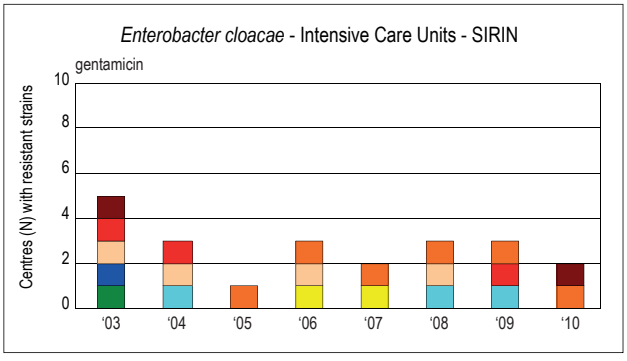

Figure 4.32. Number of centres with gentamicin-resistant *Enterobacter cloacae* on Intensive Care Units. Each color represents one specific centre.

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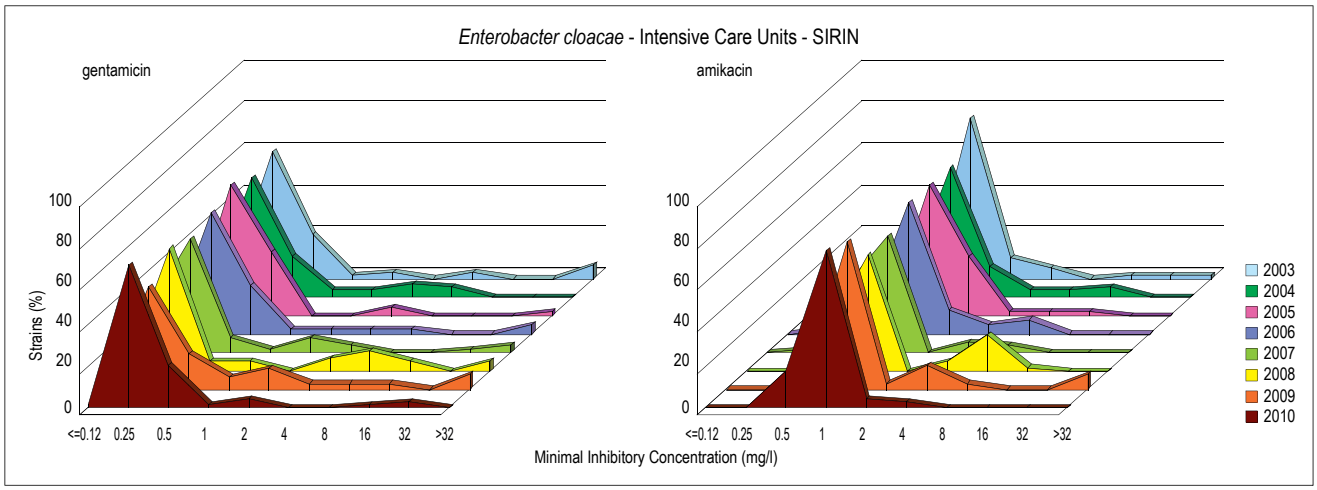

Figure 4.33. MIC distributions of aminoglycosides for *Enterobacter cloacae* from Intensive Care Units.

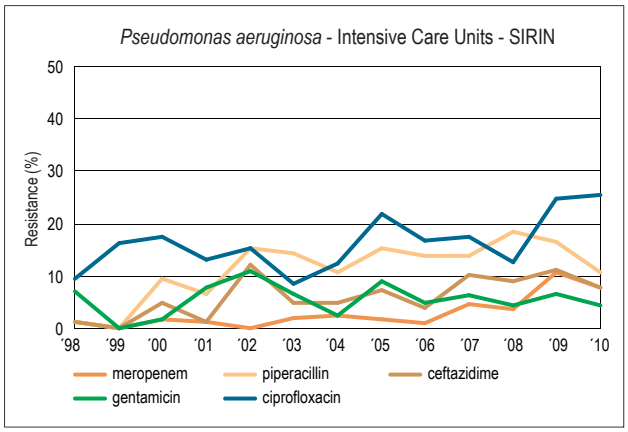

Figure 4.34. Trends in antibiotic resistance among clinical strains of *Pseudomonas aeruginosa* from Intensive Care Units.

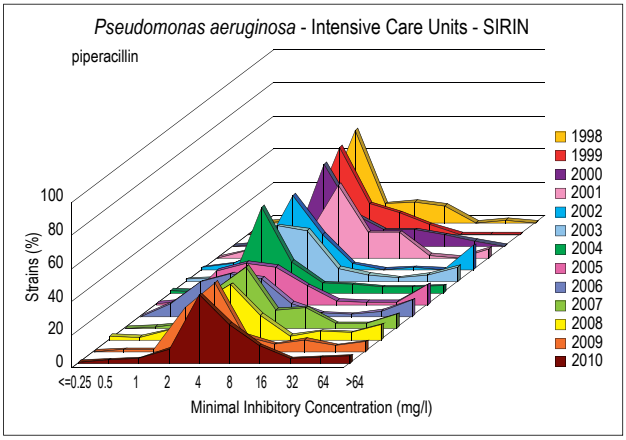

Figure 4.35. MIC distributions of piperacillin for *Pseudomonas aeruginosa* from Intensive Care Units.

was complete cross resistance with tobramycin, but not with amikacin (21% of strains). The MIC distribution for gentamicin was bimodal with a susceptible subpopulation (MIC < 2 mg/l) and a small resistant one (MIC > 16mg/l) (figure 4.33). Small subpopulations with MIC 4- 8 mg/l appeared from 2004 on; the appearance of less susceptible strains is a warning signal of emerging resistance and underlines the importance of the longitudinal evaluation of the MIC distributions. Overall amikacin resistance was sporadic.

Proteus mirabilis
Isolates tested (N): 549
Trends. The resistance trends for *P. mirabilis* strains were evaluated from 2003 onward. Amoxicillin resistance was stable at 27%; co-amoxiclav resistance was low. No resistance to imipenem, meropenem was observed; resistance to cefuroxime (3-7%), cefotaxime (2%) or ceftazidime (2%) were rather stable over the years with some exceptions in some Intensive Care Units. Gentamicin and tobramycin resistances fluctuated (0-9%) and were overall 4% in 2010, amikacin resistance was lower (0-2%). The fluctuations were related to certain Intensive Care Units and do not reflect a general trend. Co-trimoxazole resistance was 26% in 2010 and quinolone resistance was 4-5% until 2009, but increased to 11% in 2010.

Pseudomonas aeruginosa
Isolates tested (N): 1579
Trends. Piperacillin resistance among *P. aeruginosa* was not found until 2000, it increased to 15% in 2002 and stabilized around 10-15% during the following years (figure 4.34). The MIC distributions of piperacillin (and piperacillin-tazobactam) became bimodal after 2000 with emergence of a small resistant subpopulation (MIC > 64 mg/l) (figure 4.35).
Meropenem resistance was less than 2% until 2006, increased then to 8% in 2010 (figure 4.34). Resistant strains were found in 10 centres throughout the study years, but never more than in four centres yearly. Meropenem resistance appeared to be a local temporary

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Table 4.8. MIC distribution (% of strains) of meropenem for *Pseudomonas aeruginosa* from Intensive Care Units

Minimal Inhibitory Concentration (mg/l)										
Year	<=0.12	0.25	0.5	1	2	4	8	16	32	>32
'98	10.6	22.4	34.1	18.8	5.9	3.5	3.5	1.2	-	-
'99	6.8	37.8	27.0	16.2	6.8	4.1	1.4	-	-	-
'00	25.4	27.0	14.3	15.9	7.9	6.3	1.6	1.6	-	-
'01	27.3	40.3	15.6	6.5	5.2	2.6	1.3	1.3	-	-
'02	35.2	23.1	26.4	5.5	5.5	2.2	2.2	-	-	-
'03	47.6	23.8	10.5	7.6	6.7	1.0	1.0	-	-	1.9
'04	59.0	15.6	17.2	0.8	1.6	2.5	0.8	0.8	-	1.6
'05	50.8	18.5	8.1	5.6	11.3	1.6	2.4	1.6	-	-
'06	58.8	14.7	8.8	4.9	6.9	2.0	2.9	-	1.0	-
'07	38.5	23.9	17.4	5.5	4.6	2.8	2.8	3.7	0.9	-
'08	43.0	23.7	14.1	5.2	5.9	2.2	2.2	1.5	-	2.2
'09	33.7	16.0	15.4	9.5	8.9	1.8	4.1	0.6	4.1	5.9
'10	40.4	21.3	14.9	5.0	1.4	7.1	2.1	7.1	0.7	-

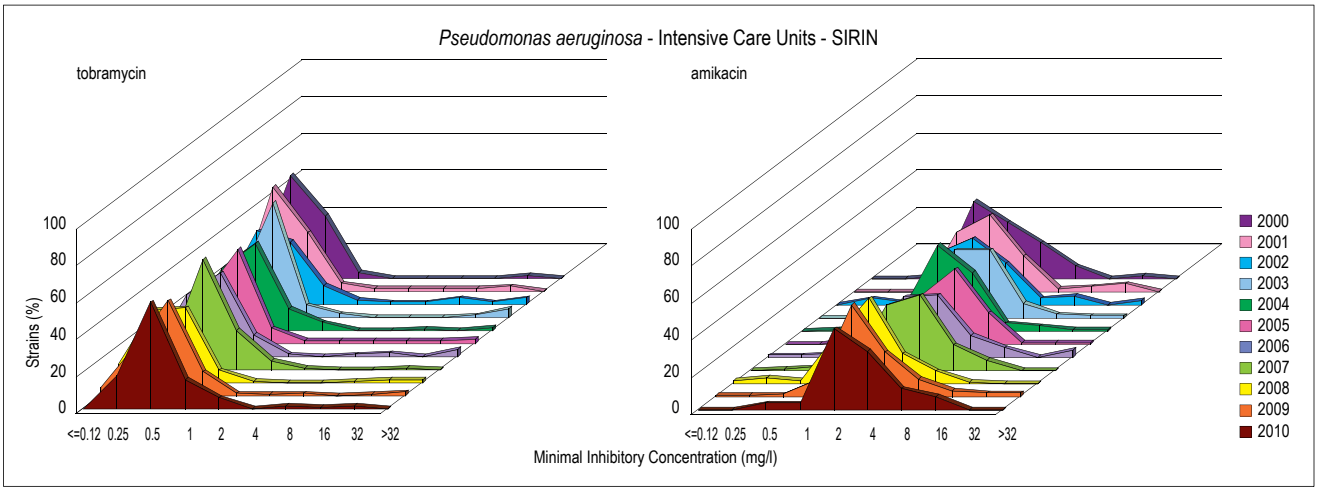

Figure 4.36. MIC distributions of aminoglycosides for *Pseudomonas aeruginosa* from Intensive Care Units.

problem for most centres and is not representative for The Netherlands as a whole. Concern is the increase of strains with MIC 1-8 mg/l (table 4.8), which are categorized susceptible, but may require higher dosing of meropenem. Ceftazidime resistance fluctuated but the trend was increasing from 1% in 1998 to 11 % in 2010. Like for meropenem, resistance was recorded in a part of the centres (N=5-6), so the current resistance levels reflect local problems with highly resistant populations rather than a general problem. Gentamicin resistance was recorded in 13 centres during the whole study period, but never more than in six centres per year; this may explain the yearly variations in the total resistance level of 4-10% (figure 4.34). The same was found for tobramycin; amikacin resistance varied from 0-4%. A total 63% of gentamicin-resistant strains were tobramycin-resistant and 27% were amikacin-resistant; 84% of tobramycin-resistant strains were gentamicin-resistant and 31% were amikacin-resistant. The MIC distributions of gentamicin (not shown) and tobramycin were bimodal with one subpopulation with MICs over a broad range (0.12-4 mg/l and a very small resistant subpopulation (MIC > 16 mg/l, figure 4.36). The MIC distribution of amikacin was unimodal over a broad range from 0.5-> 16 mg/l.

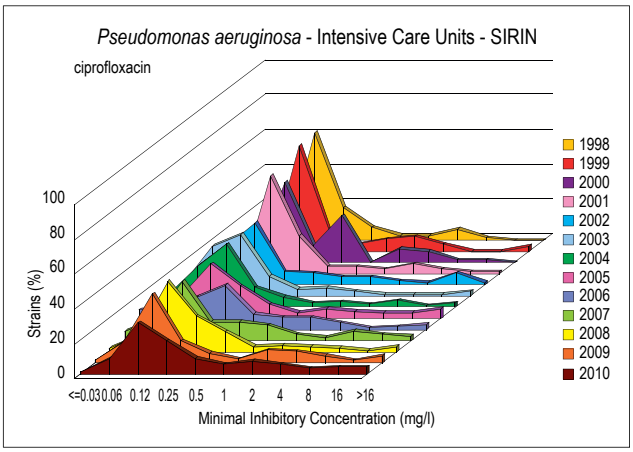

Figure 4.37. MIC distributions of ciprofloxacin for *Pseudomonas aeruginosa* from Intensive Care Units.

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Table 4.9. Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa in Intensive Care Units in 2010.

Antibiotic	*E. coli*	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
amoxicillin	60			27	
co-amoxiclav	44	32	100	2	
piperacillin	43	39	30	6	11
piperacillin-tazobactam	3	10	14	0	12
carbapenem	0	0	0	0	8
cefuroxime	20	33		2	
cefotaxime/ceftriaxone	8	17	33	2	
ceftazidime	6	13	32	0	11
ceftibuten	13	10	38	2	
cefixime	20	17	58	2	
cefepime	4	6	2	0	
gentamicin	6	16	4	4	4
tobramycin	8	19	4	4	4
amikacin	1	0	0	2	0
trimethoprim	34	33	12	39	
co-trimoxazole	32	25	11	26	
norfloxacin	19	24	11	15	
ciprofloxacin	17	18	8	11	25
levofloxacin	16	15	4	2	32
moxifloxacin	17	16	10	15	
nitrofurantoin	1	19	0	61	

increasing since 2005
decreasing since 2005
stable since 2005

Tobramycin had the highest intrinsic activity with MICs two-fold lower than those of gentamicin and four-fold lower than those of amikacin.
Quinolone resistance fluctuated strongly (figure 4.34) during the study period and stabilized in 2010 at 25% for ciprofloxacin (figure 4.34) and 32% for levofloxacin (not shown). The MIC distributions (figure 4.37) remained unimodal over a broad range of MICs, but the whole population moved slowly to higher MICs during the study period: 90% of strains were susceptible to 0.5 mg/l ciprofloxacin or less in 1998 versus 70% in 2010. These figures were 70% and 45%, respectively for levofloxacin.

Enterococcus faecalis
Isolates tested (N): 1091
Trends. A total of 19 isolates of *E. faecalis* appeared amoxicillin resistant (2%); these strains were found occasionally in six centres during 1-4 years; 18 were also resistant to imipenem. two were vancomycin resistant of which one was also teicoplanin resistant. The latter strain was co-resistant to linezolid, quinupristin/dalfopristin and chloramphenicol.
Imipenem resistance fluctuated between 1% and 14% without a real trend; such strains were incidentally found in 11 centres over the years. It is therefore a local problem rather than a national phenomenon.

Vancomycin resistance was found in one centre in 2003 and in another in 2007, teicoplanin resistance was not found.

Staphylococcus aureus
Isolates tested (N): 1466
Trends. MRSA strains were occasionally isolated (1.2%). Eight of 17 MRSA strains were co-resistant to ciprofloxacin, six also to clarithromycin, and one also to gentamicin.

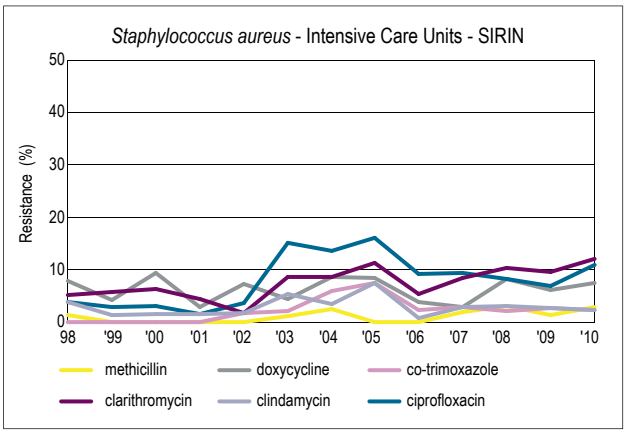

Figure 4.38. Trends in antibiotic resistance among clinical strains of *Staphylococcus aureus* from Intensive Care Units.

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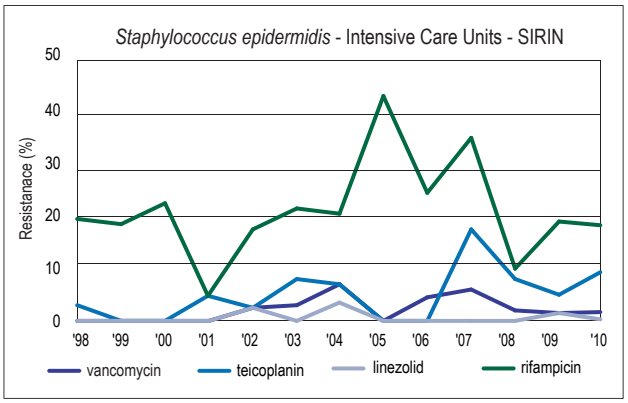

Figure 4.39. Trends in antibiotic resistance among clinical strains of *Staphylococcus epidermidis* from Intensive Care Units.

Clarithromycin resistance increased from 5% in 1998 to 12% in 2010 (figure 4.38). Clindamycin resistance fluctuated around 2-4% over the years without a shift or clear trend, indicating that MLSb resistance is stable, and the increase of macrolide resistance is due to efflux or other mechanisms. Doxycycline resistance remained 6-8% during the last years, co-trimoxazole resistance was low (2-3%).
Gentamicin resistance remained less than 1% (not shown). Ciprofloxacin resistance increased from 4% in 1998 to 16% in 2005, decreased to 9% in 2006 and remained at that level since (figure 4.38). Moxifloxacin resistance followed this trend at a lower level (8% in 2010). MIC_{90} was 8 mg/l for ciprofloxacin and 0.5 mg/l for moxifloxacin in 2010.
Resistance rates to rifampicin, linezolid and quinupristin/dalfopristin were less than 1% (not shown). Vancomycin resistance was once recorded in 2006 and 2010, teicoplanin resistance once in 2003.

Staphylococcus epidermidis
Isolates tested (N): 694
Trends. About 80% of all strains of *S. epidermidis* were methicillin-resistant; 75% of methicillin-resistant strains were co-resistant to erythromycin, clarithromycin, gentamicin and ciprofloxacin. Mean resistance levels for clarithromycin were 77%, clindamycin 60%, gentamicin 74%, ciprofloxacin 74% and for moxifloxacin 52%. Rifampicin resistance fluctuated around 20% (figure 4.39). Vancomycin-resistance was occasionally found in 1-2 centres per year since 2002. Three vancomycin-resistant strains were also teicoplanin-resistant (MIC 8, 64, 256 mg/l, respectively). Teicoplanin resistance was observed intermittently in nine centres with 10% overall resistance in 2010. Linezolid resistance was sporadic. High resistance levels among *S. epidermidis* from Intensive Care Units are common and reflect the high selective pressure in these wards. They belong to specific populations circulating in Intensive Care Units and their resistance levels may differ from Unit to Unit. Such populations may serve as a reservoir for multiresistance with the risk of exchange of resistance factors to other micro-organisms in the commensal flora of patients and health care workers.

Intensive Care Units - Conclusion
(see also table 4.9)

1. Increasing and/or high resistance to co-amoxiclav, piperacillin and cephalosporins among *Enterobacteriaceae* except in *P. mirabilis*.
2. Carbapenem resistance was rare among *Enterobacteriaceae*, but occasionally among *P. aeruginosa* in some centres. Concern for strains with MIC 1-8 mg/l, reflecting creeping MIC values which often remain unnoticed in routine susceptibility testing.
3. Aminoglycoside resistance not in all centres; if found: low and stable in *E. coli*, increasing in *K. pneumoniae*, decreasing in *E. cloacae*.
4. Co-trimoxazole- and quinolone resistance was high, increasing in *K. pneumoniae* and *P. mirabilis.*
5. Increasing multiresistance among *E. coli* and *K. pneumoniae.*
6. Empiric therapy for suspected Gram-negative infections in Intensive Care Units requires wide-spectrum antibiotics in most centres such as combination therapy or carbapenems.
7. Amoxicillin resistance among *E. faecalis* is low (2%) and centre-related; vancomycin- en teicoplanin resistance not found in 2010.
8. Resistance among *S. aureus* low for all antibiotics tested, MRSA 1.2%.
9. Resistance among *S. epidermidis* high for most antibiotics.
10. The MIC distributions over time identified significant "MIC creeps" for cephalosporins *(K. pneumoniae)*, meropenem *(P. aeruginosa)*, quinolones *(K. pneumoniae, P.aeruginosa)* and aminoglycosides *(K. pneumoniae, E. cloacae)* which predict upcoming resistance in the following years.
11. High resistance levels to aminoglycosides, ceftazidime, meropenem and quinolones among *Enterobacteriaceae*, *P. aeruginosa* and multiresistance are centre-related. This underlines the need of both local and national surveillance.

4.2.5 Urology Services - SIRIN

Escherichia coli
Isolates tested (N): 8149
Trends. Amoxicillin resistance increased to 47% in 2010 (figure 4.40); co-amoxiclav resistance was steadily increasing from 19% in 1998 to 30% in 2009 en 2010. Piperacillin- (40%) and piperacillin-tazobactam (2%)

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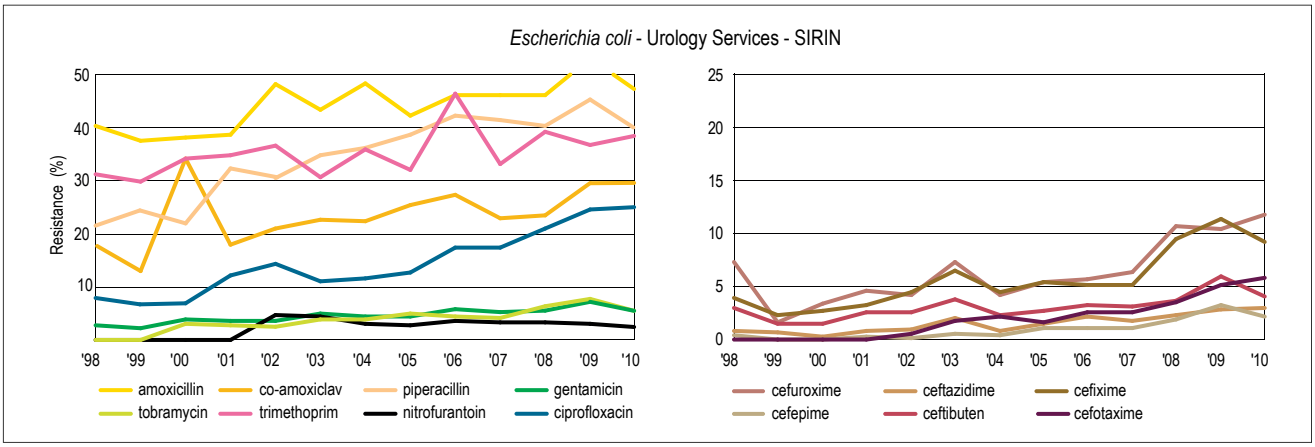

Figure 4.40. Trends in antibiotic resistance among clinical strains of *Escherichia coli* from Urology Services.

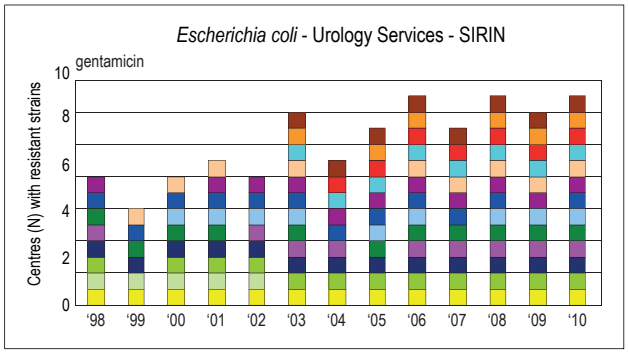

Figure 4.41. Number of centres with gentamicin-resistant *Escherichia coli* on Urology Services. Each color represents one specific centre.

resistances were similar to those found in Intensive Care Units. Carbapenem resistance was rare.
Cephalosporin resistance levels were consistently lower than those recorded for Intensive Care Units, but slowly increasing with resistance to cefuroxime 12%, cefotaxime 6%, ceftazidime 3%, cefixime 9% and ceftibuten 4%.
Gentamicin- and tobramycin resistance was found in all centres since 2003 at low levels (figure 4.40 and 4.41).
Amikacin resistance remained less than 1%.
Trimethoprim resistance increased from 31% to 38% in 2002 and remained around that level, co-trimoxazole resistance showed the same trend at a lower level (36% in 2010), both levels being consistently higher compared to those in Intensive Care Units.
Ciprofloxacin resistance level increased to 25% in 2010. The same levels were found for norfloxacin, levofloxacin

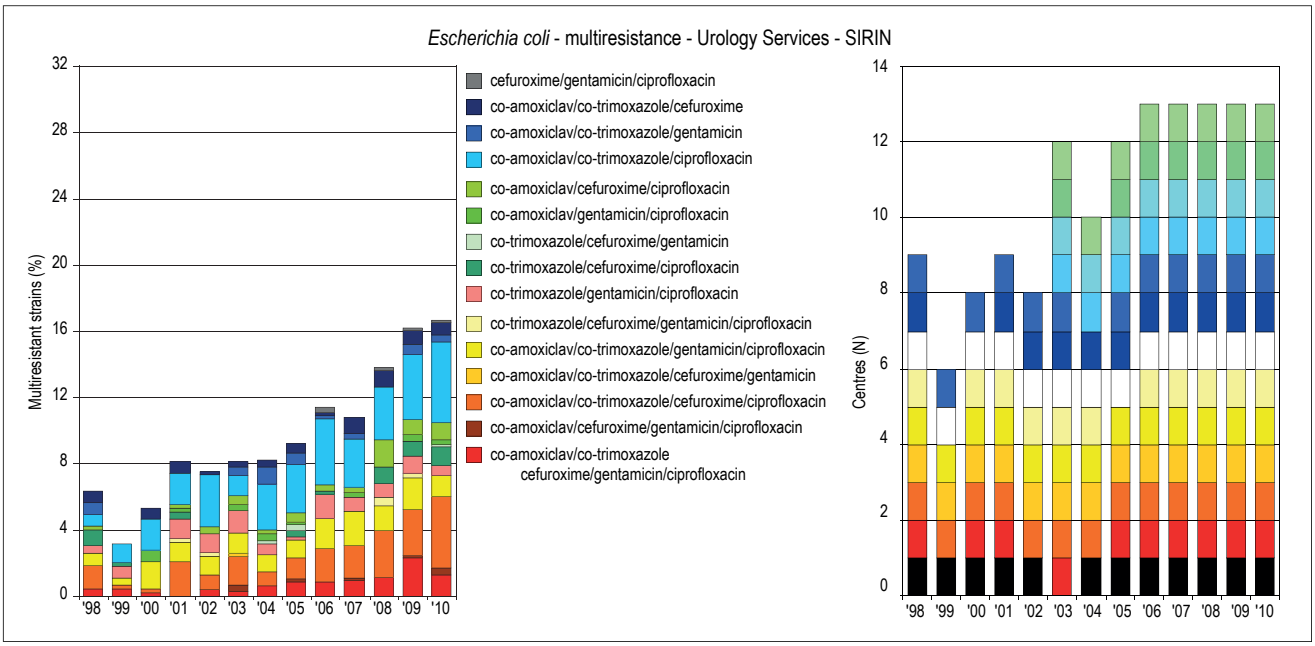

Figure 4.42. Trends in multiresistance among *Escherichia coli* from Urology Services and the number of centres. Each color represents one specific centre.

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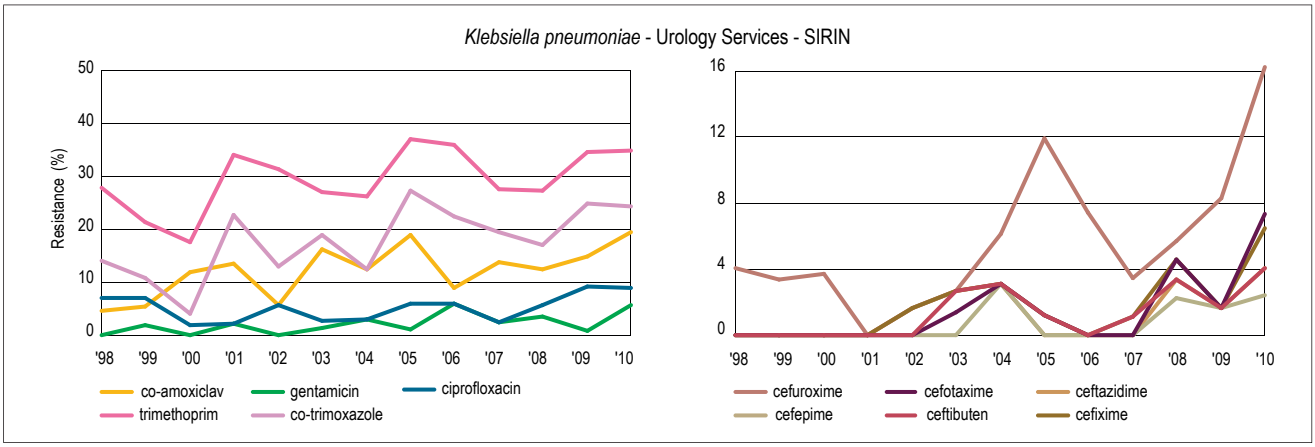

Figure 4.43. Trends in antibiotic resistance among clinical strains of *Klebsiella pneumoniae* from Urology Services.

and moxifloxacin. The MICs showed the same distributions as found in strains from Intensive Care Units. Nitrofurantoin resistance increased from 0% in 1998 to 5% in 2002 and stabilized at 3% since 2003 (figure 4.40).

Multiresistance of *Escherichia coli* in Urology Services
Surprisingly a higher rate of multiresistance was found in Urology Services compared to Intensive Care Units (figure 4.42), increasing from 6% of all strains in 1998 to 16.7% in 2010. A total of 696 multiresistant strains were isolated during the whole study period (9.8% of all). Resistance to three classes increased from 2.9% in 1998 to 9.4% in 2010, to four classes from 1.9% to 6% and to five classes from 0.4% to 1.3%. The combination co-amoxiclav/co-trimoxazole/ ciprofloxacin was most prominent. Multiresistance was found in almost all centres since 2006 (figure 4.42); reflecting a national problem.

Klebsiella pneumoniae
Isolates tested (N): 1082
Trends. Co-amoxiclav resistance increased from 4% in 1998 to 20% in 2010 (figure 4.43).

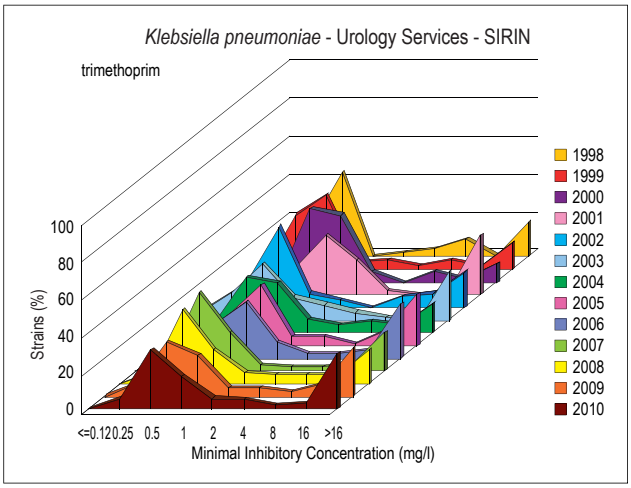

Figure 4.44. MIC distributions of trimethoprim for *Klebsiella pneumoniae* from Urology Services.

Piperacillin-tazobactam resistance fluctuated at a low level (0-6%) and was only found in a few centres. So the piperacillin-tazobactam resistance found did not reflect the resistance level for Urology Services as a whole. Carbapenem resistance was not found.

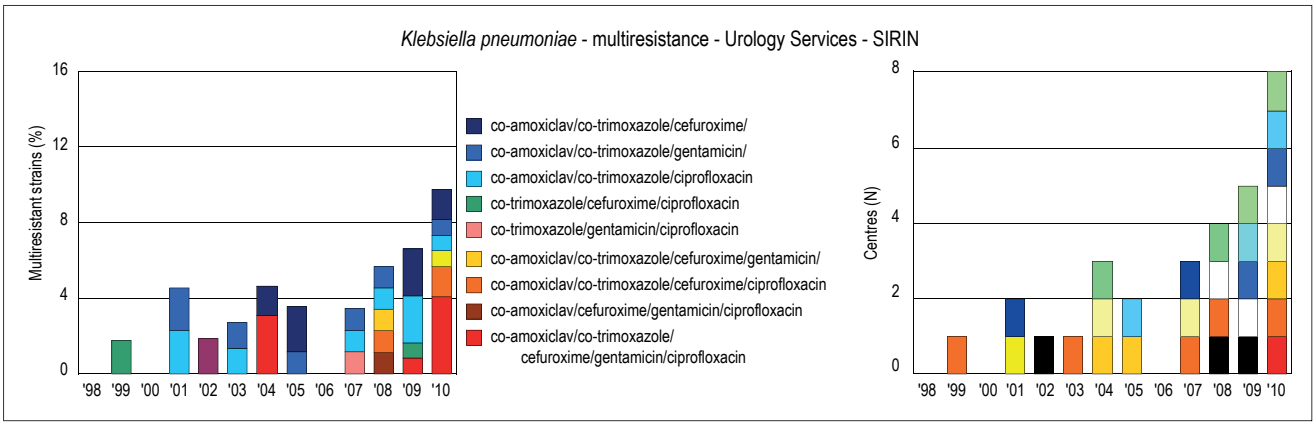

Figure 4.45. Trends in multiresistance among *Klebsiella pneumoniae* and number of centres with multiresistant Klebsiella from Urology Services.

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Resistance to cefuroxime increased since 2007 (3.5%) to 16% in 2010 (figure 4.43). Ceftazidime and cefotaxime resistance were slightly increasing to 4% and 7% respectively.
Trimethoprim resistance increased slowly from 29% in 1998 to 34% in 2001 and remained stable with some fluctuations, which might be explained by the existence of strains with MICs 4-8 mg/l, which is around the breakpoint for resistance (figure 4.44). The resistance to co-trimoxazole followed the trend of trimethoprim with a total increase from 12% in 1998 to 24% in 2010.
Gentamicin and tobramycin resistance was less than 6% in 2010 and not common in all Urology Services. Amikacin resistance was not found.
Nitrofurantoin was stable at 23-27% throughout the years. Quinolone resistance increased slowly to 21% for norfloxacin and 9% for ciprofloxacin. The MIC distributions of all quinolones were comparable to those found for strains from Intensive Care Units (figure 4.27)

Multiresistance of *Klebsiella pneumoniae* in Urology Services
Multiresistance in Urology Services increased to 9.8% in 2010, which is much less frequent than in Intensive Care Units. The rate of multiresistance found in *Klebsiella* in Urology Services was lower than the rate found in *E. coli* strains (figure 4.45).

Proteus mirabilis
Isolates tested (N): 1.104
Trends. Amoxicillin resistance increased from 18% in 1998 to 37% in 2004 and decreased subsequently to 21% in 2010 (figure 4.46). The distribution of MICs was bimodal with a susceptible subpopulation over a small range in most years (MIC 0.5-1.0 mg/l) and a resistant one with MICs >8 mg/l (figure 4.47). Co-amoxiclav resistance increased from 1% in 1998 to 5% in 2010. The unimodal MIC distribution (figure 4.47) showed a broadening of the susceptible subpopulation (MIC 0.25-8 mg/l) from 2000 onward, flattening of the peak at 1 mg/l and appearance of small and growing subpopulations with MIC 8 ->16 mg/, resulting in resistance in the following years. Such creeping MICs are predictable for upcoming resistance.
Piperacillin resistance fluctuated at low levels (4% in 2010); no resistance to piperacillin-tazobactam was recorded. Imipenem- and meropenem resistance was not found.
Cefuroxime resistance fluctuated from 0-4%; resistance to the other cephalosporins was sporadically found.
Trimethoprim resistance was fluctuating and high with 50% of all strains resistant until 2007, thereafter a decrease followed to 39% in 2010. Co-trimoxazole resistance levels were lower and decreased to 18% in 2010 (not shown).
Gentamicin resistance increased from 3% in 1998 to 8% in 2008 and decreased to 3% in 2010 (figure 4.46). These fluctuations were due to emergence of resistant strains in 2-6 centres each year, underlining the value of local surveillance.

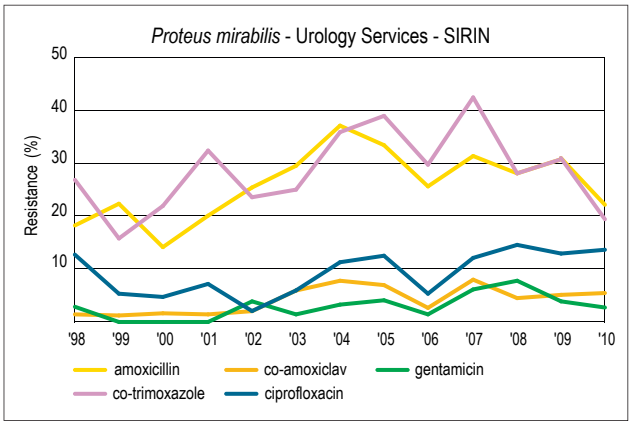

Figure 4.46. Trends in antibiotic resistance among clinical strains of *Proteus mirabilis* from Urology Services.

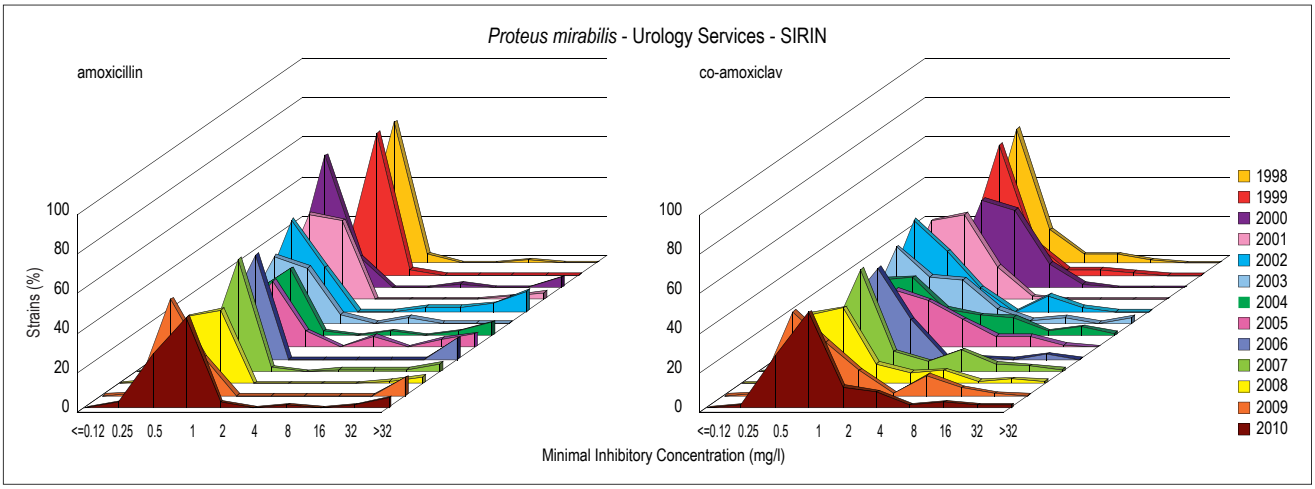

Figure 4.47. MIC distributions of amoxicillin and co-amoxiclav for *Proteus mirabilis* from Urology Services.

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Table 4.10. Resistance levels (%) among Enterobacteriaceae and Pseudomonas aeruginosa in Urology Services participating in SIRIN in 2010.

Antibiotic	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
amoxicillin	47		21	
co-amoxiclav	30	20	5	
piperacillin	40		4	7
piperacillin-tazobactam	2	7	0	
carbapenem	0	0	0	2
cefuroxime	12	16	1	
cefotaxime/ceftriaxone	6	7	0	
ceftazidime	3	4	0	2
ceftibuten	4	4	1	
cefixime	9	7	1	
cefepime	2	2	0	
gentamicin	6	6	3	3
tobramycin	6	5	3	4
amikacin	1	0	2	0
trimethoprim	38	35	39	
co-trimoxazole	36	24	18	
norfloxacin	26	21	16	
ciprofloxacin	25	9	14	18
levofloxacin	25	9	4	24
moxifloxacin	26	11	17	
nitrofurantoin	3	28	52	

increasing since 2005
decreasing since 2005
stable since 2005

Quinolone resistance fluctuated considerably, which was exclusively due to the existence of strains with MICs 1-2 mg/l (figure 4.48), which is near the breakpoints of norfloxacin (1 mg/l), ciprofloxacin (1 mg/l) and levofloxacin (2 mg/l). The MIC distributions of the quinolones are similar, but the difference in breakpoints resulted in a 16% resistance to norfloxacin, 14% for ciprofloxacin and 4% for levofloxacin in 2010

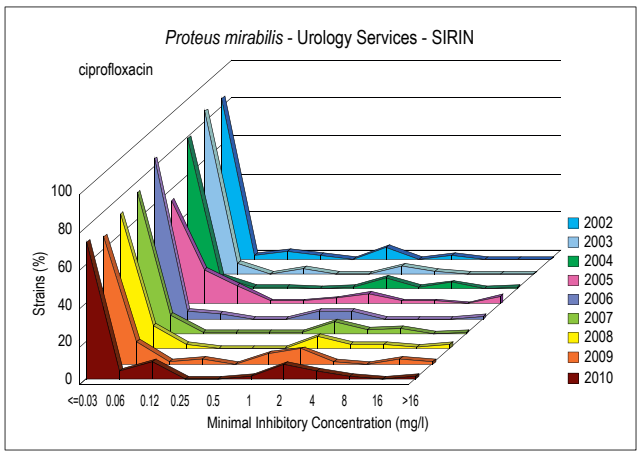

Figure 4.48. MIC distributions of ciprofloxacin for *Proteus mirabilis* from Urology Services.

Pseudomonas aeruginosa
Isolates tested (N): 610
Trends. Piperacillin resistance was occasionally found, fluctuating between 0% and 7%. Meropenem resistance was found only once in 2003. Ceftazidime resistance was consistently low (0-5%) without a trend. Aminoglycoside resistance was found sporadically. Ciprofloxacin resistance levels calculated (0-25%) fluctuated strongly because of the existence of many strains with MIC around the breakpoint. From the levels calculated one may conclude that resistance increased since 2006, but no significant change in the shape of the MIC distributions was observed during the study period, which shows that resistance percentages without data from quantitative methods are not reliable for demonstration of trends.

Enterococcus faecalis
Isolates tested (N): 1.656
Trends. Amoxicillin resistance was found in 17 strains (1%) from eight centres between 2002 and 2007, only once per centre. Seven (out of 17) were co-resistant to vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin. Vancomycin resistance (12 strains) was found once in three centres in 2003 and 2004. All but one was also teicoplanin resistant, which is evidence for clonal spread of a VanA gene positive strain. MICs for both drugs were >128 mg/l.

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Urology Services - Conclusion (see also table 4.10)

1. High and increasing resistance to penicillins, cefuroxime, trimethoprim, co-trimoxazole and quinolones among *Enterobacteriaceae* is matter of concern.
2. Aminoglycoside resistance remained low (max 6%)
3. Multiresistance is increasing and found in all centres
4. The MIC distributions over time identified significant "MIC creeps" for co-amoxiclav *(P. mirabilis)* and quinolones *(K. pneumoniae)*, which predict upcoming resistance in the following years.
5. Nitrofurantoin is still the drug of 1st choice for uncomplicated *E. coli* infections
6. Empiric therapy with any of the existing oral drugs cannot be advised in complicated UTI, except for suspected infection by *P. mirabilis* (co-amoxiclav).

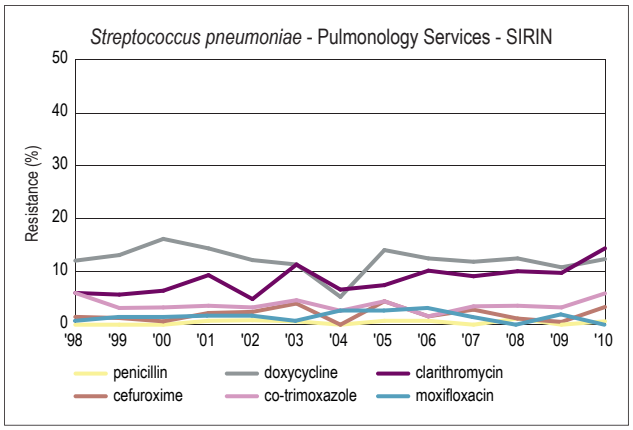

Figure 4.49. Trends in antibiotic resistance among clinical strains of *Streptococcus pneumoniae* from Pulmonology Services.

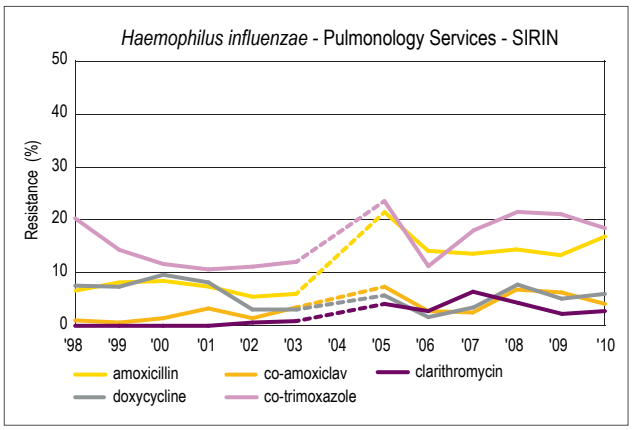

Figure 4.51. Trends in antibiotic resistance among clinical strains of *Haemophilus influenzae* from patients of Pulmonology Services.

4.2.6 Pulmonology Services - SIRIN

Streptococcus pneumoniae
Isolates tested (N): 1883
Trends. Penicillin resistance among *S. pneumoniae* was less than 1% during the whole study period with 0.5% in 2010 (figure 4.49).
Cefaclor resistance increased further to 59% in 2010 with a significant shift to the right side within the susceptible area, beginning in 2000 (MIC_{90} 0.25-0.5 mg/l) and moving many strains into the resistant area (MIC > 1mg/l) from 2007 on, resulting in MIC_{90} = 2mg/l in 2010 (figure 4.50). Cefuroxime resistance remained less than 4% (figure 4.50). Clarithromycin resistance increased further to 14% (figure 4.49), clindamycin resistance fluctuated around 6% from 2005 onward, indicating that the increase in macrolide resistance is mainly due to efflux mechanisms. (not shown). Doxycycline resistance level was stable at 12% during the whole study period (figure 4.49). The MIC distribution was bimodal with

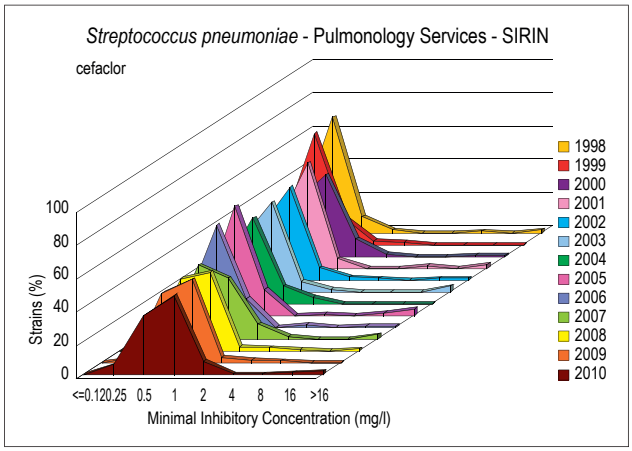

Figure 4.50. MIC distributions of cefaclor for *Streptococcus pneumoniae* from Pulmonology Services.

one susceptible (MIC < 0.5 mg/l) and one resistant subpopulation (MIC > 16 mg/l).
Co-trimoxazole resistance was 3-6% during the whole study period.
Moxifloxacin resistance was 1-3%, without significant changes over time (figure 4.49); MIC_{90} was 0.12 mg/l.

Haemophilus influenzae
Isolates tested (N): 3060
Trends. Amoxicillin resistance among *H. influenzae* increased with fluctuations from 8% in 1998 to 17% in 2010, whereas the resistance to co-amoxiclav fluctuated between 4-7% during the last years (figure 4.51).
The latter resistance mechanism is not based on beta-lactamase production. The unimodal MIC distribution (figure 4.52) of amoxicillin became bimodal in 2005 and the susceptible subpopulation moved to the right side:

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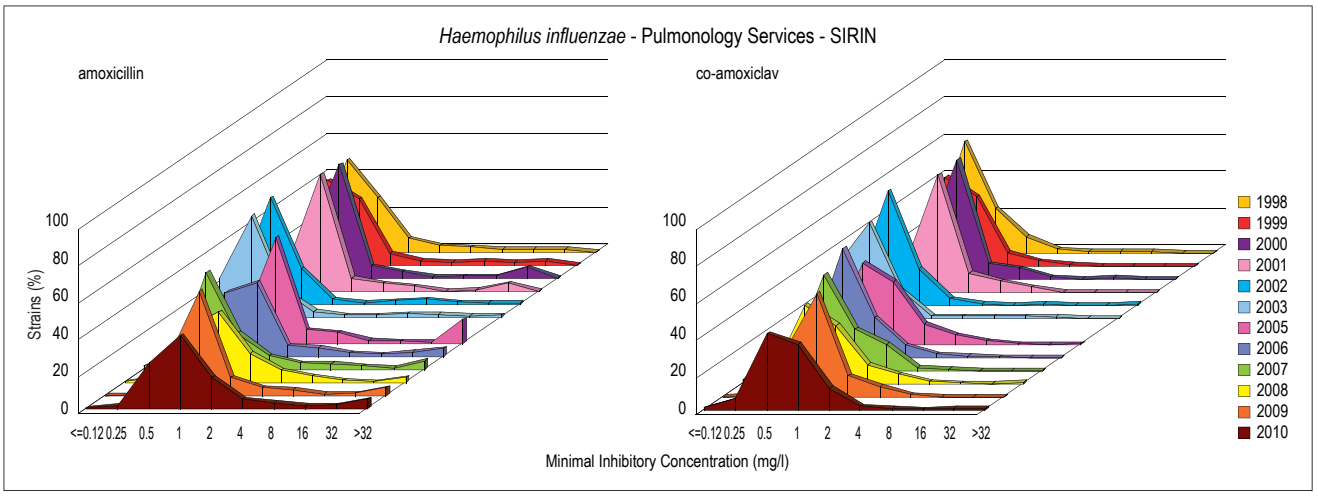

Figure 4.52. MIC distributions of amoxicillin and co-amoxiclav for *Haemophilus influenzae* from Pulmonology Services.

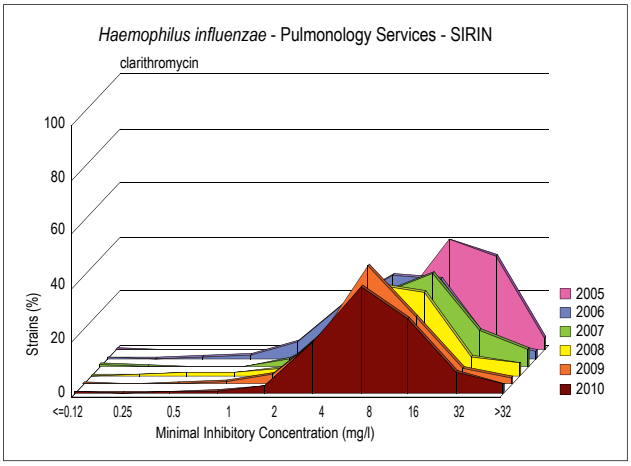

Figure 4.53. MIC distributions of clarithromycin for *Haemophilus influenzae* from Pulmonology Services.

80-90% of strains had MIC 0.5 mg/l until 2005, compared to 27% in 2010. The same shift to the right within the susceptible population was observed for co-amoxiclav. This may predict upcoming resistance and it is a matter of concern.

Clarithromycin resistance (MIC > 32 mg/l) was (3-6%). The MIC distribution (figure 4.53) showed that > 90% of strains have MIC 4-16 mg/ l (intermediate area), whereas only 1-2% can be categorized as susceptible (MIC < 1 mg/l). Doxycycline resistance was 8-10% in the first years of study and decreased to 6% in 2010.

A matter of concern is the high resistance to co-trimoxazole (19% in 2010), indicating that co-trimoxazole should not be used as empirical therapy for COPD exacerbations.

Table 4.11. Resistance levels (%) in respiratory pathogens in Pulmonology Services participating in SIRIN in 2010.

Antibiotic	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
penicillin	0.5		
amoxicillin		17	40
co-amoxiclav		5	0
cefaclor	59		100
cefuroxime	4		0
clarithromycin	14	3	1
clindamycin	6		0
doxycycline	12	6	0
co-trimoxazole	3	19	3
ciprofloxacin	3		0
moxifloxacin	2		0

increasing since 2005
decreasing since 2005
stable since 2005

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Moraxella catarrhalis
Isolates tested (N): 1197
Trends. Amoxicillin resistance among was 50-60% over the whole study period. This resistance was completely due to beta-lactamase production since resistance to co-amoxiclav was not observed.
Cephalosporin resistance was low in all Pulmonology Services. According to the new cefaclor breakpoint for resistance (> 0.12 mg/l) all *M. catarrhalis* strains were considered resistant. No resistance to cefuroxime (MIC_{90} 1 mg/l), cefotaxime (MIC90 0.5 mg/l) and ceftazidime (MIC_{90}< 0.12 mg/l) was observed and no significant changes in MIC distributions were found. Clarithromycin resistance was 1-3% and did not show any changing trend; the same was found for clindamycin. Doxycycline resistance was 4-8% until 2001 and decreased thereafter to 1% or less. Co-trimoxazole resistance was 0-3% (not shown). Ciprofloxacin resistance was found once in 2005, moxifloxacin resistance was not found.

Pulmonology Services - Conclusion
(see also table 11)
1. Penicillin or amoxicillin for treatment of respiratory tract infections by pneumococci remain first choice
2. The MIC creep for cefaclor among pneumococci, identified in 2000, resulted in progressive resistance since 2007.
3. Empiric therapy with amoxicillin is not advised when other pathogens then pneumococci may play a role. Co-amoxiclav or doxycycline may be useful alternatives.

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5 Comparison of resistance in patient populations

The resistance data of the species tested in the various patient populations in 2010 were compared for level and trend observed over the whole study period in each population.

Escherichia coli

In general: the highest resistance levels of penicillins and cephalosporins were observed in Intensive Care Units, those of co-trimoxazole and ciprofloxacin in Urology Services; resistance levels in Nursing Homes, Outpatient Departments and Unselected Hospital Departments are roughly equal and higher than in Selected GP patients whereas the latter are higher than in GP patients from the community (figure 5.1). These findings reflect specific use of these antibiotics in specific settings and previous contact/use/exposure in all study groups except GP patients from the community. Co-amoxiclav resistance was 44% in Intensive Care Units, 30% in Urology Services, 20-23% in Nursing Homes, Outpatient Departments and Unselected Hospital Departments, 16% in selected GP patients and 14% in GP patients in the community (figure 5.1). The use of co-amoxiclav for empirical therapy in any patient group should be reconsidered. Cefuroxime resistance showed the same pattern for the different populations: Intensive Care Units 20%, GP patients 6% and 10-12% for the other study groups (figure 5.1). Also resistance to cefotaxime and ceftazidime showed similar prevalence, although at lower level. Concern is the increasing resistance to all cephalosporins in all study groups, most in Intensive Care Units during the last three years and it might be interesting to investigate whether this is associated with change of antibiotic treatment and prophylaxis regimens. Carbapenem resistance is still very exceptional, but the emerging number of strains with MIC 1-8 mg/l is a matter of concern. These strains are categorized susceptible in routine susceptibility testing but may form a serious problem for the treatment of severe infections without dosing adjustments.

Aminoglycoside resistance is 5-6% in all study groups including Nursing Homes, except in the community (<1%). Gentamicin is not often used outside the hospital; this finding suggests previous use of gentamicin in all patients groups except those from the community or colonization with strains co-resistant to gentamicin after treatment with another antimicrobial drug. Aminoglycoside resistance in Intensive Care Units appeared mostly a local problem was, which underlines the importance of local surveillance.

Co-trimoxazole resistance was 36% in Urology Services, 32% in Intensive Care Units, 23-26% in Unselected Hospital Departments, Outpatient Departments and selected GP patients and 19% in Nursing Homes and the community (figure 5.1). The latter is decreasing, which may be the result of the change in the Dutch Standard for treatment of uncomplicated UTI in 2005 when trimethoprim was replaced by nitrofurantoin as a first choice.

Ciprofloxacin resistance was 25% in Urology Services, 16-17% in Intensive Care Units, Outpatient Departments and Nursing Homes, 10% in selected GP patients and 5% in the community. Extensive use of this drug in most hospital- and Outpatients Departments and Nursing Homes may lead to further increasing resistance.

A matter of concern is the increasing multiresistance in Intensive Care Units and Urology Services.

Klebsiella pneumoniae

The highest resistance levels for all antibiotics were observed in Intensive Care Units and Urology Services (figure 5.1). Further the same prevalence as for *E. coli* was observed: (1) comparable resistance levels in Selected GP patients, Outpatient Departments and Unselected Hospital Departments for co-amoxiclav, cefuroxime and co-trimoxazole; (2) resistance levels of parenteral antibiotics (cefotaxime/ceftriaxone, gentamicin) were somewhat lower in selected GP patients, Outpatient Departments compared to Unselected Hospital Departments. Carbapenem resistance remained exceptional.

Resistance to most cephalosporins is increasing, most in Intensive Care Units. Attention needs the increasing aminoglycoside resistance in Intensive Care Units and in Unselected Hospital Departments.

Increasing resistance was recorded for all quinolones in all study groups. A matter of concern is the increasing resistance to fosfomycin in selected GP patients and Outpatient Departments; this may reflect frequent use as an alternative therapy for UTI after failure of 1st choice drugs (nitrofurantoin and trimethoprim or co-trimoxazole).

Multiresistance is increasing in Intensive Care Units, although not in all, but needs careful attention.

Proteus mirabilis

Amoxicillin was replaced by co-amoxiclav for complicated UTI in the last decade because of resistance to amoxicillin and this may have led to the 10-12% resistance for co-amoxiclav now found in selected GP patients, Outpatient Departments and Unselected Hospital Departments (figure 5.1). Co-trimoxazole resistance was highest in selected GP patients and Outpatient Departments, obviously as result of previous exposure. The 7-10% resistance level of ciprofloxacin in selected GP patients, Outpatient Departments and Unselected Hospital Departments confirm the assumption that these patients have been exposed to quinolones before since no resistance was found in the community.

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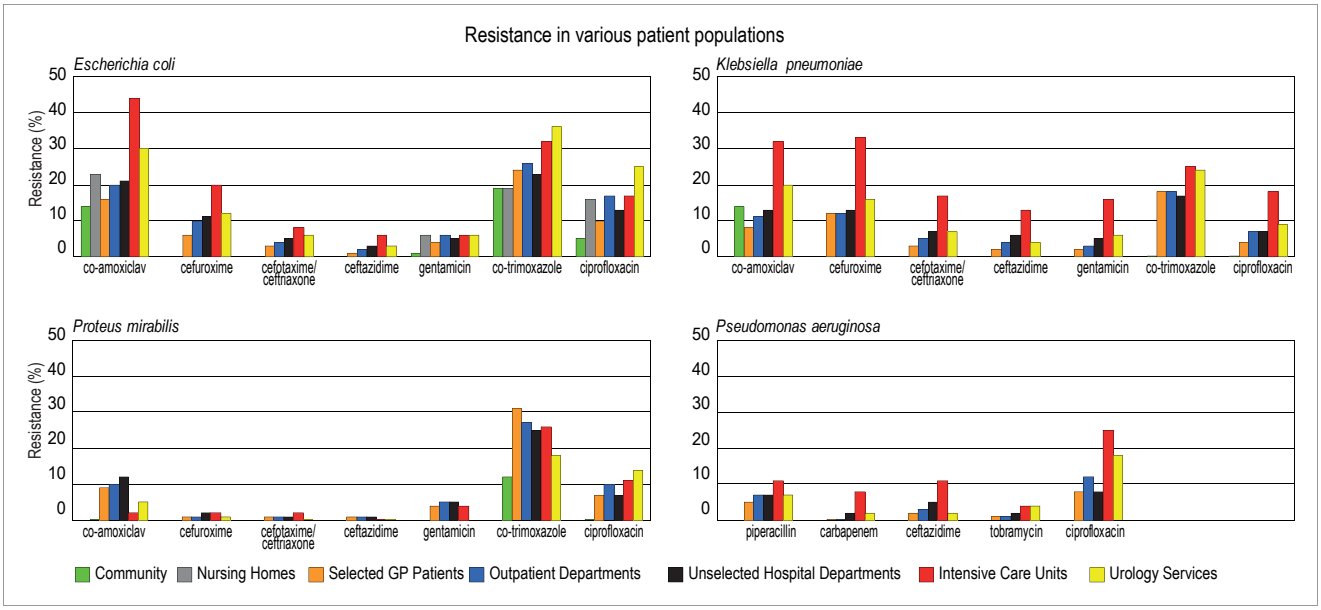

Figure 5.1. Resistance to antibiotics among *Enterobacteriaceae* and *Pseudomonas aeruginosa* in various patient populations in 2010.

Pseudomonas aeruginosa
Resistance levels to all antibiotics tested were the highest in Intensive Care Units (figure 5.1); this was most frequently due to circulation of resistant clones in a limited number of centres, which underlines again the value of local surveillance. Aminoglycoside resistance remained low in general in all study groups. Ciprofloxacin resistance was high in Intensive Care Units (25%), Urology Services (18%) and lower in Outpatients Departments (12%). Unfortunately no alternative but parenteral antibiotics in Pseudomonas infection can be advised in case of quinolone resistance.

Staphylococcus aureus
MRSA prevalence was 2.6% in Nursing Homes, compared to 1.8% in Unselected Hospital Departments and 1.1% in Intensive Care Units. Macrolide resistance is increasing to 10-11% in hospitals, which is matter of concern since a macrolide is the alternative for patients with known penicillin allergy. Ciprofloxacin resistance is high in Nursing Homes (23%) compared to 9% in hospitals. Glycopeptide resistance is exceptional.

Streptococcus pneumoniae
Penicillin resistance was higher in strains from Unselected Hospital Departments (2%) compared to that in strains from Pulmonology Services (< 1%). Macrolide- and doxycycline resistance is higher in Pulmonology Services (14%) than in Unselected Hospital Departments (9%), indicating the preference for certain antibiotics per ward. Doxycycline resistance is comparable in both wards (10-12%).

Haemophilus influenzae
Resistance levels for amoxicillin, co-amoxiclav, macrolides and doxycycline among *H. influenzae* from patients from Pulmonology Services were consistently higher than those found in Unselected Hospital Departments; in contrast 21% of strains were resistant to co-trimoxazole in Unselected Hospital Departments, whereas resistance was even increasing, that in Pulmonology Services remained stable at 19%.

Moraxella catarrhalis
Resistance levels of amoxicillin, macrolides, doxycycline and co-trimoxazole appeared higher in Unselected Hospital Departments than in Pulmonology Services. We have no explanation for this finding.

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6 Carbapenemase producing *Enterobacteriaceae*

Daan W Notermans and Jan Muilwijk

Carbapenemase producing *Enterobacteriaceae* (CPE) are increasingly recognised as an important public health threat. In 2011, a large outbreak of OXA-48 producing *Klebsiella pneumoniae* in a hospital in the city of Rotterdam became apparent, raising considerable professional and media attention.
The guidelines for laboratory detection and confirmation of carbapenemases that were available as a draft version since early 2010 were officially adopted by general assembly of the Dutch Society for Medical Microbiology (NVMM) in 2011. (1, 2)
The national surveillance of CPE was started during 2010. From patient samples taken in 2011, excluding strains directly related to the outbreak hospital, 169 strains from 142 patients were submitted by 40 hospitals to the National Institute for Public Health and the Environment (RIVM). Thirty five different CPE were detected. The majority (21) concerned OXA-48 producing *K. pneumoniae*. In 11 of the 35 cases, MICs for meropenem were within susceptible range (<= 2 mg/L) according to EUCAST criteria. As far as information was available (n=17), the origin of strains could either be traced back to the Rotterdam outbreak or to previous visits to endemic countries from North Africa or the Middle East, mainly including hospitalisation in these countries.[3]

Data from ISIS show that only for a small fraction of *Escherichia coli* and *K. pneumoniae* with initial screening MICs above the confirmation threshold, the advised phenotypic or genotypic confirmation results were available. In 2010, this was the case for only 5%. After an evaluation in the beginning of 2011 and more active feedback through ISIS, the availability of this information increased to approximately 15 % in 2011. (4)

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7 Resistance among anaerobic pathogens

Linda Veelo, Arie Jan van Winkelhoff and
John E Degener

Anaerobic pathogens isolated from patients hospitalized at the University Medical Centre Groningen during 2011 and from patients with severe periodontitis (oral strains) were included in the study. The microaerophilic *Campylobacter ureolyticus* and capnophylic *Aggregatibacter actinomycetemcomitans* were also included. Susceptibility was determined by E-test for amoxicillin, co-amoxiclav (only Gram-negative bacteria and oral strains), clindamycin, metronidazole, azithromycin (oral strains) and tetracycline (oral strains). Resistance was determined by EUCAST criteria.

Gram-negative anaerobes

The majority of *Bacteroides fragilis* sp. and *Bilophila* sp. were resistant to amoxicillin (table 7.1); one strain of *B. fragilis* sp. also to co-amoxiclav. Oral strains of *Prevotella intermedia* were less resistant to amoxicillin compared to non-oral strains of *Prevotella* sp. (4% versus 22%). Resistance to amoxicillin among other Gram-negative anaerobes was low or absent. The MIC distribution of amoxicillin for *B. fragilis* sp. was bimodal with one subpopulation with MICs 1-64 mg/l (peak at 48 mg/l) and one with > 128 mg/l. The MIC distributions of *Fusobacterium nucleatum*, and *Bilophila* sp. were unimodal over a wide range (MIC <0.016-256 mg/l); that of *C. ureolyticus* was unimodal from MIC 0.016-0.19 mg/l (figure 7.1). Remarkable was the difference in unimodal MIC distributions of amoxicillin for oral (range MIC < 0.015-4 mg/l) and non-oral strains (range MIC < 0.015-256 mg/l) of *Prevotella* sp. (figure 7.1).

Clindamycin resistance was encountered in a minority of *B. fragilis* sp., *Prevotella* sp., *C. ureolyticus* and *Parvimonas micra* (table 7.1) and 47% of *A. actinomycetemcomitans*. The MIC distribution of *B. fragilis* sp. was bimodal with one subpopulation with MIC 0.015-8 mg/l and one with MIC >256 mg/l, that of *A. actinomycetemcomitans* was unimodal and ranged from MIC 2-64 mg/l.
Metronidazole resistance was not encountered among the anaerobic bacteria tested

Gram-positive anaerobes

Amoxicillin and co-amoxiclav resistances were not encountered for Gram-positive anaerobes. The highest MIC was 1 mg/l. The MIC distributions were unimodal for all genera tested.
Clindamycin resistance was recorded in 14 % of the Gram-positive anaerobic cocci and sporadic in other Gram-positive bacteria tested (table 7.1). The MIC distributions were bimodal with one susceptible subpopulation (MIC 0.016-2 mg/l) and one resistant subpopulation (MIC 8-> 256 mg/l).
Metronidazole resistance was not found.
Susceptibility testing to azithromycin and tetracycline for oral strains (197) revealed unimodal MIC distributions over a broad range (< 0.015-4 mg/l) for both antibiotics; six strains had high MICs (8-256 mg/l) for azithromycin. EUCAST has not delivered breakpoints for azithromycin and tetracycline for anaerobes, so we cannot calculate resistance levels, although we assume that they might be categorized susceptible. None of the *A. actinomycetemcomitans* strains were resistant to azithromycin or tetracycline.

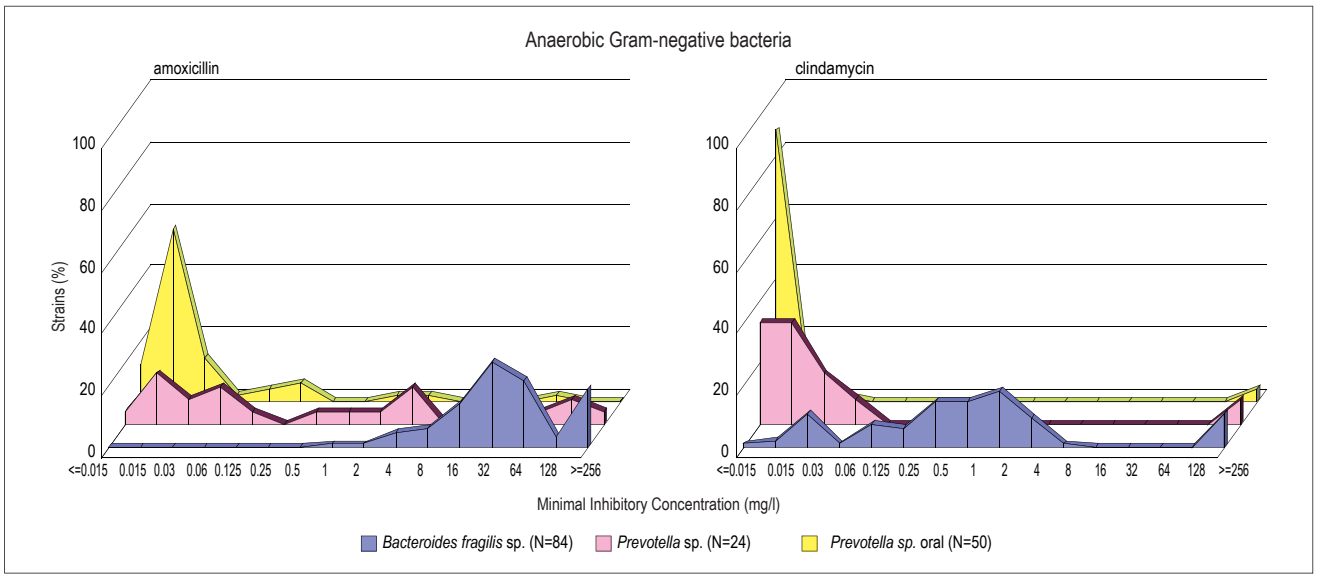

Figure 7.1. MIC distribution of amoxicillin and clindamycin for clinical strains of anaerobic Gram-negative bacteria (N= 158).

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Table 7.1. Resistance among anaerobic bacteria.

Species (N)	Antibiotic resistance N (%)			
	amoxicillin	co-amoxiclav	clindamycin	metronidazole
Gram-negative bacteria				
Bacteroides fragilis sp. (82-84)*	81 (98)	1(1)	10 (12)	0
Fusobacterium sp. (9-10)*	2	0	0	0
Fusobacterium nucleatum (50)**	1 (2)	0	0	0
Prevotella sp. (23-24)*	10 (22)	0	2 (8)	0
Prevotella intermedia (50)**	2 (4)	0	2 (4)	0
Bilophila sp. (13)	11 (85)	0	0	0
Campylobacter ureolyticus (10-11)*	0	0	1 (9)	0
Porphyromonas gingivalis (50)**	0	0	0	0
Aggregatibacter actinomycetemcomitans (47)**^	2 (4)	1 (2)	NA	NA
Gram-positive bacteria				
Gram + anaerobic cocci (57)	0	NT	8 (14)	0
Parvimonas micra (50)**	0	0	1 (2)	0
Propionibacterium sp. (31)	0	NT	1 (3)	NA
Actinomyces sp. (9)	0	NT	1	NA
Clostridium sp. (21)	0	NT	4 (19)	0

* not all strains were tested for all antibiotics

** oral strains

^ Breakpoint derived from Haemophilus influenzae

NA not available

NT not tested

Anaerobic bacteria - Conclusion

1. Amoxicillin resistance was 2% in oral Gram-negative anaerobic bacteria, 74% in non-oral bacteria and 0% in Gram-positive bacteria (oral and non-oral); co-amoxiclav resistance was not found, except for one *B. fragilis* sp. strain.
1. Clindamycin resistance was 10-12% for both Gram-positive and Gram-negative bacteria
2. All anaerobic bacteria were susceptible to metronidazole.

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8 Antifungal resistance

Dik Versteeg and Paul Verweij

Further increase of azole-resistant *Aspergillus* isolates in the Netherlands

Resistance to medical triazoles is an increasing problem in the opportunistic fungus *Aspergillus fumigatus*. This mold causes a spectrum of diseases in humans, including invasive aspergillosis in severely immunocompromised patients. In the Netherlands azole resistance emerged in 1998 when the first azole resistant isolate was recovered from a clinical specimen (1). Since then the prevalence has increased and surveillance studies indicate that azole resistance is now wide spread (1, 2). The underlying resistance mechanism is a combination of 2 changes in the *Cyp51A*-gene (TR34/L98H), which is the target for antifungal azoles. This resistance mechanism is found in more than 90% of azole-resistant isolates, and there is increasing evidence that TR34/L98H has emerged through environmental exposure to azole fungicides (3). Molecule alignment studies have shown that that there are five azole fungicides that exhibit a highly similar molecule structure to the medical triazoles (4). These compounds include, tebuconazole, epoxiconazole, difenoconazole, bromuconazole and propiconazole, and interestingly these compounds were authorized for use by Dutch Board for the Authorization of Plant Protection Products and Biocides between 1990 and 1996.

Azole resistance in the Netherlands

In the prospectively collected fungus culture collection of the Microbiology Department of the Radboud University Nijmegen Medical Centre, a continued increase was observed (figure 8.1) and in most recent years around 10% of patients with a positive culture with *A. fumigatus* azole resistance is found. This increase is partly due to the emergence and spread of a second resistance mechanism, which is believed to also be from environmental origin. This resistance mechanism involves multiple changes to the *Cyp51A*-gene (TR_{46}/Y121F/T289A) and has been found in environmental *A. fumigatus* isolates. The phenotype of this isolate is lack of activity of voriconazole, which is the recommended agent of first choice for treatment of many *Aspergillus* diseases, including invasive aspergillosis. The proportion of azole resistant isolates in Nijmegen that harbour the TR_{46}/Y121F/T289A resistance mechanism has increased from 10% in 2010 to 20% in 2011. Sequence analysis of 55 azole resistant Af isolates sent to Nijmegen in 2011 from various primary and secondary Dutch referral hospitals revealed 35 (64%) isolates with TR_{34}/L98H and 20 (36%) isolates with TR_{46}/Y121F/T289A.

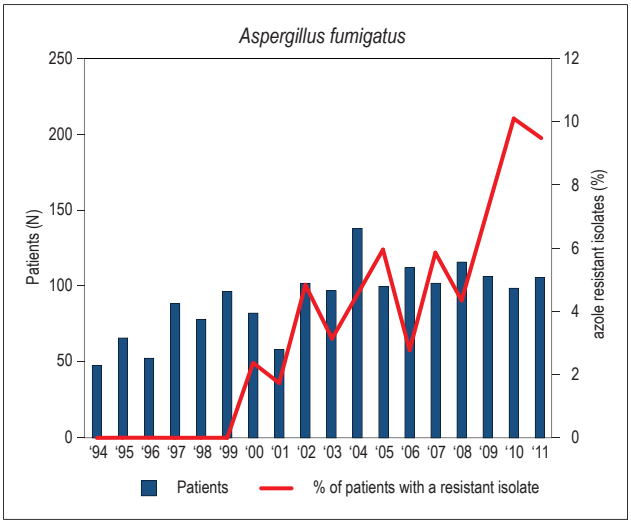

Figure 8.1. Trends in azole resistance among 2.542 clinical isolates of *Aspergillus fumigatus* in 1.647 patients. On the left Y-axis (bars) the number of patients are displayed, on the right Y-axis (line) the percentage of azole resistant isolates are displayed.

The Dutch Ministry of Health, Welfare and Sports has supported the initiation of a National Reference Laboratory situated at the RUNMC that will perform active surveillance in close collaboration with the National Centres for Infectious Disease Control (CIb).

Migration of azole resistance

International surveillance indicates that TR_{34}/L98H is also found in clinical isolates in other countries. TR_{34}/L98H was found in Belgium, France, Italy, Austria and Denmark. The molecular epidemiology of TR_{34}/L98H isolates from the Netherlands and Europe indicates that these isolates are genetically less diverse than wild type control isolates (5). The implication of this finding is that TR_{34}/L98H might have originated from a common ancestor and that migration of TR_{34}/L98H has occurred rather than de novo development of TR_{34}/L98H locally. Given the similar characteristics of isolates harboring TR_{46}/Y121F/T289A, compared to TR_{34}/L98H, one can anticipate that TR_{46}/Y121F/T289A will also spread across borders.

Over the past year the prevalence of azole resistance in *A. fumigatus* has increased further. Our current understanding of resistance development in the environment indicates that this trend will continue and that the emergence and spread of new resistance mechanisms can be anticipated.

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9 Resistance to influenza antiviral drugs

Adam Meijer and Marcel Jonges

Introduction

Infection by influenza A(H1N1), A(H3N2) or B viruses, results in substantial morbidity and excess mortality each year. Vaccination against seasonal influenza is the key control measure used in the Netherlands and Europe to minimize morbidity and mortality, especially in the risk groups for development of complications upon influenza virus infection. However, antigenic mismatch between vaccine components and circulating viruses does occur every few years requiring the vaccine to be reformulated. This together with sub-optimal vaccine uptake in recommended patient groups, non-responders to vaccination and waning immunity during the season provides the rationale for the use of antiviral drugs in the prophylaxis and treatment of influenza under special circumstances (1, 2). In addition, preparations have been made for provision of antiviral treatment and prophylaxis in case of a pandemic and the Dutch government has stockpiled oseltamivir and zanamivir. These preparations came into effect when in 2009 the first influenza pandemic of the 21th century occurred, caused by a triple reassortant virus from swine origin, the A(H1N1)pdm09 virus (3).

Prescriptions of influenza antivirals

Two classes of influenza antiviral drugs are available for treatment and prophylaxis, the M2 ion-channel blockers (M2Bs), amantadine (Symmetrel®) and rimantadine (Flumadine®, not registered in the Netherlands), and the neuraminidase inhibitors (NAIs), oral oseltamivir (Tamiflu®) and inhaled zanamivir (Relenza®). M2Bs have been available since 1964, but their usefulness have been limited because of adverse effects, rapid development of resistance (full cross-resistance for both drugs) and lack of activity against influenza B virus infections. M2Bs are also indicated for Parkinson disease. We showed previously that during influenza outbreaks there is no significant increase in amantadine prescriptions in the Netherlands, consistent with the limited usefulness of this type of influenza antiviral drugs (4).

The introduction in 1999 of NAIs, which are active against both type A and B influenza viruses, was a major breakthrough in treatment and prophylaxis of influenza using antiviral drugs. In addition, because of different molecular interactions of both drugs with the neuraminidase, a limited number of mutations result in full cross-resistance, and if resistance mutations occur these mostly adversely affect infectiveness and transmissibility of the mutated virus. According to prescription data, NAIs are not widely used in the Netherlands during seasonal epidemics (5). During the first and second wave of the A(H1N1)pdm09 pandemic use of oseltamivir was at the peak months 2-4 times higher than in October 2005 when there were concerns about spread of A(H5N1) avian influenza into Europe. However, a substantial amount of prescriptions as precaution cannot be excluded (6). After the pandemic, prescriptions dropped to levels seen before the A(H1N1) 2009 pandemic (5). In Europe the number of prescriptions of NAI by country is in general low, but the Netherlands is among the lowest (7).

Surveillance for resistance

Details about surveillance for influenza antiviral resistance has been described previously (4). Briefly, in the Netherlands, monitoring of antiviral susceptibility is since the 2005/2006 season embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinel stations, which is carried out by the NIVEL Netherlands Institute for Health Services Research and the National Influenza Centre location Bilthoven, Centre for Infectious Disease Control, National Institute for Public Health and the Environment. In special circumstances, like during the emergence of oseltamivir resistant A(H1N1) virus during the 2007/2008 season and during the 2009 A(H1N1) 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 (8, 9). Techniques used to monitor antiviral resistance in influenza viruses are determination of the 50 percent inhibitory concentration (IC50) in cell-ELISA virus growth inhibition assay or plaque reduction assay and Sanger sequencing, pyrosequencing or site-specific polymerase chain reaction (PCR) assay for known resistance markers for both the M2Bs and NAIs (10-13). For NAIs the IC50 can also be determined using an enzyme inhibition assay (14, 15). In the absence of known NAI resistance mutations detected by genotypic assays, determination of the IC50 is the only way to determine the drug susceptibility of a virus.

Resistance

Antiviral susceptibility of influenza viruses in the Netherlands since the 2005/2006 season is summarised in the table. Previously we described the emergence of M2B resistance in A(H3N2) viruses and A(H1N1) viruses, although for A(H1N1) a lineage of M2B sensitive viruses gradually replaced the resistant lineage (4,16). In addition, the emergence of oseltamivir resistant A(H1N1) viruses during the 2007/2008 season was described (4, 8). During the A(H1N1) 2009 influenza pandemic in the Netherlands enhanced molecular influenza surveillance was implemented using molecular methods to screen for mutations in the genome related to antiviral resistance

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Table 9.1. Resistance/reduced susceptibility of influenza viruses to NAIs and M2Bs in the Netherlands, 2005/2006 - 2010/2011[1].

	A(H3N2)		A(H1N1) seasonal		A(H1N1)pdm09		B
Season	NAI	M2B	NAI	M2B	NAI	M2B	NAI
2005/2006	1/39 (3%)[2]	29/39 (74%)	NA	NA	NA	NA	2/48 (4%)[3]
2006/2007	0/50	38/51 (75%)	0/5	0/6	NA	NA	0/3
2007/2008	0/10	12/12 (100%)	47/172 (27%)[4]	0/49	NA	NA	1/81 (1%)[2]
2008/2009	5/74 (7%)[5]	8/8 (100%)	5/5 (100%)	ND	0/492	8/8 (100%)	0/19
2009/2010	ND	1/1 (100%)	NA	NA	20/627 (3%)[6]	54/54 (100%)	NA
2010/2011	0/2	2/2 (100%)	NA	NA	0/57	40/40 (100%)	0/64

[1] Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NA = not applicable as there were no viruses of the given type or subtype tested; ND = viruses available, but analysis was not done.

[2] The resistant virus had an extreme outlier IC_{50} for oseltamivir and mild outlier IC_{50} for zanamivir.

[3] Both resistant viruses had outlier IC_{50} values for oseltamivir as well as zanamivir.

[4] Viruses resistant to oseltamivir only. Viruses were sensitive to zanamivir and M2Bs.

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

[6] Nineteen viruses were resistant to oseltamivir and not to zanamivir with H275Y mutation. One other virus had a 3-fold increased IC_{50} for oseltamivir and a 5-fold increased IC_{50} for zanamivir.

directly from a clinical specimen (9). Because no virus isolate is needed this reduces the analysis time by 1-1 ½ week, which is of benefit for a rapid response. A total of 19 cases with the H275Y resistance amino acid substitution in the neuraminidase were detected, 18 of them associated with oseltamivir therapy (9). In the 2010/2011 season no viruses resistant against the NAIs were detected (Table).

All the A(H1N1)pdm09 influenza viruses analysed since the 2008/2009 season of which the M2 gene was sequenced had the S31N amino acid substitution characteristic for resistance against the M2 blockers (Table). All A(H3N2) viruses analysed since the 2007/2008 season were also resistant against the M2 blockers with the same substitution (Table). Therefore, there is since 2009 no place for M2 blockers in the treatment or prophylaxis of influenza.

The pandemic likely caused the seasonal A(H1N1) viruses to disappear, and with that the natural resistance of this subtype influenza viruses against oseltamivir. Although sporadic resistance against oseltamivir is detected in A(H1N1)pdm09 influenza viruses, these viruses do not harbour the same permissive amino acid substitutions in the neuraminidase that allowed the emergence of transmissible oseltamivir resistant seasonal A(H1N1) viruses (17).

Influenza B viruses in general are less susceptible to NAI than type A influenza viruses. However, over the period 2005-2011 only very sporadically an influenza B virus that was resistant against NAIs or had a reduced susceptibility for NAIs has been detected in the Netherlands (Table) (16).

Conclusion

Emergence of oseltamivir resistance in A(H1N1) pdm09, A(H3N2) and B influenza viruses is since the replacement of seasonal A(H1N1) by A(H1N1) pdm09 viruses sporadic. However, the emergence of natural resistance against oseltamivir in seasonal A(H1N1) viruses during the 2007/2008 season has set the scene. Therefore, continuous alertness using sentinel surveillance and close monitoring of patients under therapy and their contacts is needed for early warning and timely action to limit spread of resistant viruses.

Acknowledgements

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MARAN 2012

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2010/2011

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Colophon

This report is published under the acronym MARAN-2012 by the Central Veterinary Institute of Wageningen University and Research Centre in collaboration with the Agricultural Economics Research Institute of Wageningen UR, the Food and Consumer Product Safety Authority, and the National Institute for Public Health and the Environment. The information presented in MARAN-2012 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-2012 is published in a combined back-to-back report with NETHMAP-2012. The combined report is available on the website of CVI-Lelystad at www.cvi.wur.nl. Detailed information on the usage of antibiotics per animal species, based on a collation of data from on-going monitoring, is available on the MARAN website www.maran.wur.nl.
MARAN-2012 can be ordered from the secretariat of CVI-Lelystad, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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1. Summary

Antibiotic Usage
In the years 2009-2011 the total sales of antibiotics licenced for therapeutic usage in the Netherlands decreased by nearly 32%, from 495 tonnes in 2009 to 338 tonnes in 2011. This means that the policy objective for 2011, a 20% reduction in 2011, compared with 2009, was more than reached. Moreover, using 2007 as reference year with highest antibiotic usage the decrease in antibiotic usage up to 2011 was even 40%.

Antimicrobial resistance
As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most prevalent serovars isolated from humans in the Netherlands, represented by 29.5% and 28.1% of all isolates respectively, followed by the antigenic monophasic variant of *S*. Typhimurium: *S. enterica* serovar 1,4,5,12:i:-. The contribution of this variant increased from 6.2% in 2008/2009 to 14.2% in 2010/2011 among human isolates, but increased also in animals. Other remarkable shifts in *Salmonella* serovars from animals are the strong increase for *S.* Derby in pigs (from 16.7% in 2008/2009 to 31.2 in 2010/2011), the replacement of *S*. Dublin (34.6%) as most prevalent serovar in cattle by *S.* Typhimurium (36.2%) and the appearance of *S.* Braenderup in laying hens (15.7%).
S. Typhimurium has acquired resistance against a number of antimicrobials. The most common resistance pattern is ASTSuCipNalFC. In addition, ESBL producing strains (cefotaxime reduced susceptible with MIC > 0.5 mg/l) amounted for 0.5% of the Typhimurium isolates. The group 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, 12.6% of *Salmonella* isolates demonstrated a non-wild type phenotype for ciprofloxacin, while 1.1% showed MICs larger that the clinical breakpoint (1 mg/l). The serovars of these ciprofloxacin resistant isolates were predominantly travel related *S*. Kentucky (67%) or *S*. Java (12%). The remaining 20% consisted of a diversity of serovars merely represented by a single strain. In 2010/2011, the total number of cefotaxime reduced susceptible (MIC > 0.5 mg/l) ESBL suspected *Salmonella* isolates was 52 (1.2%), among 17 different serovars. A substantial part of the isolates belonged to the serovar *S*. Java (19 of 52 isolates) and *S*. Heidelberg (7 isolates).
In animals and poultry meat, a very high proportion of *Campylobacter* was resistant to ciprofloxacin, whereas lower levels were observed for erythromycin and gentamicin. High resistance was also recorded for commonly used antimicrobials such as ampicillin and tetracyclines. Similarly, a high proportion of *Campylobacter* in humans was resistant to ciprofloxacin, whereas low resistance was recorded for erythromycin.
Among indicator (commensal) *E. coli* isolates from meat and animals, resistance to ampicillin, streptomycin, tetracyclines, sulfonamides and trimethoprim was commonly detected in all host species except dairy cattle. Reduced susceptibility to ciprofloxacin and nalidixic acid was highest for *E. coli* isolates from broilers (59.4% and 59.6% respectively) and turkeys (31.6% and 27.4% respectively). Resistance to third-generation cephalosporins was observed in indicator *E. coli* isolates from poultry, pigs and cattle and varied from very low levels in dairy cattle (0.4%) to 13.2% in broiler chickens in 2010/2011.
For the first time, data for enterococci from turkey were included. Although isolates from only a limited number of farms (n=20) were included, resistance levels were high in comparison to other farm animals, notably for tetracycline, erythromycin, ampicillin, chloramphenicol and ciprofloxacin. Resistance to vancomycin continued to be detected at low levels in enterococci from animals.
ESBL/AmpC-genes are detected in high prevalences in all food-producing animals, pets and wild-birds. This demonstrates that the Dutch ecosystem is heavily contaminated. However in broiler chickens the occurrence of ESBL producing *E. coli* in broilers is shows a tendency to decrease from 2010 onwards, the moment when the use of ceftiofur at Dutch hatcheries was banned.
It can be concluded that antibiotic sales data show a steady and substantial decrease since the top year 2007. In 2008 measures to limit antibiotic usage were initiated. Its effect on the occurrence of resistance is not yet noticeable except for the occurrence of cefotaxime resistance in broilers, which show a tendency to decrease after the ban of ceftiofur in poultry hatcheries in 2010.

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2. Usage of antibiotics in animal husbandry in the Netherlands

2.1 Total sales of veterinary antibiotics in the Netherlands 2011

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, reports the total number of kilograms of antibiotics (active ingredient) sold in the Netherlands in 2011 at the level of pharmacotherapeutic groups. The data about use of active substances are based on sales data of members of FIDIN and are estimated to cover about 98% of all sales in the Netherlands. Actual use can be different from the amounts sold as a result of stock piling and cross border use.
The European Medicines Agency (EMA) collects harmonised antibiotic usage data based on overall sales of veterinary antimicrobial agents, as well as per animal species. The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project was launched by EMA in September 2009. To ensure that the data provided by the Member States are harmonised, an ESVAC Data Collection Protocol (ESVAC template) has been developed and a call for data has been sent to most EU member states. To fully implement the ESVAC protocol FIDIN had to adjust the levels of active ingredients for several products, taking into account the salt and ester formulations and calculation factors of active ingredients expressed in international units. These corrections led to a reduction of the calculated total amount of active substance by approximately 4%. The sales figures of 2009, 2010 and 2011 were based on the ESVAC template; the figures of 1999 to 2008 were re-calculated and corrected accordingly. The sales data in this report give information about the total sales for all animals, not per individual animal species. Detailed information about antibiotic usage per animal species can be found on the MARAN website www.maran.wur.nl.

To adjust for trends in the size of the animal population the sales of antibiotics were also expressed as grams of active ingredient per kilogram live animal weight (Figure ABuse01). For this purpose the annual total sales data published by FIDIN were related to the total live weight of the average number of animals present in the Dutch livestock farming sector (pigs, poultry, veal calves, other cattle and sheep). For this analysis the following average weights were used: veal calves 172 kg (i.e. the weighted average of white veal calf 164 kg and rosé veal calf 192 kg), other cattle 500 kg, turkeys 6 kg, other poultry 1 kg, fattening pigs 70 kg, sows 220 kg, piglets (<20 kg) 10 kg, sheep 60 kg. This yields information about the trend in the sales of antibiotics in grams per kilogram of live animal weight present in the Netherlands over the years, thus taking into account yearly fluctuations in the size of the animal population. The yearly average

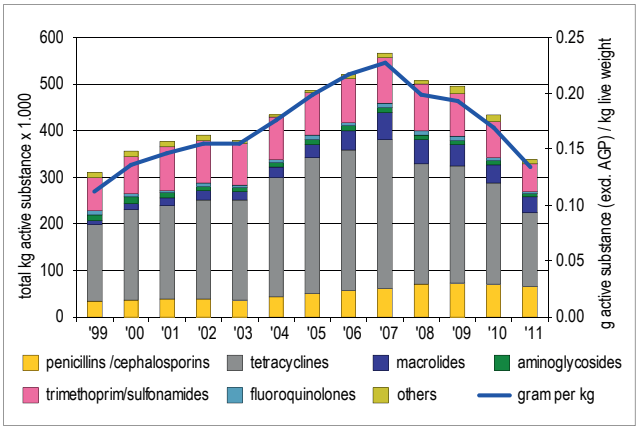

Figure ABuse01. Veterinary therapeutic sales from 1999-2011 (FIDIN, 2012; vertical bars). The line presents the the trends in grams of active ingredients used per kg live weight.

numbers of animals and its conversion into live weight are given in Table ABuse01 and Table ABuse02.

2.1.2 Trends in total sales

Figure ABuse01 shows the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands, also expressed in grams of active substance per kg of live weight present. It reveals that in the years 2009-2011 the total sales decreased by nearly 32%, to a total of 338 tonnes in 2011. This means that the policy objective for 2011, a 20% reduction in 2011, compared with 2009, was more than reached. Moreover, using 2007 as reference year with the highest antibiotic usage, the decrease in usage up to 2011 was even 40%.

Discussion

The total sales volume amounted to 338 tonnes in 2011, which is below the level of the year 2000. However, at that time an additional 205 tonnes of antimicrobial growth promoters were used (see Table ABuse03). Almost all classes of antibiotics showed a decrease.

Tetracyclines

The sales data show a decrease of tetracyclines from 251 tonnes in 2009 to 157 tonnes in 2011 (-37%), of which 102 tonnes of oxytetracycline (-41%) and 54 tonnes of doxycycline (-31%). The true exposure to tetracyclines decreased by an estimated 35%, roughly assuming a constant livestock population and an average dosage of 30 mg per kg of animal for oxytetracycline and 10 mg per kg for doxycycline.

Fluoroquinolones

In 2011 the fluoroquinolones represented 1.52% of the total veterinary antibiotic sales in the Netherlands, of which 0.43% ‘newer’ fluoroquinolones (danofloxacin, difloxacin,

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Table ABuse01. Trends in livestock in the Netherlands in numbers (thousands).

Number of animals * 1.000	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Piglets (less than 20 kg)	4791	4935	4422	4225	3896	4300	4170	4470	4680	4555	4809	4649	4797
Sows	1320	1272	1161	1140	1052	1125	1100	1050	1060	1025	1100	1098	1106
Fattening pigs	7028	6615	5931	5789	5818	5715	5730	5700	5970	6155	6199	6459	6200
Turkeys	1544	1544	1523	1451	1112	1238	1245	1140	1232	1222	1246	1167	1167
Other poultry	53,453	53,453	58,475	62,066	42,991	43,854	45,525	42,529	44,487	50,270	52,323	54,367	57,811
Veal calves	800	756	676	692	748	775	813	824	860	913	886	921	919
Cattle (excl. veal calves)	3297	3134	3166	3088	2986	2984	2933	2849	2960	3083	3112	3039	2993
Sheep	1152	1250	1250	1300	1476	1700	1725	1755	1715	1545	1091	1211	1113

Sources: Eurostat (pigs, cattle, sheep, laying hens before 2008), FAO (other poultry), CBS (laying hens 2008-2011); For poultry, FAO Poultry figures 2011 were not yet available; and 2010 data were used.

Table Abuse02. Trends in livestock in the Netherlands in live weight (tonnes).

Live weight *1.000 kg	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
pigs	831,676	793,563	715,996	699,438	678,789	691,693	685,946	675,840	699,094	703,131	725,260	741,472	726,530
Poultry	62,717	62,717	67,613	70,772	49,663	51,282	52,995	49,369	51,879	57,602	59,799	61,369	64,813
Veal calves	137,920	130,334	116,542	119,301	129,024	133,610	140,161	142,058	148,264	157,401	152,746	158,780	158,436
Cattle (excl. veal calves)	1,648,500	1,567,000	1,583,000	1,544,000	1,493,150	1,492,000	1,466,500	1,424,500	1,480,000	1,541,500	1,556,000	1,519,500	1,496,500
Sheep	69,120	75,000	75,000	78,000	88,560	102,000	103,500	105,300	102,900	92,700	65,460	72,660	66,780
Total	**2,749,933**	**2,628,614**	**2,558,152**	**2,511,511**	**2,439,186**	**2,470,585**	**2,449,102**	**2,397,067**	**2,482,137**	**2,552,334**	**2,559,265**	**2,553,781**	**2,513,059**

Table ABuse03. Antibiotic sales from 1999-2011 in tonnes (FIDIN, 2012).

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Penicillins /cephalosporins	35	36	38	38	36	43	51	57	61	70	73	71	66
Tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157
Macrolides	10	15	17	19	17	23	28	42	55	52	46	39	34
Aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	9	7
Fluoroquinolones	7	7	6	6	5	7	8	7	9	8	8	7	5
Trimethoprim/Sulfonamides	71.5	80.4	92.1	92.1	88.2	91.1	91.1	93.1	99	100	92	78	58
Others	11	12	11	11	7	6	6	8	8	7	15	13	10
Total therapeutic sales	310	356	376	390	378	434	487	519	565	506	495	433	338
Antimicrobial growth promoters (AGP)	250	205	180	140	120	80	40	0	0	0	0	0	0
Total sales including AGP	**560**	**561**	**556**	**530**	**498**	**514**	**527**	**519**	**565**	**506**	**495**	**433**	**338**

enrofloxacin and marbofloxacin). In the years 2009-2011 the sales of fluoroquinolones decreased substantially, by 34%. The sales of the newer fluoroquinolones showed a slight increase of 6%.

Cephalosporins

The cephalosporins represented 0.23% of the total sales, of which 0.17% third and fourth generation cephalosporins (cefoperazon, cefovecin, cefquinome, ceftiofur). In 2009-2011 the sales of third and fourth generation cephalosporins decreased by 37%.

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3. Resistance data

In this chapter susceptibility test results are presented as determined in 2010 and 2011 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*

Salmonella is an important zoonotic pathogen, which can be isolated from the intestinal content of a broad range of animal host species. More than 2500 serovars of zoonotic *Salmonella* have been recognised, and the prevalence of these different serovars differs among host species and can change over time. The present convention is to capitalize the serovar designation and indicate it (without italics) after the genus name. As an example, *Salmonella enterica* subsp. *enterica* serovar Typhimurium is described as *S.* Typhimurium.
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.

3.1.1.1 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=2708 in 2010/2011) concerned a selection from first 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=159) and cattle (N=109), including calves were sent to the RIVM by the Animal Health Service from a diversity of surveillance programs and clinical *Salmonella* infections. Those from chickens (broilers, including poultry products, N= 146; layers, reproduction animals and eggs, N=110) concerned mainly nonclinical *Salmonella* isolations 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.).
As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most prevalent serovars isolated from humans in the Netherlands, represented by 29.5% and 28.1%

Highlights

1. As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most prevalent serovars isolated from humans in the Netherlands, represented by 29.5% and 28.1% of all isolates respectively, followed by the antigenic monophasic variant of S. Typhimurium: *S. enterica* serovar 1,4,5,12:i:-. The contribution of this variant increased from 6.2% in 2008/2009 to 14.2% in 2010/2011 among human isolates, but also increased in animals. Other remarkable shifts in *Salmonella* serovars from animals are the strong increase for *S.* Derby in pigs (from 16.7% in 2008/2009 to 31.2 in 2010/2011), the replacement of *S.* Dublin (34.6%) as most prevalent serovar in cattle by *S.* Typhimurium (36.2%) and the appearance of *S.* Braenderup in laying hens (15.7%).
2. *S.* Typhimurium has acquired resistance against a number of antimicrobials, most commonly resistance pattern is ASTSuCipNalFC. In addition, ESBL producing strains (cefotaxime reduced susceptible with MIC > 0.5 mg/l) amounted for 0.5% of the Typhimurium isolates.
3. In *S.* Java characteristic findings are high level resistance against trimethoprim which is characteristic of the clone predominating in the Netherlands in poultry, in combination with acquired resistance against the quinolones and third generation cephalosporins cefotaxime and ceftazidime.
4. The group of fluoroquinolones is widely regarded as the treatment of choice for salmonellosis in adults. Using the epidemiological cut off value of 0.06 mg/l, 12.6% of *Salmonella* isolates demonstrated a non-wild type phenotype for ciprofloxacin, while 1.1% showed MICs larger that the clinical breakpoint (1 mg/l). The serovars of these ciprofloxacin resistant isolates were predominantly travel-related *S.* Kentucky (67%) or *S.* Java (12%). The remaining 20% consists of a diversity of serovars merely represented by a single strain.
5. In 2010/2011, the total number of cefotaxime reduced susceptible (MIC > 0.5 mg/l) ESBL suspected *Salmonella* isolates was 52 (1.2%), among 17 different serovars. A substantial part of the isolates belonged to the serovar *S.* Java (19 of 52 isolates) and *S.* Heidelberg (7 isolates).

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Table S01. Most prevalent Salmonella sero- and phage types isolated in 2010/2011 and 2008/2009 from humans, pigs, poultry, broilers and layers and the % travel related human infections from 2008 – 2011.

	N	Travel	Humans		Pigs		Cattle		Poultry		Broiler		Layer	
		'08/11	'08/09	'10/11	'08/09	'10/11	'08/09	'10/11	'08/09	'10/11	'08/09	'10/11	'08/09	'10/11
N total		10%	3130	3096	1137	279	179	127	1004	459	641	198	109	134
N tested			2502	2708	386	159	149	109	639	352	400	146	75	110
Typhimurium	*1797*	3%	34.6	28.1	14.2	19.4	20.1	36.2	2.6	4.8	1.6	3.0	6.4	6.0
SI 1,4,5,12:i:2-	*623*	3%	6.2	14.4	4.6	15.4	3.9	10.2	1.6	2.4	1.2	3.0		3.0
Enteritidis	*1822*	13%	31.8	29.5	1.4	0.4	0.6	0.8	11.3	12.9	7.8	8.6	33.9	31.3
Pt 1	*218*	15%	3.0	4.4					0.3	0.2	0.3		0.9	1.5
Pt 21	*255*	9%	4.8	2.4				0.8	2.3	1.7	1.9	1.5	7.3	3.0
Pt 4	*530*	7%	7.2	9.9	0.1				3.0	3.1	1.6	1.5	11.0	6.7
Pt 6	*131*	33%	2.7	1.6	0.1				0.3	1.3	0.3	1.0		3.0
Pt 7	*32*	29%	0.2	0.4					0.6	0.7			3.7	2.2
Pt 8	*370*	7%	7.1	5.0	0.7		0.6		1.9	1.5	1.6	0.5	3.7	7.5
Pt 14b	*91*	18%	1.8	1.1						0.7				2.2
Anatum	*41*	17%	0.2	0.3	2.8	0.4	0.6		0.6	0.7	0.9			1.5
Bareilly	*27*	14%	0.2	0.3					0.2	0.4			1.8	4.5
Bovismorbificans	*31*	0%	0.4	0.4	3.2		1.7							
Braenderup	*59*	15%	0.2	0.5	0.1			0.8	0.1	4.6	0.2			15.7
Brandenburg	*87*	0%	0.4	0.5	5.6	10.0	0.6	0.8	0.3	0.7		0.5	0.9	1.5
Bredeney	*14*	---		0.1	1.5	2.5			0.2	0.9	0.2			
Corvallis	*69*	29%	0.9	1.0					0.2	2.0	0.2	1.5		3.0
Cubana	*7*	---			0.1		2.2		0.2	0.4	0.3			1.5
Derby	*169*	7%	0.5	0.6	16.7	31.2	0.6	1.6	0.3	1.1	0.2	2.0	1.8	
Dublin	*169*	0%	0.5	0.6	0.2		62.6	34.6						
Hadar	*54*	18%	0.4	0.3	0.3				1.8	0.2	2.5	0.5		
Havana	*9*	---	0.03	0.1					0.3				1.8	
Heidelberg	*62*	5%	0.6	0.4	0.9				1.2	1.7	1.1	2.5	1.8	
Indiana	*47*	0%	0.1	0.1	0.1			1.6	1.9	4.4	2.0	7.1		0.7
Infantis	*221*	15%	1.5	1.2	4.1	1.4			7.3	5.4	6.4	8.6	3.7	1.5
Paratyphi B. var. Java	*705*	0%	0.6	0.9	0.1		1.1		46.6	32.7	57.3	40.4	0.9	6.7
Kottbus	*21*	13%	0.3	0.2	0.2			0.8	0.8		0.2		1.8	
Livingstone	*77*	20%	0.2	0.1	6.1	1.4			1.5	2.4	0.8	2.0	4.6	3.7
London	*60*	0%	0.4	0.4	10.9	1.4								
Mbandaka	*82*	17%	0.2	0.4	0.7		0.6	0.8	4.1	2.4	6.1	4.5	0.9	
Minnesota	*54*	---		0.1					1.9	3.5	1.6	6.1		
Molade	*6*	---		0.1					0.2	0.2		0.5	1.8	
Montevideo	*30*	38%	0.3	0.3	0.3		2.8	2.4	0.2	0.2	0.3	0.5		
Newport	*104*	21%	1.3	2.0	0.3				1.2	0.9	0.8	0.5		
Oranienburg	*33*	13%	0.5	0.5					0.1				2.8	
Poona	*24*	33%	0.3	0.4					0.1				1.8	
Rissen	*31*	33%	0.1	0.4	0.9	1.1			0.3	0.9	0.2		0.9	2.2
Senftenberg	*22*	10%	0.3	0.03	0.4		0.6		0.9		0.6		1.8	
Tennessee	*24*	0%	0.2	0.1	0.1			2.4	0.7	0.7	0.5	1.0	3.7	0.7
Goldcoast	*42*	0%	0.2	0.3	6.2	11.1		3.9		0.2				0.7
(Para)Typhi (A B C)	*68*	32%	1.2	1.1										
SI 9,12:NM	*6*	33%	0.1	0.3			1.1	0.8	0.2				1.8	
SI 4,5,12:d:2-	*10*	---	0.03	0.03	2.6	0.7			0.2	0.4	0.3	0.5		
Other	*1029*	18%	15.4	14.0	15.7	3.6	1.1	2.4	11.1	13.1	7.0	6.6	24.7	15.7

Typing results of the Dutch Salmonella Reference Laboratory (RIVM, Bilthoven). Isolates are from different sources and programs. Poultry: all chicken categories together; Broilers: including chicken products; Layers: including reproduction animals and eggs.

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of all isolates respectively, followed by the antigenic monophasic variant of *S*. Typhimurium: *S. enterica* serovar 1,4,5,12:i:-. The contribution of this variant increased from 6.2% in 2008/2009 to 14.4% in 2010/2011.
Major shifts in the occurrence of *Salmonella* serotypes in food animal and humans are regularly seen. Currently the global spread of a multidrug-resistant antigenic monophasic variant of *S*. Typhimurium: *S. enterica* serovar 1,4,5,12:i:-, both in animals and humans is a cause of concern. In the Netherlands, this serotype has shown a sudden, dramatic increase in humans since 2008. Also in animals, mostly pigs and cattle, the relative contribution seems to be increasing although the absolute numbers are much smaller (Table S01)
In pigs, an evident increase in *S*. Derby was apparent (from 16.7% in 2008/2009 to 31.2 in 2010/2011), while traditionally *S*. Typhimurium used to be the most common serotype. Also the monophasic variant of *S*. Typhimurium was considerably more common compared to previous years (increasing from 4.6% of all porcine isolates in 2008/2009 to 15.4% in 2010/2011).
In cattle, *S*. Dublin has for years been the most recovered serovar, but has been surmounted by *S*. Typhimurium (34.6% and 36.2 respectively 2010/2011). Also the rise of the monophasic variant of *S*. Typhimurium should be noted, which covered 10.2% of the bovine isolates as opposed to 3.9% in 2008/2009. Another finding is the presence of *S*. Goldcoast in the top four most prevalent types (3.9%), which was not found in previous years in cattle.
In poultry *S*. Paratyphi B var. Java (*S*. Java) was again the most predominant serovar in broilers, in which species it represented 40.4% of all isolates. The most common serovar in laying hens was *S*. Enteritidis (31.3%).
A notable finding in laying hens was the appearance of *S*. Braenderup in 2010/2011, consisting of 15.7% of the isolates. This serovar was also frequently detected in poultry in 1998 to 2000, but has not been recovered in the period in-between for no apparent reason.
Depending on the sero/phagetype, travel contributed up to 38% of the cases of human salmonellosis from 2008 onwards. This substantial contribution was noted for *S*. Montevideo, but also for a number of other serovars about one third of the cases was travel related (*S*. Enteritidis Pt6; *S*. Poona; *S*. Rissen; *S*. SI 9,12:NM; and typhoidal *Salmonella* cases). 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.

3.1.1.2 Resistance levels

Antimicrobial susceptibility testing was performed on 4404 isolates. Table S02 presents MIC-distributions and resistance percentages of all *Salmonella*'s tested for susceptibility in 2010/2011 (n = 4404). Highest levels of resistance were observed for tetracycline, sulfamethoxazole, ampicillin, streptomycin and to a lesser extend trimethoprim, ciprofloxacin, and nalidixic acid.

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, 12.6% of *Salmonella* isolates demonstrated a non-wild type phenotype for ciprofloxacin, while 1.1% showed MICs larger than the clinical breakpoint (1 mg/l). The serovars of these ciprofloxacin resistant isolates were predominantly travel related *S*. Kentucky (67%) or *S*. Java (12%) in poultry. The remaining 20% consists of a diversity of serovars merely represented by a single strain.

ESBL

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 2010/2011, the total number of cefotaxime reduced susceptible (MIC > 0.5 mg/l) ESBL suspected *Salmonella* isolates was 52 (1.2%), among 17 different serovars. A substantial part of the isolates belonged to the serovar *S*. Java (19 of 52 isolates). Of the *S*. Java isolates for which information on the origin was available, all but one were recovered from poultry. A single isolate was recovered from a human sample, with a resistance profile distinctive from the multiresistant poultry type. In total, 11% of *S*. Java isolates were suspected ESBL producers. Remarkably, among *S*. Heidelberg this percentage was even higher as seven of the 21 isolates (33%) that were tested were cefotaxime resistant, all from poultry origin. Of all the ESBL producing *Salmonella* isolates, 25% were recovered from human samples, 52% from poultry or poultry products, 6% from meat or meat products (other than poultry), and 6% were from other food samples. For 10% the source was unknown. Resistance against cefotaxime in isolates from poultry is associated with transfer of ESBLs between *E. coli* and *Salmonella* in the GI-tract of Dutch poultry (see appendix 1).

Resistance profiles vary considerably among serovars as shown in Table S03. This table presents resistance percentages for the thirteen most prevalent serovars isolated in the Netherlands in 2010/2011. Highest resistance levels are observed in *S*. Typhimurium, the monophasic *S. enterica* serovar 1,4,[5],12:i:-, *S*. Java, and to a lesser extend in *S*. infantis.
Generally, *S*. Typhimurium has acquired resistance against a number of antimicrobials. The most common resistance pattern was ASTSuCipNalFC. In addition, ESBL producing strains (cefotaxime reduced susceptible with MIC > 0.5 mg/l) amounted for 0.5% of the isolates. The monophasic *S*. Typhimurium 1,4,5,12:i:- typically has a multidrug resistance phenotype, with resistance

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against amoxicillin, streptomycin, sulfamethoxazole, and tetracycline, referred to as resistance type ASSuT.
In *S*. Java characteristic findings are high level resistance against trimethoprim which is characteristic of the clone, in combination with acquired resistance against the quinolones and third generation cephalosporins cefotaxime and ceftazidime.

_S_. Typhimurium
As shown in Table S01, *S*. Typhimurium represented 28.1% of all human *Salmonella* isolates as characterized by the RIVM in 2010/2011. Also 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. It is also present in poultry, although to a lesser extent.
Resistance in *S*. Typhimurium was very high for ampicillin, tetracycline and sulphonamides and high for streptomycin (Table S02), which is also related to the frequent occurrence of the monophasic *S*. Typhimurium variant. Resistance to the fluoroquinolones and third generation cephalosporins, regarded as clinically important drugs in human medicine, was moderate (ciprofloxacin and nalidixic acid) or low (ceftazidime). Resistance to chloramphenicol and florfenicol was common.
In previous years, resistance patterns for different phage types were presented. However, at the RIVM phage typing has in 2010 been replaced by MLVA typing as the routine typing system. For this reason, in this report data on the different subtypes of *S*. Typhimurium are no longer presented.
Generally, the typical resistance patterns for *S*. Typhimurium (ASTSuCipNalFC) is irrespective of phage type and also evident when isolates are classified according to host species. Nevertheless, some variation is evident for the different antimicrobials tested as shown in Table S04. In addition, ESBL resistance is occasionally present in isolates recovered from human samples and in samples from poultry origin, but has not been detected in cattle and swine.
With regard to trends, resistance levels in *S*. Typhimurium isolates from human samples appear to increase over the years. This is probably influenced by the emergence and spread of multidrug resistant clones like DT104 (2008, concerning a foodborne outbreak of which the origin was suspected to be abroad) and the recent upswing 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. The trend analysis is further affected by the differences in proportion of multi drug resistant phage types per category and per year. In this respect, especially the results in poultry isolates (i.e. the apparent decrease in resistance levels for a number of antimicrobials in 2011 compared to 2010) should be interpreted with caution.

***S. enterica* serovar 1,4,5,12:i:- (monophasic *S.* Typhimurium 1,4,[5],12:i:-)**
The number of *S. enterica* serovar 4,[5],12:i:- isolates has increased dramatically over the last years, not only in the Netherlands, but also in other EU member states (EFSA, EU summary report on antimicrobial resistance, 2010). *S. enterica* serovar 4,[5],12:i:- is considered a monophasic variant of serovar Typhimurium (4,[5],12:i:1,2) due to antigenic and genotypic similarities between the two serovars. Pigs have been suggested as a likely source of infection as cases of infection have been particularly linked to pigs and pork products. Interestingly, the antibiotic susceptibility profiles of the *Salmonella* 4,5,12:i:– isolates recovered around the world show considerable variation. In general, isolates from Europe appear to harbour more resistance traits as opposed to isolates from North and South America and Asia. Dutch strains also show a multidrug resistance phenotype, with typically resistance against ampicillin, streptomycin, sulfamethoxazole, and tetracycline, referred to as resistance type ASSuT. (Table S03).

_S._ Enteritidis
In the Netherlands, human infections caused by *S*. Enteritidis are predominantly related to the consumption of raw shell eggs. However, the difference in phage types isolated from Dutch broilers and humans and the moderate resistance of strains from human infections compared to the lack of resistance in Dutch layers indicates that other sources of infection exist. These are considered to be consumption of imported eggs and travel abroad (Table S01).
In Dutch poultry the prevalence of *S*. Enteritidis is substantially lower than *S*. Java (12.9% and 32.7% respectively of all galline *Salmonella* isolates) as shown in Table S01. Although *S*. Enteritidis prevalence varies over the years, it is traditionally higher in layers than in broiler chickens. In 2010/2011, *S*. Enteritidis was by far the most common serotype, consisting of 31.3% of all *Salmonella* isolates recovered from layers. In broilers, *S*. Enteritidis covered 8.6% of all *Salmonella* isolates and was, together with *S*. Infantis, the second most prevalent serotype.
In Table S05, resistance percentages for *S*. Enteritidis are specified according to host and most prevalent phage types. As in previous years, Pt4 was the most frequently recovered *S*. Enteritidis phage type among human isolates (9.9%). Other common phage types were Pt8 (5.0 %), Pt1 (4.4%), Pt21 (2.4%), Pt6 (1.6%), and Pt14b (1.1%), together representing 87.5% of all *S*. Enteritidis isolates examined. Among *S*. Enteritidis isolated from Dutch poultry, the most commonly found phage types were Pt4, Pt8 and Pt 21, with slightly different relative prevalences in layers as opposed to broilers.

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Table S02. MIC distribution (in %) and resistance percentages (R%) for all Salmonella's (N = 4404) tested for antibiotic susceptibility during 2010 and 2011.

Salmonella N = 4404	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin						1.0	35.1	31.5	2.9	0.2	0	0	29.2						29.5	28.1 - 30.8
Cefotaxime			31.5	55.9	10.3	1.1	0	0	0	1.1									1.2	0.8 - 1.5
Ceftazidime					63.5	32.1	3.0	0.2	0.2	0.1	0.2	0.6							1.1	0.8 - 1.4
Gentamicin					31.7	55.7	9.5	1.5	0.1	0.2	0.7	0.3	0.2						1.5	1.1 - 1.9
Kanamycin									95.7	2.6	0.3	0.1	0	0	1.2				1.7	1.3 - 2.1
Streptomycin								5.5	20.3	26.6	16.6	7.7	4.3	3.5	15.4				23.3	21.9 - 24.5
Tetracycline							4.5	59.0	5.9	0.7	0.2	2.4	3.5	23.9					30.0	28.5 - 31.3
Sulfamethoxazole										49.7	16.8	3.0	0.3	0.1	0	0	0.1	29.9	30.0	28.6 - 31.3
Trimethoprim						87.1	1.4	0	0	0	0	0	11.4						11.5	10.5 - 12.4
Ciprofloxacin	27.5	57.8	2.0	1.5	6.0	2.5	1.4	0.3	0	0.2	0.5								12.6	11.5 - 13.5
Nalidixic acid									81.3	5.8	1.2	0.4	0.1	11.1					11.6	10.6 - 12.5
Chloramphenicol								0.1	7.1	77.7	7.7	0.2	0.2	6.9					7.4	6.5 - 8.1
Florfenicol								0.6	43.1	47.2	3.0	3.0	2.1	1.0					6.1	5.3 - 6.8

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the epidemiological cut-off values we used to calculate the resistance percentages, the dashed bars indicate clinical breakpoints.

Table S03. Resistance (%) of the thirteen most prevalent Salmonella serovars isolated in the Netherlands in 2010 and 2011.

	Enteritidis (948)	Typhimurium (1008)	serovar 1,4,[5],12:i:- (459)	Paratyphi B var Java (166)	Derby (99)	Cubana (91)	Infantis (91)	Dublin (75)	Senftenberg (71)	Mbandaka (70)	Newport (69)	Livingstone (54)	Brandenburg (52)
Ampicillin	3.7	59.2	91.7	51.2	6.1	0	8.8	4.0	2.8	4.3	15.9	1.9	1.9
Cefotaxime	0.1	0	0.9	11.4	0	0	2.2	0	1.4	2.9	1.4	0	0
Ceftazidime	0.1	0.4	0.9	10.8	0	0	2.2	0	1.4	2.9	1.4	0	0
Gentamicin	0.1	0.6	1.5	3.6	0	1.1	0	0	0	0	5.8	1.9	0
Kanamycin	0.2	1.0	1.7	10.2	1.0	1.1	6.6	0	0	0	5.8	0	0
Streptomycin	0.5	42.5	91.7	27.1	5.1	0	6.6	6.7	1.4	2.9	7.2	9.3	3.8
Tetracycline	1.6	62.5	92.4	19.9	8.1	1.1	23.1	4.0	1.4	0	14.5	3.7	11.5
Sulfamethoxazole	1.3	59.5	92.0	53.0	6.1	0	25.3	2.7	1.4	0	11.6	5.6	5.8
Trimethoprim	0.7	23.6	6.1	81.3	4.0	0	14.3	0	1.4	0	7.2	0	3.8
Ciprofloxacin	9.2	16.0	2.4	50.6	2.0	0	23.1	4.0	1.4	0	14.5	1.9	0
Nalidixic acid	9.3	15.3	1.7	49.4	0	0	23.1	4.0	0	0	13.0	1.9	0
Chloramphenicol	0.2	26.1	4.1	1.8	0	0	5.5	1.3	2.8	0	5.8	1.9	0
Florfenicol	0	24.2	1.3	0	0	0	1.1	0	1.4	0	5.8	0	0

Compared to other *Salmonella* serovars, resistance in *S.* Enteritidis are generally low. Highest levels are seen for the quinolones, showing some variation between phage types as shown in Table S05. Most extensive levels are observed in Pt1 and Pt14b (31.3% and 23.5% respectively), both of which are travel related in humans. 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 phage 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.

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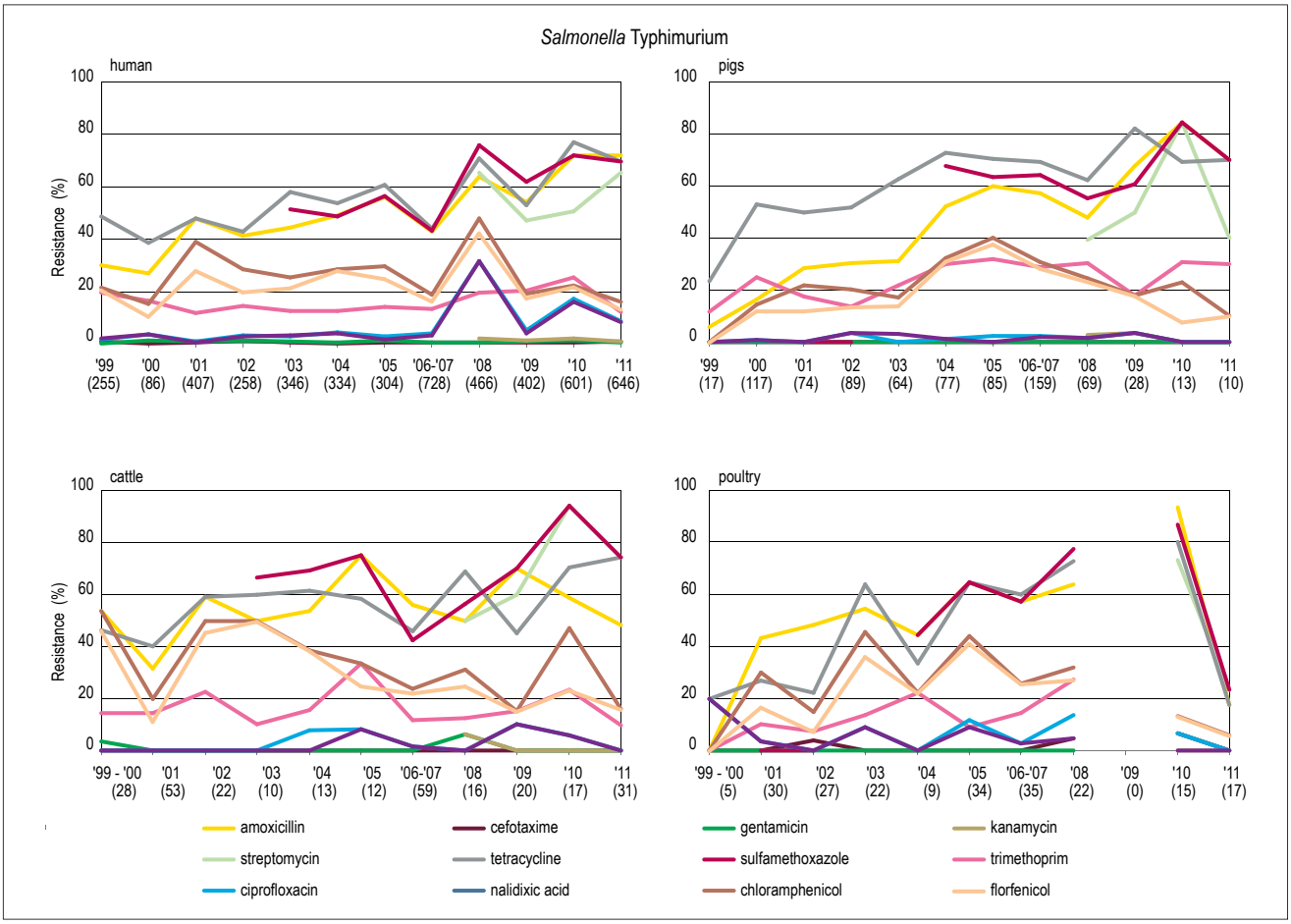

Figure S01. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals from 1999 – 2011.

Table S04. Resistance percentages of S. Typhimurium isolated from different sources in 2010/2011.

	S. Typhimurium			
	humans (1247)	cattle (48)	pigs (23)	poultry (32)
Ampicillin	71.9	52.1	78.3	53.1
Cefotaxime	0.6	0	0	3.1
Ceftazidime	0.6	0	0	3.1
Gentamicin	0.8	0	0	3.1
Tetracycline	73.3	72.9	69.6	46.9
Sulfamethoxazole	70.7	81.3	78.3	53.1
Trimethoprim	18.3	14.6	30.4	3.1
Ciprofloxacin	12.7	2.1	0	3.1
Nalidixic acid	11.9	2.1	0	0
Chloramphenicol	18.9	27.1	17.4	9.4
Florfenicol	17.2	18.8	8.7	9.4
Streptomycin	58.2	81.3	65.2	46.9
Kanamycin	1.4	0	0	0

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

As in previous years, in 2010/1011 *S.* Java was the most predominant serovar isolated in broiler production. Roughly one third of all *Salmonella* strains isolated from poultry were identified as such (Table S01, Figure S03). From poultry 138 *S.* Java strains were isolated. With the exception of five isolates, all harboured the phenotype typical for the clone, which is characterized by high level resistance against 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 (59.4%) and nalidixic acid (58.0%); Resistance to cefotaxime (ESBL-producers) was detected in 13.0% of the isolates from poultry, which is slightly less than in previous years (22.9% in 2009 and 20.9 in 2008). A number of *S.* Java strains were isolated from human infections in 2010 (10) and 2011 (18). All but two strains were trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related.

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Table S05. Resistance (%) of S. Enteritidis isolated from different sources and phage types 4, 8, 1, 21, 6, and 14b in 2010/2011.

	Total (948)	Humans (842)	Layers (35)	Other poultry (22)	Pt4 (289)	Pt8 (164)	Pt1 (134)	Pt21 (87)	Pt6 (54)	Pt14b (34)
Ampicillin	3.7	3.8	0	4.5	1.0	0.6	6.7	9.2	3.7	0
Cefotaxime	0.1	0.1	0	0	0	0	0	0	0	0
Ceftazidime	0.1	0.1	0	0	0	0	0	0	0	0
Gentamicin	0.1	0.1	0	0	0	0	0	1.1	0	0
Kanamycin	0.2	0.2	0	0	0	0	0	0	0	0
Streptomycin	0.5	0.4	0	4.5	0.7	0	0.7	1.1	0	0
Tetracycline	1.6	1.8	0	0	0	0	0.7	0	3.7	0
Sulfamethoxazole	1.3	1.1	0	9.1	1.4	0	0.7	1.1	1.9	0
Trimethoprim	0.7	0.7	0	0	0	0	0.7	1.1	0	0
Ciprofloxacin	9.2	9.7	2.9	13.6	3.1	3.7	31.3	6.9	11.1	23.5
Nalidixic acid	9.3	9.9	2.9	13.6	3.1	3.7	31.3	6.9	11.1	23.5
Chloramphenicol	0.2	0.2	0	0	0.3	0	0	0	1.9	0
Florfenicol	0	0	0	0	0	0	0	0	0	0

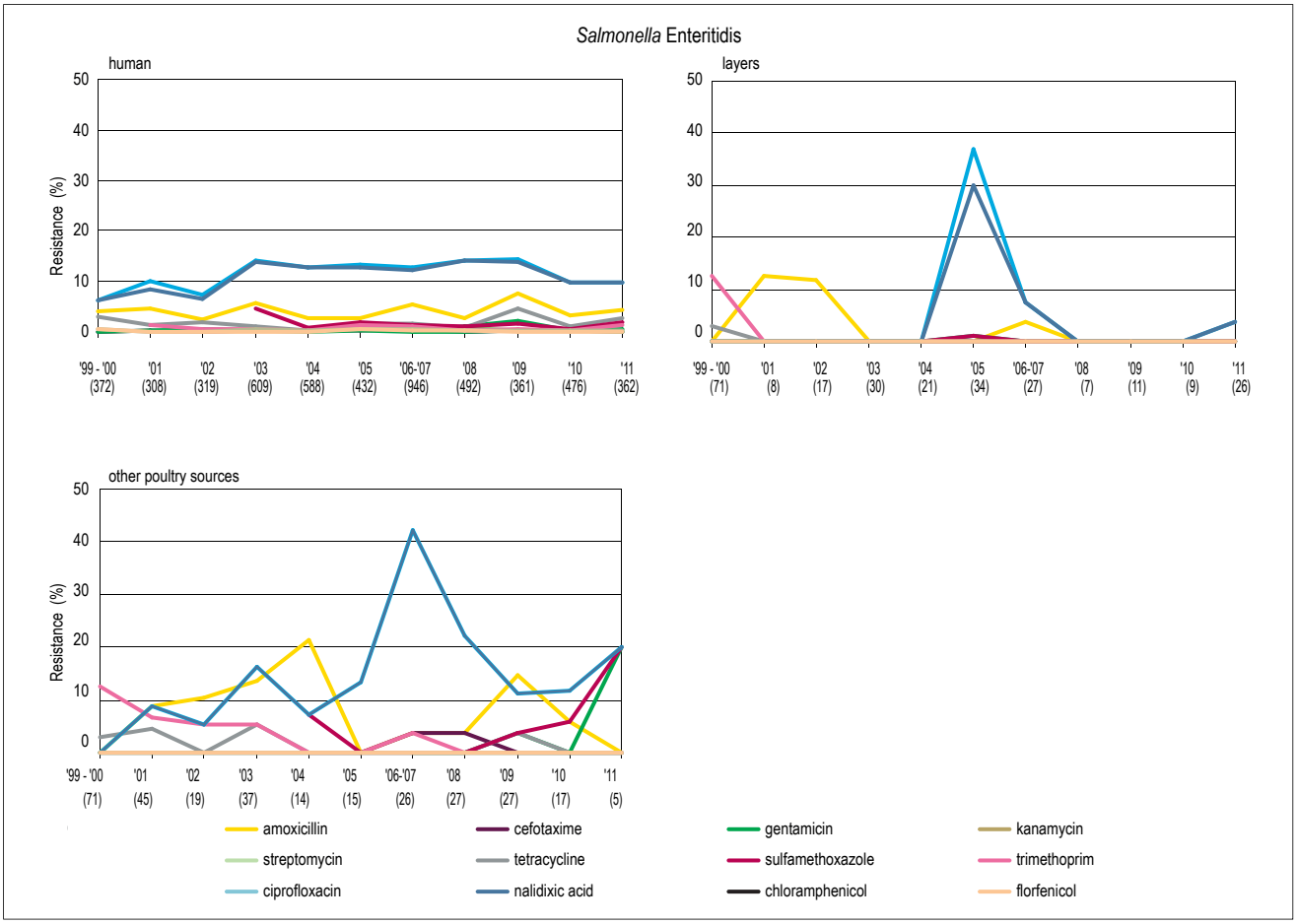

Figure S02. Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other poultry sources from 1999 – 2011.

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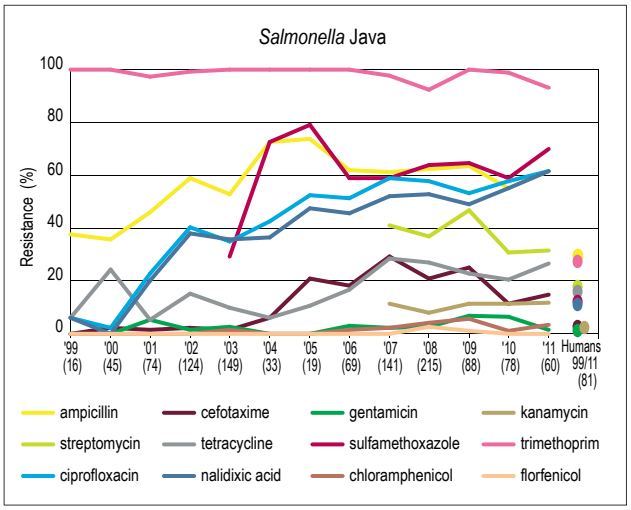

Figure S03. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2011 and humans (Separate data on the right indicate all human *S.* Java isolates from 1999 – 2011).

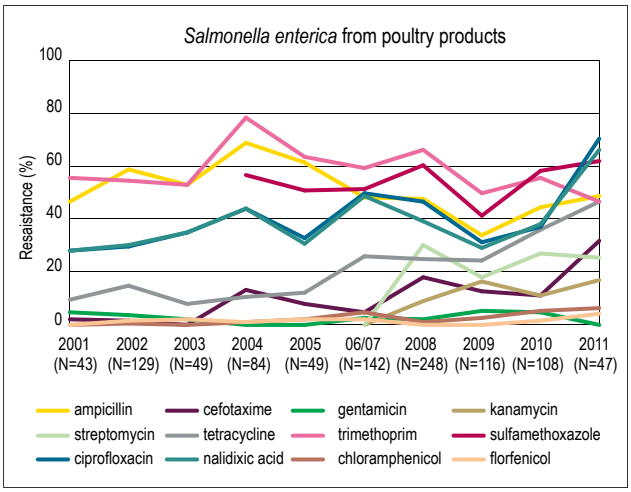

Figure S04. Trends in resistance (%) of *Salmonella* enterica isolated from poultry meats in the Netherlands from 2001 – 2011.

3.1.1.3 Salmonella in raw meats at retail

Resistance data are presented for poultry meat only, because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Table S06). As expected, in poultry meat samples *S.* Java was the most prevalent *Salmonella* serovar encountered (49%). Other serovars regularly included were *S.* Infantis (9%), *S.* Minnesota (8%), *S.* Heidelberg (5%), *S.* Enteritidis (5%) and *S.* Typhimurium (3%). 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 *S.* Heidelberg, *S.* Minnesota, *S.* Infantis and S. Mbandaka. Also resistance to the quinolones was relatively high, which mainly can be attributed to the presence of *S.* Heidelberg and *S.* Infantis, the relatively high level of kanamycin resistance to *S.* Minnesota. From "exotic meat", 19 *Salmonella* serovars were isolated which are infrequently seen. Only two serovars isolated from crocodile meat show resistance, to streptomycin, sulfamethoxazole, tetracycline and trimethoprim. All other isolates, mainly from kangaroo meat were fully susceptible for the antibiotics tested. 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.

Table S06. Resistance (%) of Salmonella enterica isolated from raw meats from poultry, and other raw meat sources in 2010/2011.

	poultry meat *S.* Java N = 76	poultry meat other serovars N = 79	other raw meat sources N = 116
Ampicillin	59.2	32.9	34.5
Cefotaxime	18.4	16.5	3.4
Ceftazidime	18.4	16.5	2.6
Gentamicin	5.3	1.3	2.6
Kanamycin	10.5	15.2	4.3
Streptomycin	38.2	15.2	28.4
Tetracycline	28.9	49.4	40.5
Sulfamethoxazole	64.5	54.4	40.0
Trimethoprim	100	7.6	14.7
Ciprofloxacin	68.4	26.6	18.1
Nalidixic acid	65.8	27.8	18.1
Chloramphenicol	2.6	8.9	16.4
Florfenicol	2.6	2.5	13.8

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3.1.1.4 Salmonella in other European countries

The annual report on trends and sources of zoonoses, issued by the European Food Safety Authority and the European Centre for Disease prevention and Control summarizing data from 27 EU member states, presents data on the occurrence of zoonoses and foodborne outbreaks as well as on antibiotic resistance in these agents. Data from 2010 show that 99,020 salmonellosis cases in humans with a decreasing trend in case numbers over the years (assumed to be due to successful *Salmonella* control programmes in poultry, in particular vaccination against *S*. Enteritidis). In foodstuffs, *Salmonella* was most often detected in fresh broiler and turkey meat (The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010, EFSA Journal 2012;10(3):2597; http://www.efsa.europa.eu/en/efsajournal/pub/2597.htm).

As in previous years, *S*. Enteritidis (45.0%) and *S*. Typhimurium (22.4%) were the two most commonly reported *Salmonella* serovars of all reported confirmed human cases in 2010 in reporting member states. Monophasic *S*. Typhimurium 1,4,5,12:i:- entered the top 10 group as the fourth most commonly reported serovar. Monophasic *S*. Typhimurium was the second most commonly reported serovar in pigs and third most commonly reported serovar in cattle as well as in pig and bovine meat, following *S*. Typhimurium and *S*. Derby in pigs and *S*. Typhimurium and *S*. Dublin in cattle. EFSA data suggest that monophasic *S*. Typhimurium are increasing in poultry and poultry products and that the current tetracycline-resistant epidemic strain has spread beyond his initial porcine source in many countries since its emergence in 2006.

It should be noted that the methods and breakpoints used for *Salmonella* antimicrobial susceptibility testing differed somewhat between member states. Most countries used clinical breakpoints as provided by the Clinical and Laboratory Standards Institute (CLSI) for the interpretation of test results, whereas in this report mostly epidemiological cut-off values provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have been used. For chloramphenicol, nalidixic acid, sulfonamides and tetracycline, the CLSI and EUCAST breakpoints/cut-offs are equivalent, whereas for the remaining antimicrobials they might differ markedly, hampering comparison between countries. Although disparities in resistance were frequently observed between different member states, in general high resistance levels were recorded to ampicillin, tetracyclines and sulfonamides in *Salmonella* isolates from humans, whereas resistance to third-generation cephalosporins and fluoroquinolones remained low. In *Salmonella* isolates from fowl, pigs, cattle and meat thereof, resistance to tetracyclines, ampicillin and sulfonamides was also commonly detected, whereas resistance to third-generation cephalosporins was low.

Moderate to high levels of ciprofloxacin resistance were observed in *Salmonella* isolates from turkeys, fowl and broiler meat (The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. EFSA Journal 2012;10(3):2598; http://www.efsa.europa.eu/en/efsajournal/pub/2598.htm)

Compared to the reported data from other EU member states, resistance percentages of Dutch *Salmonella* isolates showed no exceptional differences.

3.1.2 *Campylobacter*

This chapter 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, slaughter pigs, veal calves and dairy cows), 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).

Highlights

1. In veal calves and poultry meat, a very high proportion of *Campylobacter* is resistant to ciprofloxacin (ranging from 58.7% in *C. jejuni* to up to 80.6% in *C. coli*).
2. Low levels were observed for erythromycin and gentamicin, with the exception of erythromycin resistance in *C. coli* (25%).
3. High resistance was also recorded for commonly used antimicrobials such as ampicillin and tetracyclines.
4. Similarly, a high proportion of *Campylobacter* in humans is resistant to the critically important antimicrobial ciprofloxacin whereas low resistance was recorded for another critically important antimicrobial, erythromycin.

3.1.2.1 Resistance levels

In Table C01 the MIC-distributions and resistance percentages are summarized for all *Campylobacter jejuni* and *C. coli* strains isolated from broilers, pigs, veal calves and dairy cows in 2010 and 2011. 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.

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Table C01. MIC distribution (in %) for all Campylobacter jejuni (N = 448, of which 201 from broilers, 132 from veal calves, 77 from dairy cows, and 38 from other sources) and C. coli (N = 454, of which 262 from pigs, 150 from veal calves, 39 from broilers, and 13 from other sources).

C. jejuni (N = 448)	MIC (%) distribution mg/L															R%	95% CI
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin			0.2	0.7	4.9	29.0	23.4	5.8	4.9	31.0						41.7	37.3-46.4
Gentamicin		90.0	6.5	3.1		0.2				0.2						0.4	0-1.1
Neomycin			88.4	4.2	1.1		1.1	1.6	2.2	0.7	0.7					7.4	5.1-9.8
Streptomycin				92.4	2.5	0.9	0.9	1.6	0.4	0.2		1.1				5.1	3.1-7.4
Tetracycline			35.0	4.9	3.6	0.9	0.4	0.9	2.2	9.2	42.9					56.5	51.8-60.9
Sulfamethoxazole							1.1	9.2	20.1	36.4	24.8	5.4	1.6	1.1	0.4	3.1	1.6-4.9
Ciprofloxacin	42.9	5.4	1.8	0.9	0.9	0.7	17.9	17.9	11.8							49.1	44.4-53.8
Nalidixic acid					6.3	29.9	12.9	2.7	0.7	0.7	13.8	33.0				48.2	43.5-52.9
Erythromycin			25.2	48.0	19.2	6.7	0.7		0.2							0.9	0.2-1.8
Clarithromycin			17.9	38.8	29.7	10.7	2.7		0.2							0.2	0-0.7
Tulathromycin			83.5	12.5	2.2	0.7	0.2	0.4	0.2		0.2					0.4	0-1.1
Chloramphenicol					37.1	44.0	12.7	5.6	0.4	0.2						0.7	0-1.6

C. coli (N = 454)	MIC (%) distribution mg/L															R%	95% CI
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin		0.4	0.4	0.2	2.8	10.6	29.3	26.1	10.3	19.8						30.2	26.1-34.5
Gentamicin		35.8	58.0	3.9	1.1				0.4	0.9						1.3	0.4-2.4
Neomycin			53.2	33.0	2.8	1.3	0.6	0.2	0.4	0.9	7.5					11.0	8.2-14
Streptomycin				23.7	3.7	1.9	1.3	24.6	31.0	6.3	1.1	6.5				70.7	66.6-74.8
Tetracycline			8.4	3.4	1.7	0.4	0.6	0.4	1.5	3.9	79.5					86.4	83.2-89.4
Sulfamethoxazole							9.7	15.7	12.7	9.1	2.4	3.7	23.5	17.2	6.0	46.8	42.2-51.3
Ciprofloxacin	39.4	17.2	1.9	0.2		2.6	11.4	17.7	9.5							41.2	36.6-45.7
Nalidixic acid				0.2	0.2	20.7	31.9	5.4	0.2	0.4	17.5	23.5				41.4	36.9-45.9
Erythromycin			3.4	13.6	30.6	21.3	5.2	0.9	0.4	1.1	23.5					25.0	21.1-29.1
Clarithromycin			5.2	8.6	29.5	23.7	6.7	1.3	1.3	0.4	23.3					23.7	19.8-27.6
Tulathromycin			47.4	21.6	4.1	1.3	0.4	0.2	3.2	6.9	14.9					25.0	21.1-29.1
Chloramphenicol					4.7	36.4	47.4	9.5	1.7			0.2				1.9	0.9-3.2

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

National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure C03, and Tables C03 and C04. 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.

In 2010/2011 high resistance levels in *C. jejuni* from animals are seen for tetracycline (56.5%), the quinolones ciprofloxacin and nalidixic acid (49.1% and 48.2% respectively), and ampicillin (41.7%). Resistance levels in *C. coli* are traditionally higher compared to *C. jejuni*, with very high levels of resistance for tetracycline (86.4%) and streptomycin (70.7%). However resistance is also commonly seen for sulfamethoxazole (46.8%), ciprofloxacin and nalidixic acid (41.2% and 41.4% respectively), ampicillin (30.2%), as well as for the macrolides (erythromycin, tulathromycin, clarithromycin).

Quinolones

For years, there is an increasing trend in the percentage of isolates resistant to the quinolones, both in strains

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Table C02. Resistance percentages of Campylobacter jejuni and C. coli isolated from raw meat from poultry and from faecal samples of broilers, veal calves, dairy cows (only C. jejuni) and pigs (only C. coli) in 2010/2011.

	C. jejuni				*C. coli*			
	poultry products	broilers	veal calves	dairy cows	poultry products	broilers	veal calves	pigs
N	254	201	132	77	103	39	150	262
Ampicillin	53.5	59.2	30.3	7.8	42.7	30.8	41.3	23.3
Gentamicin	0	0.5	0.8	0	1.0	0	4.0	0
Neomycin	2.4	1.0	19.7	3.9	8.7	2.6	27.3	3.4
Streptomycin	3.1	2.5	10.6	0	13.6	15.4	72.7	79.8
Tetracycline	47.6	48.8	84.8	15.6	76.7	48.7	96.7	87.4
Sulfamethoxazole	2.8	4.5	1.5	0	18.4	20.5	43.3	54.2
Ciprofloxacin	58.7	60.7	50.8	15.6	80.6	66.7	86.7	9.5
Nalidixic acid	58.7	58.7	51.5	16.9	80.6	66.7	86.7	9.9
Erythromycin	1.6	1.0	1.5	0	32.0	7.7	30.0	24.0
Tulathromycin	1.6	0	1.5	0	32.0	7.7	30.0	24.0
Clarithromycin	1.6	0.5	0	0	31.1	2.6	30.0	22.9
Chloramphenicol	1.2	0	0.8	0	1.0	0	4.7	0

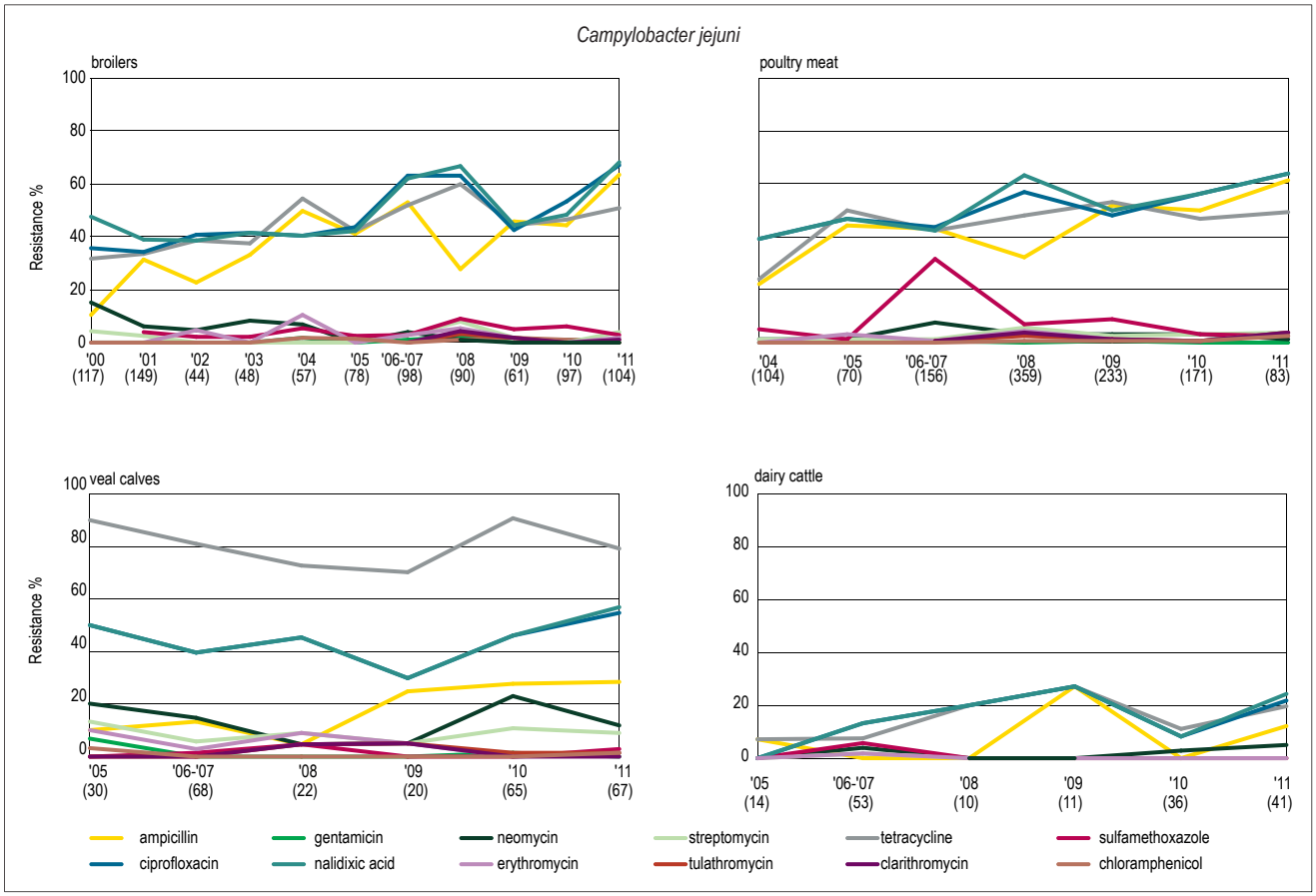

Figure C01. Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers, poultry meat, veal calves and dairy cattle in recent years in the Netherlands.

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Figure C02. Trends in resistance (%) of *Campylobacter coli* isolated from broilers, poultry meat, slaughter pigs and veal calves in recent years in the Netherlands.

Table C03. Percentage Campylobacter jejuni and C. coli isolates from humans resistant against fluoroquinolones, tetracycline and erythromycin from 2002 to 2011.

	percentage of resistant Campylobacter isolates					
	2002/05	2007	2008	2009	2010	2011
Fluoroquinolones	35.2	45.2	50.5	51.4	53.3	57.0
Tetracycline	20.2	23.9	17.2	20.3	22.1	24.8
Erythromycin	1.5	2.9	2.4	2.6	2.7	3.7

Table C04. Domestically acquired and travel related resistance in C. jejuni and C. coli isolated from humans from 2002 - 2011 from all 16 PHLS covering >50% of the Dutch population.

	2009-2011								2002-2005							
	C. jejuni				*C. coli*				*C. jejuni*				*C. coli*			
	Domestically acquired		Travel related		Domestically acquired		Travel related		Domestically acquired		Travel related		Domestically acquired		Travel related	
	R%	(n)	R%	(n)	R%	(n)	R%	(n)	R%	(n)	R%	(n)	R%	(n)	R%	(n)
Fluoroquinolones	53.1	(8673)	67.2	(454)	51.1	(630)	56	(50)	32.7	(6792)	53.5	(600)	36.3	(386)	50	(56)
Tetracycline	20.5	(5164)	34.4	(90)	34.7	(398)	40	(10)	18.5	(5028)	27.1	(425)	22.7	(353)	20.4	(49)
Erythromycin	2.4	(7339)	4.4	(338)	8.1	(540)	14.3	(35)	1.2	(5735)	1.6	(511)	3.0	(372)	0	(52)

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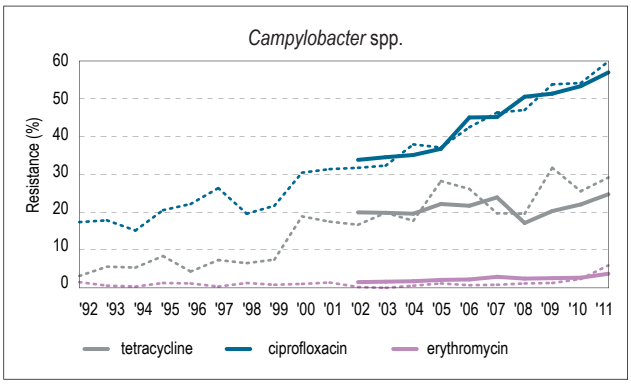

Figure C03. Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2011 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900 – 2900.

from animal origin (Figure C01) as in those from human patients (Figure C02). This is a worrisome development as ciprofloxacin is the second-choice drug for treatment of campylobacteriosis and resistance evolves rapidly, not only in the Netherlands, but worldwide. In 2010 and 2011, 60.7% of *C. jejuni* and 66.7% of *C. coli* isolates from broilers were resistant, and 57% of the human *Campylobacter* isolates.

Macrolides

Erythromycin or other suitable macrolides, are the first-choice drugs for the treatment of campylobacteriosis in humans. The level of resistance for erythromycin reported in animals and humans is low for *C. jejuni*, on average 0.9% in strains from animal origin in 2010 and 2011(n=448) and 2.4% in human isolates from 2009-2011 (n=7339). It should be noted that for human isolates more sensitive breakpoints for resistance have been applied (≥ 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* levels are much higher and importantly, seem to be steadily increasing in recent years (Figure C01) in isolates from animals. In veal calves, levels as high as 30% and 24% in pigs (Table C02) are noted.

3.1.2.1.1 Broiler chickens and poultry meat

In *Campylobacter* from poultry, resistance profiles for isolates recovered from animals as well as from meat samples are available. *Campylobacter* is seldom isolated from other animal meat, except turkey. As shown in Table C02, high levels of resistance are observed for the quinolones, tetracycline and ampicillin, while resistance to the other antimicrobial drugs is (very) low. In general, resistance levels in isolates from both sample categories are comparable. One exception is the higher level of macrolide resistance in *C. coli* from poultry meat (31.1% for clarithromycin, 32.0% for erythromycin and tulathromycin) compared to the percentages observed in caecal samples from poultry taken at abattoirs (2.6% for clarithromycin, 7.7% for erythromycin and tulathromycin). This might be due to the relatively low numbers of isolates tested or to possible bias in sampling.

3.1.2.1.2 Pigs

In *C. coli* from pigs, highest resistance levels were observed for tetracycline (87.4%), followed by streptomycin (79.8%), and sulfamethoxazole (54.2%). Resistance to nalidixic acid and ciprofloxacin was relatively low (9.9% and 9.5% respectively) compared to levels in broilers (66.7%) and veal calves (86.7%), probably reflecting the low use of quinolones in swine. Resistance to macrolides occurred fairly common with 22.9-24% of the isolates resistant. Over the last 5-10 years, levels have remained relatively stable.

3.1.2.1.3 Veal calves

Data for both *C. jejuni* and *C. coli* isolated from veal calves are included in this report. *C. coli* isolates were more resistant than *C. jejuni* for all antimicrobial drugs included in the test panel. In both species highest levels were observed for tetracycline (84.8% in *C. jejuni* and 96.7% in *C. coli*) and ciprofloxacin and nalidixic acid (50.8% and 51.5% respectively in *C. jejuni* and 86.7% in *C. coli*). The high level of macrolide resistance (30%) is comparable to that observed in pigs. There is also a strong tendency to increase over the last six years.

3.1.2.1.4 Dairy cows

In *C. jejuni* form dairy cattle the highest levels of resistance were observed for the quinolones (15.6% for ciprofloxacin and 16.9% for nalidixic acid) and tetracycline (15.6%). A lower level of resistance was recorded for ampicillin (7.8%) and neomycin (3.9%) while for the other antimicrobials tested no resistance was found.
Due to the relative low number of isolates, the levels over the years show some variation. Nevertheless, no obvious changes have been observed.

3.1.2.2 Campylobacter in humans

Data on resistance levels are available for ciprofloxacin, erythromycin and tetracycline and are summarized in Table C03 and Figure C03. High levels of resistance are observed in 2010 and 2011 for the critically important antimicrobial ciprofloxacin (53.3% and 57.0%) and also resistance to tetracycline was commonly detected (22.1% and 24.8% respectively). Erythromycin resistance, another critically important antimicrobial, was low in both years, although the percentage in 2011 (3.7%) tended to be somewhat higher than in previous years.
The trends as shown in Figure C03 indicate that resistance levels for ciprofloxacin and tetracycline show a constant tendency to increase, most outspokenly for ciprofloxacin.
In Table C04 resistance levels have been specified

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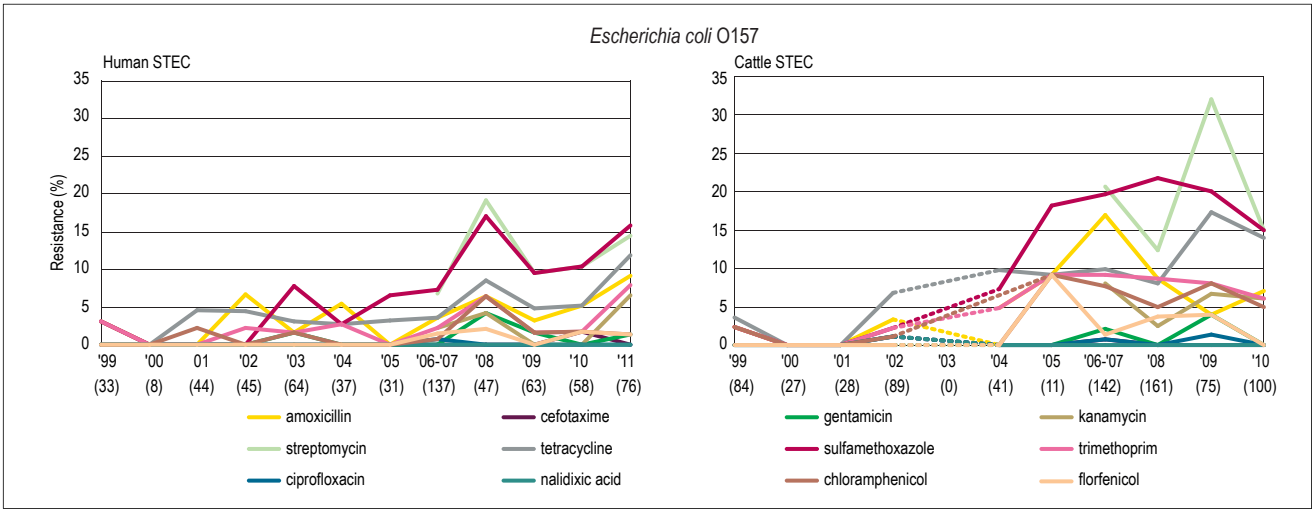

Figure STEC01 Trends in resistance percentages of E. coli O157 (STEC) isolated in The Netherlands from 1999 – 2011.

according to the most probable infection route, i.e. whether the infection was either acquired domestically or abroad. In *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.

3.1.2.3 Campylobacter in other European countries

The EU notification rate of campylobacteriosis has followed an increasing trend since 2006, making it by far the most frequently reported zoonosis in humans in the EU with over 200,000 reported cases in 2010 in 27 countries (The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010, EFSA Journal 2012; 10(3):2597; http://www.efsa.europa.eu/en/efsajournal/pub/2597.htm). In the EU member states, the high occurrence of resistance against ciprofloxacin is of concern, commonly observed in *Campylobacter* isolates from poultry, as well as from pigs and cattle, at levels ranging from 37% to 84%.

Resistance to erythromycin was detected in *Campylobacter* isolates from poultry, poultry meat and pigs at levels of 0.5% to 25%. Among isolates of *Campylobacter* from meat and animals, resistance against ciprofloxacin, nalidixic acid and tetracyclines was commonly detected at levels of 21% to 84%, whereas in general much lower levels of resistance against erythromycin and gentamicin were reported.

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

Highlights

1. In the last five years, resistance levels in Shiga-toxin producing *E. coli* (STEC) from human patients and from cattle have seen in increase, although there seems to be a time lag in trends from human isolates compared to bovine isolates.
2. Resistance levels in cattle are generally higher for streptomycin, tetracycline, sulfamethoxazole, trimethoprim and chloramphenicol. Nevertheless, the similarity in MIC data of STEC from cattle and humans isolated in 2010 has led to the suggestion that cattle, via the food chain, might be a likely source of at least some human infections.
3. Based on MIC profiles, one extended spectrum beta-lactamases (ESBL) suspected isolate was found in humans, but not in cattle.antimicrobial, erythromycin.

In 2010 and 2011, 234 Shiga-toxin producing *E. coli* O157 (STEC) isolates were tested for susceptibility. Isolates were obtained from human patients in 2010 (N=58) and 2011 (N=76), from cattle isolates were available only for 2010 (N=100). MIC results are shown in Table STEC01. Of the bovine isolates, 65 were recovered from veal calves and 35 from dairy cattle.

3.1.3.1 Trends in resistance

Over the last ten years, MIC profiles seem to have a tendency to increase as shown in Figure STEC01. Traditionally, resistance levels in *E. coli* O157 have been very low, both in human as in bovine strains. From 2004 onwards, resistance in cattle strains occurred more commonly. Most striking increases have been noted for

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Table STEC01. MIC distribution (in %) and resistance percentages (R%) for E. coli O157 isolated from humans (N=134) and cattle (N=100) in The Netherlands in 2010 and 2011.

Humans N = 134	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin								17.2	76.1				7.5						7.5	2.9 - 12
Cefotaxime			91.8	7.5						0.7									0.7	0 - 2.2
Ceftazidime					98.5	0.7						0.7							0.7	0 - 2.2
Gentamicin					20.1	58.2	19.4	2.2		0.7									0.7	0 - 2.2
Kanamycin									93.3	3.0	0.7				3.0				3.7	0.4 - 7.0
Streptomycin								3.0	47.8	36.6	0.7	0.7	3.0	1.5	7.5				12.7	6.9 - 18.4
Tetracycline								66.4	24.6	0.7			0.7	8.2					9.0	4 - 13.8
Sulfamethoxazole										85.1	1.5							13.4	13.4	7.5 - 19.3
Trimethoprim						94.0	0.7						5.2						5.2	1.3 - 9.0
Ciprofloxacin	67.2	33.6																	0	0 - 0.03
Nalidixic acid									100.0										0	0 - 0.03
Chloramphenicol									3.7	85.1	10.4			1.5					1.5	0 - 3.5
Florfenicol									17.2	78.4	3.7			1.5					1.5	0 - 3.5

Cattle N = 100	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin									92.0	1.0		7.0							7.0	1.8 - 12.1
Cefotaxime			100.0																0	0 - 0.04
Ceftazidime					99.0	1.0													0	0 - 0.04
Gentamicin					18.0	75.0	6.0	1.0											0	0 - 0.04
Kanamycin									92.0	2.0					6.0				6.0	1.2 - 10.7
Streptomycin									64.0	19.0	2.0	3.0	5.0	3.0	4.0				15.0	7.8 - 22.1
Tetracycline								19.0	67.0				2.0	12.0					14.0	7.0 - 20.9
Sulfamethoxazole										36.0	32.0	17.0						15.0	15.0	7.8 - 22.1
Trimethoprim						92.0	2.0						6.0						6.0	1.2 - 10.7
Ciprofloxacin	46.0	54.0																	0	0 - 0.04
Nalidixic acid									96.0	4.0									0	0 - 0.04
Chloramphenicol										94.0	1.0		5.0						5.0	0.6 - 9.3
Florfenicol									28.0	72.0									0	0 - 0.04

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 cut-off values used as breakpoints. Dashed bars indicate the clinical breakpoints.

tetracycline, sulfamethoxazole and trimethoprim and to a lesser extend for ampicillin, chloramphenicol and florfenicol. Resistance to the aminoglycosides kanamycin and streptomycin is also commonly found.
Likewise a moderate increase can be noted in human isolates from 2006/2007, although in general resistance levels are lower. The similarity in MIC data of Shiga-toxin producing *E. coli* (STEC) from cattle and humans isolated in 2010 has been interpreted as confirmatory data for cattle, via the food chain, being a likely source of at least some human infections (The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010, EFSA Journal 2012;10(3):2598). Although resistance data may provide a powerful tool to investigate and speculate on likely epidemiology of infection, for the confirmation of epidemiological relationships such data should preferably be used in combination with other typing methods.

Beta-lactamases (ESBLs)
In 2010, for the first time resistance to third generation cephalosporins (cefotaxime 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. No ESBL suspected isolates have been recovered from bovine samples in this period, although a cefotaxime and ceftazidime resistance has been recorded in 2006/2007 (among 142 isolates tested).

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3.2 Commensal indicator organisms

This chapter 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 animals (broiler chickens and pigs) 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 monitoring, using the same samples that were taken at farms to determine the prevalence of *Salmonella*, *E. coli* O157 and *Campylobacter*. From 2010 onwards, samples of dairy cattle were obtained at slaughter houses. In 2011, for the first time samples of turkeys (taken at farms), turkey meat, herbs, vegetables and fruits were included in the surveillance.
It should be noted however, that the sampling strategy implies that this method is inherently insensitive for detecting resistance as only one randomly selected isolate is tested from a single sample taken from one animal per epidemiological unit (herd or flock). The total set of selected isolates is intended to represent the *E. coli*, or *Enterococcus* species population of each animal species of the entire country. One per cent resistance in e.g. *E. coli* indicates that in all animals 1% of the *E. coli* bacteria are resistant. Because each animal harbours about 10^6 cfu/g faeces *E. coli* in its gut, 1% would be approximately 10^4 cfu/g faeces. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in relatively small numbers in individual animals.

3.2.1 *Escherichia coli*

In this chapter information is presented on resistance in *E. coli* from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-negative bacteria present in the gastro-intestinal tract of food-producing animals. It should be noted that resistant isolates were defined by using epidemiological cut-off values 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. If available, the epidemiological cut-off values as well as the clinical breakpoints are shown in Table Eco01.

Highlights

1. Among indicator (commensal) *E. coli* isolates 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- and fourth generation cephalosporins, was also observed in the indicator *E. coli* isolates.
2. Reduced susceptibility to ciprofloxacin and nalidixic acid was highest for *E. coli* isolates from broilers (59.4% and 59.6% respectively) and turkeys (31.6% and 27.4% respectively). These high proportions of isolates exhibiting resistance to ciprofloxacin are of concern.
3. Resistance to third-generation cephalosporins was observed in indicator *E. coli* isolates from poultry, pigs and cattle and varied from very low levels in dairy cattle (0.4%) to 13.2% in broiler chickens. More extensive information on ESBL producing *E. coli* in broilers is presented in appendix I, which shows that cefotaxime resistance shows a tendency to decrease from 2009 onwards.

3.2.1.1 Resistance levels

MIC distributions concerning a total of 2098 isolates selected from chicken, pig, cattle, and turkey are summarized in Table Eco01. Trends in resistance levels over time according to host animal species are shown in Figure Eco01 and information on multidrug resistance is shown in Figure Eco02. Additionally, data are presented on 1091 isolates collected and tested by the Dutch Food and Consumer Product Safety Authority. These consist of MIC distributions of *E. coli* isolates from beef, veal, pork, poultry and turkey meat. This year for the first time data are included on *E. coli* isolated from vegetables, fruits and herbs in 2011 (Table Eco02). Trends in resistance of *E. coli* isolated from poultry meat products, beef and pork in the Netherlands from the period 1998 to 2011 are presented in Figure Eco03. Finally, results for the Netherlands are related to the situation in other EU member states.
Table Eco01 presents the MIC distributions and percentages for *E. coli* strains expressing reduced susceptibility from farm animals in 2010 and 2011and specified for broiler chickens, slaughter pigs, veal calves

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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Table Eco01 MIC distribution (in %) and resistance percentages (R%) for E. coli isolated as indicator organism from intestines of broiler chicken (N = 567), slaughter pigs (N=569), veal calves (N=338), dairy cattle (N=529) in The Netherlands in 2010 and 2011 and turkey (N=95) in 2011.

Broilers N = 567	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							0.5	10.6	16.2	1.4	0.2		71.1						71.3	67.4 - 75
Cefotaxime			66.8	17.6	2.3	0.2	0.7	0.4	0.5	11.5									13.2	10.3 - 16
Ceftazidime					80.6	6.5	2.3	2.1	2.6	2.6	2.3	0.9							12.9	10 - 15.6
Gentamicin					2.6	51.3	32.6	5.1	1.1	1.6	3.2	1.4	1.1						8.3	5.9 - 10.6
Kanamycin									77.8	7.6	1.6	0.2			12.9				14.6	11.6 - 17.6
Streptomycin								0.2	7.1	23.3	4.9	6.2	8.6	8.8	40.9				64.6	60.5 - 68.5
Tetracycline							4.9	23.1	15.3	0.9	0.2	0.5	7.2	47.8					55.7	51.5 - 59.9
Sulfamethoxazole										32.6	0.2					0.9	0.2	66.1	67.2	63.2 - 71.1
Trimethoprim						40.6	1.1		0.4				58.0						58.4	54.2 - 62.5
Ciprofloxacin	30.9	8.8	0.9	3.7	32.8	11.8	3.9	0.5	0.4	3.9	2.5								59.4	55.3 - 63.5
Nalidixic acid									39.9	0.2	0.4	1.1	4.2	54.3					59.6	55.4 - 63.7
Chloramphenicol								0.4	6.3	58.6	11.5	3.0	7.1	13.2					23.3	19.7 - 26.8
Florfenicol								0.4	10.6	65.6	19.9	3.2	0.4						3.5	1.9 - 5

Slaughter pigs N = 569	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							2,5	23,2	36,4	3,5		0,2	34,3						34,4	30.4 - 38.4
Cefotaxime			84,4	14,2	0,2	0,4		0,2		0,7									1,2	0.3 - 2.1
Ceftazidime					94,0	4,4	0,9	0,2	0,2		0,4								1,6	0.5 - 2.6
Gentamicin					7,6	51,1	33,4	6,2	1,4	0,4									1,8	0.6 - 2.8
Kanamycin									88,0	8,1	1,6	0,5			1,8				3,9	2.2 - 5.4
Streptomycin								0,2	9,3	24,4	9,5	10,7	10,0	10,2	25,7				56,6	52.4 - 60.7
Tetracycline							4,2	21,1	9,0	0,5	1,2	1,4	12,8	49,7					65,2	61.2 - 69.1
Sulfamethoxazole										45,2	0,2						0,2	54,5	54,7	50.4 - 58.8
Trimethoprim						51,0	0,5	0,4	0,2			0,2	47,8						48,2	43.9 - 52.3
Ciprofloxacin	81,2	17,6	0,2	0,2	0,4	0,4					0,2								1,1	0.1 - 1.9
Nalidixic acid									97,4	1,8	0,2			0,7					0,7	0 - 1.4
Chloramphenicol								0,2	10,0	67,7	10,2	4,2	2,1	5,6					12,0	9.2 - 14.6
Florfenicol								1,2	14,6	71,0	12,1	0,9		0,2					1,1	0.1 - 1.9

Veal calves N = 338	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							1,8	16,9	34,0	1,8	0,3		45,3						45,6	40.1 - 50.9
Cefotaxime			75,1	18,3	4,1	0,9	0,3			1,2									2,4	0.7 - 4
Ceftazidime					91,4	6,5	1,2	0,9											2,1	0.5 - 3.6
Gentamicin					4,1	45,0	32,5	7,4	1,2	0,6	3,0	3,3	3,0						10,9	7.5 - 14.3
Kanamycin									68,9	7,4	0,9	0,6	0,6		21,6				23,7	19 - 28.2
Streptomycin								0,3	13,3	25,1	6,2	4,1	4,7	9,2	37,0				55,0	49.6 - 60.4
Tetracycline							3,8	9,5	13,3	0,3		0,6	10,1	62,4					73,1	68.2 - 77.9
Sulfamethoxazole										45,6								54,4	54,4	49 - 59.8
Trimethoprim						54,4	1,2						44,4						44,4	38.9 - 49.7
Ciprofloxacin	63,0	15,1	0,6	1,8	7,4	3,3	1,2	0,3		0,9	6,5								21,3	16.8 - 25.7
Nalidixic acid									77,2	2,4	0,6			19,8					19,8	15.4 - 24.1
Chloramphenicol									6,8	53,6	11,8	0,6	4,1	23,1					27,8	22.9 - 32.6
Florfenicol								0,3	10,4	58,3	11,5	3,8	0,6	15,1					19,5	15.2 - 23.8

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Table Eco01 (continued).

Dairy cattle N = 529	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							3,2	22,5	65,8	7,0			1,5						1,5	0.4 - 2.5
Cefotaxime			79,8	18,9	0,9	0,2	0,2												0,4	0 - 0.9
Ceftazidime					96,2	3,6	0,0	0,2											0,2	0 - 0.5
Gentamicin					6,6	61,6	25,3	5,7	0,6				0,2						0,8	0 - 1.5
Kanamycin									93,0	5,9	0,8								1,1	0.2 - 2
Streptomycin								0,9	32,9	58,6	4,9	0,4	0,6	0,9	0,8				2,6	1.2 - 4
Tetracycline							6,8	52,9	36,5	0,8			0,4	2,6					3,0	1.5 - 4.5
Sulfamethoxazole										97,4	0,4	0,4						1,9	1,9	0.7 - 3
Trimethoprim						98,1	1,1	0,2					0,6						0,6	0 - 1.2
Ciprofloxacin	79,0	20,2	0,4		0,2					0,2									0,4	0 - 0.9
Nalidixic acid									97,4	2,3				0,4					0,4	0 - 0.9
Chloramphenicol								0,2	5,3	77,9	15,7	0,2	0,2	0,6					0,9	0.1 - 1.7
Florfenicol								0,2	7,2	80,9	11,0	0,4		0,4					0,8	0 - 1.5

Turkey N = 95	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							1,1	7,4	11,6				80,0						80,0	71.7 - 88.2
Cefotaxime			62,1	29,5	2,1		1,1	2,1	2,1	1,1									6,3	1.3 - 11.3
Ceftazidime					89,5	4,2			3,2		2,1	1,1							6,3	1.3 - 11.3
Gentamicin						43,2	35,8	12,6	1,1	1,1	2,1	3,2	1,1						8,4	2.7 - 14.1
Kanamycin									75,8	10,5	1,1	2,1			10,5				13,7	6.6 - 20.7
Streptomycin									1,1	36,8	6,3	14,7	12,6	10,5	17,9				55,8	45.5 - 65.9
Tetracycline							4,2	7,4	8,4		2,1	1,1	15,8	61,1					80,0	71.7 - 88.2
Sulfamethoxazole										32,6	1,1							66,3	66,3	56.6 - 76
Trimethoprim						60,0	1,1						38,9						38,9	28.9 - 48.9
Ciprofloxacin	55,8	12,6		2,1	8,4	8,4	2,1	1,1			9,5								31,6	22 - 41.1
Nalidixic acid									69,5	1,1	2,1		1,1	26,3					27,4	18.2 - 36.5
Chloramphenicol									6,3	45,3	5,3	16,8	12,6	13,7					43,2	32.9 - 53.3
Florfenicol									10,5	54,7	29,5	5,3							5,3	0.6 - 9.8

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

and dairy cows. For most drugs or drug classes there are notable variations in resistance levels between the different animal species, most strikingly for ampicillin ranging from 1.5% in dairy cattle to 80% in turkey isolates.
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.

Quinolones
Reduced susceptibility to quinolones is most commonly encountered in *E. coli* isolated from broiler chickens; 59% of all isolates that were tested showed non-wild type susceptibility[2] to nalidixic acid and ciprofloxacin. In 2010/2011 high level resistance (MIC >1 mg/l) to ciprofloxacin in broiler chickens was detected in 7.2%, compared to 5.4% in 2009 and 6.3% of the isolates in 2008.
The percentage of *E. coli* with acquired resistance mechanisms to ciprofloxacin was also high among turkey (31.6%) and veal calves (21.3%) compared to 1.1% in pigs and 0.4% in dairy cattle. This likely reflects the use of quinolones in various animal husbandry systems.

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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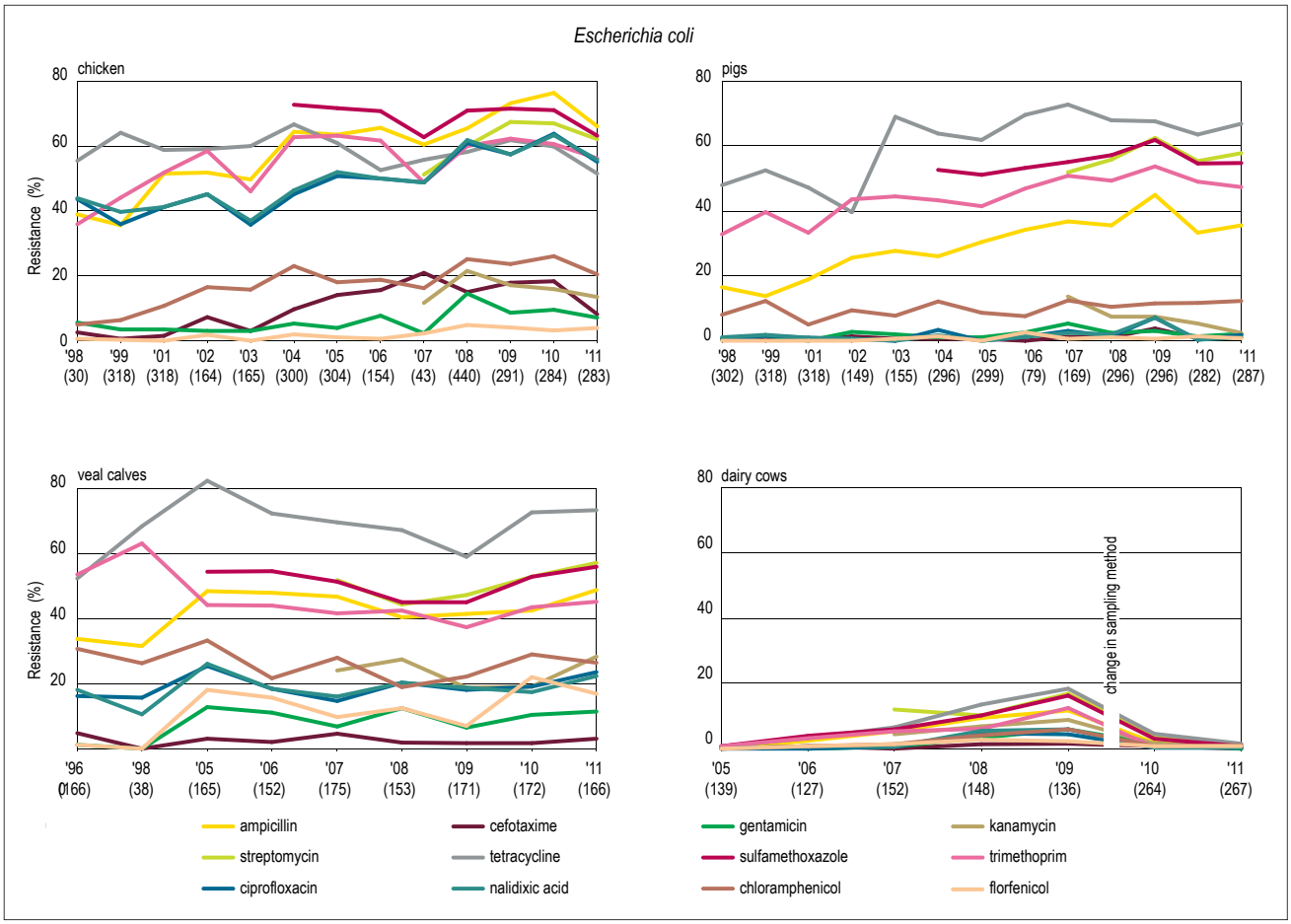

Figure Eco01. Trends in resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves, and dairy cattle in the Netherlands from 1998 – 2011. *For dairy cows changes in sampling strategy have been implemented between 2009 and 2010; Until 2009 samples were collected at farm level, in 2010 and 2011 individual animals were randomly sampled at slaughterhouses.*

Table Eco02. Resistance (in %) of E. coli isolated from raw meat products at retail in the Netherlands in 2010/2011

	Poultry meat N = 468	Pork N = 186	Veal* N = 31	Beef N = 262	Turkey meat* N = 46	Herbs* N = 33	Vegetables and fruits N = 65
Ampicillin	65.4	22.6	32.3	11.1	76.1	33.3	1.5
Cefotaxime	20.3	1.6	3.2	1.5	2.2	0	0
Ceftazidime	19.0	2.2	0	1.5	2.2	3.0	1.5
Gentamicin	10.3	4.8	0	0.4	13.0	6.1	0
Kanamycin	14.7	5.4	6.5	5.3	21.7	3.0	0
Streptomycin	54.1	25.8	35.5	14.1	50.0	36.4	1.5
Tetracycline	54.9	31.2	45.2	14.1	69.6	42.4	3.1
Sulfamethoxazole	60.5	33.3	32.3	20.6	56.5	33.3	1.5
Trimethoprim	44.2	24.2	29.0	12.6	34.8	33.3	1.5
Ciprofloxacin	48.1	2.7	6.5	3.8	39.1	33.3	1.5
Naladixic acid	45.7	2.2	6.5	3.8	37.0	21.2	1.5
Chloramphenicol	17.7	5.9	12.9	3.1	23.9	24.2	0
Florfenicol	2.1	2.2	9.7	1.1	0	18.2	0

*Data only from 2011

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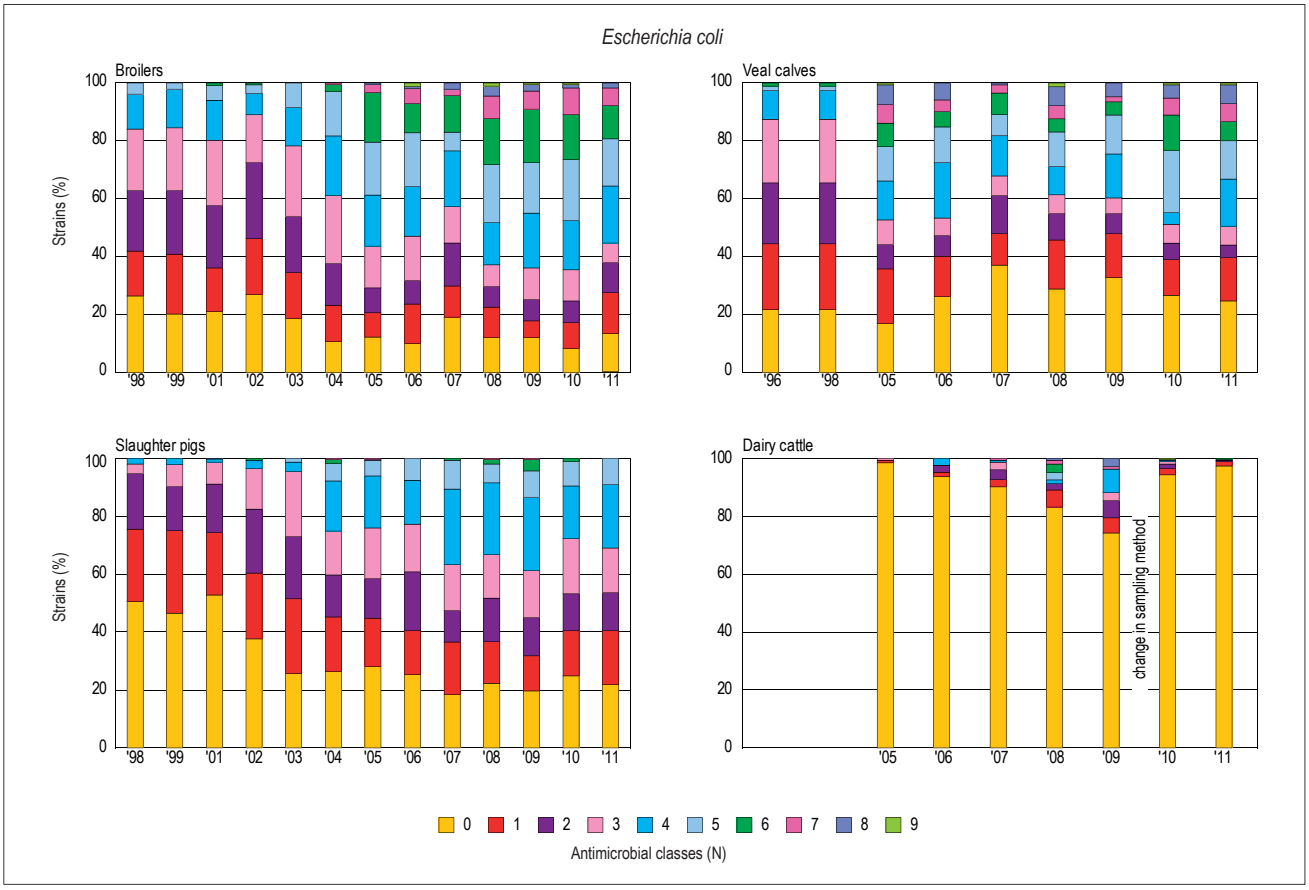

Figure Eco02. Resistance (%) to 0-9 antimicrobial classes among E. coli strains from broiler chickens, slaughter pigs and veal calves in the Netherlands from 1998 - 2011.

For dairy cows changes in sampling strategy have been implemented between 2009 and 2010; Until 2009 samples were collected at farm level, in 2010 and 2011 individual animals were randomly sampled at slaughterhouses.

Beta-lactamases (ESBL)

Resistance to third generation cephalosporins, indicative of ESBL producing E. *coli*, was detected in all animal host species included in this survey. Reduced susceptibility levels for cefotaxime ranged from 0.4% in samples from dairy cattle to 13.2% in broiler samples from 2010/ 2011 (Table Eco01). Among *E. coli* isolated from meat, levels were as high as 20.3% (n=468) in poultry meat as shown in Table Eco02. More extensive information on ESBL producing *E. coli* in broilers is presented in appendix I, which shows that cefotaxime resistance shows a tendency to decrease from 2009 onwards.

3.2.1.1.1 Broilers

In commensal *E. coli* isolated from caecal samples from broiler chickens resistance to all antimicrobials tested was common as summarized in Table Eco01. Very high levels of reduced susceptibility were observed for ampicillin (71.3%), sulfamethoxazole (67.2%), streptomycin (64.6%), trimethoprim (58.4%), the quinolones nalidixic acid (59.6%) and ciprofloxacin (59.4%) and tetracycline (55.7%). Also resistance to chloramphenicol, cefotaxime and ceftazidime, kanamycin and gentamicin was commonly found.

Overall, the resistance levels in 2011 were generally slightly lower than those in previous years for all of the antimicrobials tested (Figure Eco01)

3.2.1.1.2 Slaughter pigs

Highest levels of resistance in swine isolates in 2010/2011 were recorded for tetracycline (65.2%), streptomycin (56.6%), sulfamethoxazole (54.7%) and trimethoprim (48.2%).

E. coli isolated from slaughter pigs has shown an on-going increasing trend in resistance for most antibiotics since approximately the turn of the century. Especially with regard to beta-lactam antibiotics (ampicillin, cefotaxime and ceftazidime), fluoroquinolones (nalidixic acid and ciprofloxacin), trimethoprim and sulphamethoxazole an increase was noted as shown in Figure Eco01. However, levels from recent years appear to stabilize or even show a slight tendency to decrease. Resistance to the beta-lactam antibiotics was observed; 34.4% of the isolates resistant to ampicillin, 1.2% and 1.6% of the isolates being resistant to cefotaxime and ceftazidime respectively. The prevalence of suspected ESBL-producers was somewhat lower in 2010/2011 compared to 2009 when 3.7% of the isolates

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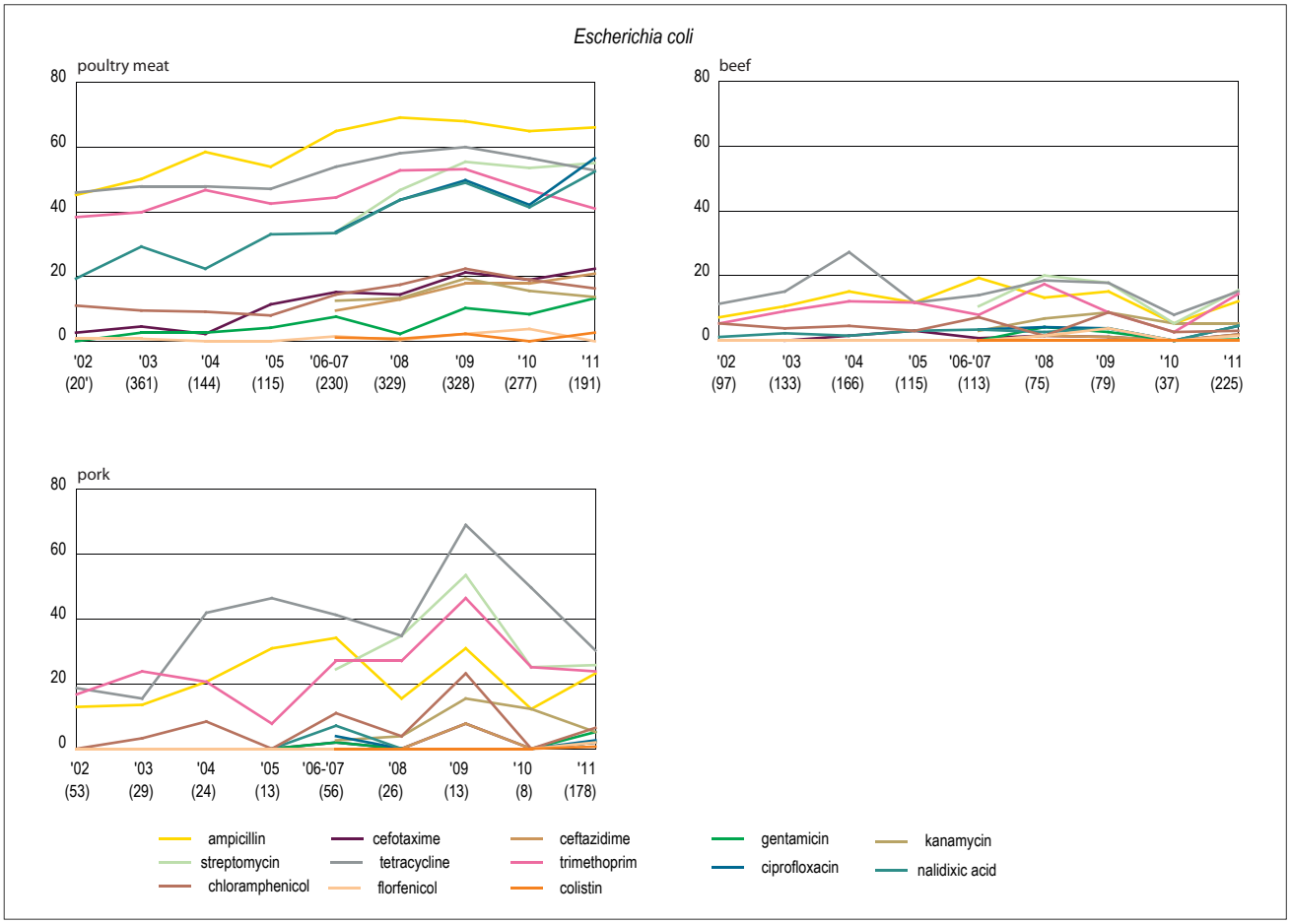

Figure Eco03. Trends in resistance (in%) of *E. coli* isolated from raw poultry meat products, beef, and pork, in the Netherlands from 2002 – 2011.

were cefotaxime resistant. Resistance is variable for the aminoglycoside class antibiotics. Streptomycin resistance is common (56.6% in 2010/2011), while 1.8% of the *E. coli* strains were resistant to gentamicin and 3.9% to kanamycin.
Resistance to chloramphenicol and florfenicol has remained stable over the years, with an average rate around 10% for chloramphenicol and below 1% for florfenicol.

3.2.1.1.3 Veal calves
Figure Eco01 illustrates the trends in resistance in *E. coli* isolated from veal calves. Resistance levels have been relatively stable over time, although there is a tendency to increase in the last two years. For instance for tetracycline, after a decrease from 82.4% in 2005 to 59.1% in 2009, levels in 2010 and 2011 had again increased to 72.7 and 73.5% respectively.
For beta-lactam antibiotics, resistance to ampicillin is commonly seen (45.6% in 2010/2011), while, resistance to 3rd generation of cephalosporins like cefotaxime and ceftazidime remains relatively low (2.4% and 2.1% respectively).
With regard to fluoroquinolone resistance, about 21.3% of *E. coli* from veal calves showed reduced susceptibility to ciprofloxacin, 7.7% were considered clinically resistant with MIC values >1 mg/l.
Resistance to both chloramphenicol and florfenicol is common, data from 2010/2011 show resistance percentages of 27.8% for chloramphenicol and 19.5% for florfenicol.

3.2.1.1.4 Dairy cattle
In general, resistance in *E. coli* isolated from dairy cattle is low compared to resistance levels seen in pigs, broilers and veal calves. Highest level of resistance was recorded for tetracycline (3.0% of the isolates). Although only 1.5% of the isolates were resistant to ampicillin, still cefotaxime resistance was expressed by 0.4% of the isolates.
The trends in resistance as illustrated in Figure Eco01 have shown a gradual increase for a number of antimicrobials since 2005 with highest levels in 2009. Data from the last two years show a sharp decrease with very low levels again. Changes in sampling strategy have been implemented in 2010 (from collection of faecal samples at farm level to randomly sampling of individual animals at slaughterhouses) which will have affected the detection level of resistance determinants in *E. coli*. Therefore, these trends have to be interpreted

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with caution, knowing the frequent occurrence of ESBL-producing *E. coli* in individual animals at slaughter (appendix 1). From 2012 onwards, sampling is again conducted at farm level.

3.2.1.1.5 Turkey
In 2011, for the first time data on turkey are included. In the Netherlands in 2010, 56 turkey farms were registered with a total of one million animals. Data in this report are based on a total of 100 samples collected from 20 individual farms (five samples per farm).
Similar to the situation in broiler chickens, resistance levels are extensive, with highest levels for ampicillin and tetracycline (80% of the isolates). The level of suspected ESBL producing isolates in 2011 was 6.3%, which is slightly lower than *E. coli* from broilers in 2011 (8.1 % of the isolates resistant to cefotaxime).
With 31.6% of the turkey *E. coli* isolates showing decreased susceptibility to ciprofloxacin this, together with broilers, represents the animal sectors with the most extensive level of resistance to quinolones.
Another remarkable finding is the high level of resistance to chloramphenicol (43.2%). Chloramphenicol has not been used for many years in veterinary medicine other than for topical use and the high level of resistance probably reflects either use of drugs conferring cross resistance or persistence of chloramphenicol resistance genes in the bacterial population.

Multidrug resistance
The overall increase in resistance is also reflected in de multidrug resistance data as shown in Figure Eco02. The highest level of multidrug resistance was present among *E. coli* originating from broilers. In 2010, 82.7% of the commensal *E. coli* strains from broiler chickens were resistant to two or more classes of antimicrobials included in the survey, which represents the highest level recorded in the past thirteen years. In 2011, this percentage was 72.8%. Also among *E. coli* from veal calves and pigs, multidrug resistance was widespread; in veal calves, 58.1% and 60.2% of commensal *E. coli* isolates was resistant to at least two classes of antibiotics in 2010 and 2011 respectively. Among *E. coli* from swine this was the case for 59.6% of the isolates, in both years.
For *E. coli* from dairy cattle multidrug resistance was only incidentally seen in 2010 and 2011, with 3.4% and 1.1% respectively resistant to two or more antibiotics. After an apparent increase in percentage multiresistant isolates up to 2009, the level has shown a tendency to decrease again in the last two years. In both 2010 and 2011, 1% of all randomly selected *E. coli* isolates from veal calves showed reduced susceptibility to all nine tested antimicrobials or classes (represented by ampicillin, cefotaxime, gentamicin, tetracycline, sulfamethoxazole, trimethoprim, nalidixic acid, chloramphenicol, and kanamycin). In broiler chickens, only one such multiresistant strain was detected in 2010, but not in 2011.

3.2.1.2 E. coli in raw meat products of food-animals
Table Eco02 shows resistance percentages of *E. coli* strains isolated from raw meat products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (VWA), as well as strains isolated from vegetables, fruits and herbs.
Overall, resistance percentages of *E. coli* strains as indicator organisms isolated from poultry products had a tendency to be slightly lower compared to those isolated from faecal samples of Dutch broiler chickens (Table Eco01 and TableEco02). One exception was the percentage of *E. coli* showing resistance to the cefotaxime and ceftazidime, which seem to be somewhat higher in isolates from meat products than in isolates from live animals for poultry.
For the first time, MIC data on *E. coli* isolated from herbs, vegetables and fruits are included in this report. Resistance levels in isolates from vegetables and fruits are generally very low. Highest level is observed for tetracycline with 3.1% of the isolates showing resistance. In contrast, levels in herbs are much higher. Resistance has been observed for all but one of the antimicrobials included in the test panel. Although no resistance to cefotaxime was found, resistance to ceftazidime was present in one of the 33 isolates.
Interpretation of data from both pork and veal 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 Eco03. Trends in resistance percentages from *E. coli* isolated from poultry meat show a tendency to increase, similar to resistance percentages from indicator bacteria isolated from faecal samples. Resistance rates of *E. coli* from beef samples are stable over the years.

3.2.1.3 E. coli in other European countries
In 2010, information on antimicrobial resistance in indicator *E. coli* isolates from animals and food was reported by seven to ten countries related to broilers, pigs and cattle. Data were analysed by the European Food Safety Authority together with the European Centre for Disease Prevention and Control. The information in this heading has been adopted from The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010 (EFSA Journal 2012;10(3):2598).
Microbiological resistance to antimicrobials was regularly observed in isolates of indicator (commensal) *E. coli* and enterococci from animals and food in the EU. In the case of many of the antimicrobials, there were large differences in the level of resistance in different member states. The Netherlands tended to have comparatively high levels of resistance for most antimicrobials, compared to other countries. No major changes in resistance in *E. coli* were observed compared with previous years. However, gradual but continuous increases in resistance for antimicrobials typically used in animals,

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such as ampicillin, streptomycin, sulphonamides and tetracyclines, have been observed in some EU member states in the last five years or more.

3.2.1.3.1 Poultry

In *E. coli* isolates from broilers, the occurrence of resistance to tetracyclines, ampicillin and sulfonamides for all reporting member states was 31%, 35% and 34%, respectively. Similarly, the level of resistance to ciprofloxacin was 29%, to nalidixic acid 26% and to cefotaxime 5%. There were wide variations in the level of resistance to these antimicrobials among different countries. Mostly relatively stable situations in the resistance levels were observed in *E. coli* isolates from broilers over the years 2005–2010, although statistically significant national trends have been reported (mostly increasing).

Compared to other EU member states, the Dutch data on poultry isolates with respect to resistance to cefotaxime, but also for chloramphenicol, sulphonamides, and streptomycin were exceptionally high.

3.2.1.3.2 Pigs

Among *E. coli* isolates from pigs, resistance levels in the reporting group of member states were 48% for tetracyclines, 21% for ampicillin and 37% for sulphonamides and 44% for streptomycin. The level of resistance to both ciprofloxacin and nalidixic acid was 2%. Cefotaxime resistance was 1% and varied from 0% to 5%. As in broilers, there were differences in the occurrence of resistance to each of these antimicrobials in different countries, with the exception of cefotaxime resistance, which was recorded by all reporting member states as low, very low or not detectable. Over the years 2005–2010 mostly stable resistance was observed in *E. coli* isolates from pigs, with only minor fluctuations and no apparent general trends.

Compared to other EU member states, the Dutch data on swine isolates were moderate to high.

3.2.1.3.3 Cattle

In indicator *E. coli* isolates from cattle, resistance levels in the reporting group of member states were 38% for tetracyclines, 28% for ampicillin and 34% for sulfonamides. Resistance to ciprofloxacin and nalidixic acid was 15% and 13% respectively, while the level of resistance to cefotaxime was 3%. Again, the occurrence of resistance was variable among the different member states for all antimicrobials except cefotaxime, the level of resistance to which was generally low.

Some countries showed statistically significant decreasing national trends in resistance to some antimicrobials (i.e. streptomycin and sulphonamides) in the 2005–2010 period.

Compared to other EU member states, the Dutch data on cattle isolates were moderate to high.

3.2.2 *Enterococcus faecalis* and *E. faecium*

This chapter presents information on resistance in *Enterococcus* species from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-positive bacteria. *Enterococcus faecalis* and *E. faecium* isolates were selected from fecal samples of chickens, pigs, cattle and turkey. Supplementary to isolates from live animals, susceptibility profiles of *E. faecalis* and *E. faecium* isolated from raw meat are presented as well as from vegetables, fruits and herbs.

Highlights

1. As in former years, high rates of resistance were observed for tetracycline, erythromycin and also for streptomycin in both *E. faecalis* and *E. faecium* isolates. Streptomycin resistance was highest in broilers, in which also a very high level of resistance was noted for salinomycin in *E. faecium* (80.8%). In contrast, salinomycin resistance in *E. faecium* from pigs has shown a strong and steady decrease to a low level in 2011 (5.4%).
2. For the first time, data for enterococci from turkey were included. Although isolates from only a limited number of farms (n=20) were included and results should be interpreted with caution, resistance levels were high in comparison to other farm animals, notably for tetracycline, erythromycin, ampicillin, chloramphenicol and ciprofloxacin.
3. Resistance levels in enterococci from dairy cows were generally low, with the exception of quinu/dalfopristin in *E. faecium* (58.3%). Similar levels were observed in *E. faecium* from veal calves (65.5%) as well as from beef (57.8%).
4. Overall, in enterococci from meat samples, resistance levels were lower than in isolates from life animals. Similar as for broiler chickens, salinomycin resistance levels in meat was high (29.1% in *E. faecalis*, 57.0% in *E. faecium*), with a strong tendency to increase in the last decade. Also ciprofloxacin and erythromycin resistance levels in *E. faecium* showed a tendency to increase in 2011.
5. Resistance to vancomycin continued to be detected at low levels in enterococci from animals.

In 2010/2011 MIC values have been determined for 477 *E. faecalis* and 952 *E. faecium* strains isolated from fecal samples of animals at abattoirs as well as for 1368 *E. faecalis* and 552 *E. faecium* isolates from different meat samples. In Table Ent01 MIC distributions are summarized for all *E. faecalis* and *E. faecium* strains isolated from live animals. Table Ent02 presents information on resistance rates in different animal species, specified for broiler chickens, slaughter pigs,

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Table Ent01. MIC distributions (in %) for Enterococcus faecalis (N = 477) and E. faecium (N =952) isolated in food producing animals in the Netherlands in 2010 and 2011.

E. faecalis (n=477)	MIC (%) distribution mg/L														R%	95% CI
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin			86.4	13.4	0.2										0.0	0 - 0
Linezolid		1.9	34.8	63.1		0.2									0.2	0 - 0.6
Tetracyline		19.5	6.1	0.2		0.2	0.4	15.9	26.0	31.7					74.2	70.2 - 78.2
Erythromycin			17.8	12.8	4.2	2.3	2.3	2.5		0.8	57.2				65.2	60.8 - 69.5
Vancomycin			54.9	40.9	4.0					0.2					0.2	0 - 0.6
Ciprofloxacin		19.9	71.9	4.6	0.4		0.4	2.7							3.1	1.5 - 4.7
Quinu/dalfopristin		0.2	0.4	0.6	1.9	38.2	54.9	3.4	0.4						0.4	0 - 1.0
Salinomycin		10.1	40.7	13.4	31.2	4.6									4.6	2.6 - 6.5
Streptomycin								0.4	4.8	44.7	7.3	0.4	0.2	42.1	42.3	37.8 - 46.8
Gentamicin					1.3	22.0	71.3	2.5					2.9		2.9	1.3 - 4.4
Chloramphenicol					7.1	81.6	2.1		6.5	2.7					9.2	6.5 - 11.8
Florfenicol			0.6	28.3	70.9					0.2					0.2	0 - 0.6

E. faecium (n=952)	MIC (%) distribution mg/L														R%	95% CI
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin			17,5	30,4	25,4	17,2	1,3	0,7	2,1	3,9	1,5				26,7	23,8 - 29,5
Linezolid		0,2	8,1	84,0	7,4	0,3									0,3	0 - 0,6
Tetracyline		34,7	0,3	0,1	0,4	0,3	0,6	1,4	18,7	43,5					64,5	61,3 - 67,5
Erythromycin			17,1	19,3	7,7	2,5	0,7	0,4	0,2	0,3	51,7				55,9	52,6 - 59,1
Vancomycin		57,4	35,7	5,8	0,8					0,3					0,3	0 - 0,6
Ciprofloxacin		5,3	22,0	21,4	42,3	8,6	0,4								9,0	7,1 - 10,8
Quinu/dalfopristin	0,1	10,4	10,5	14,3	48,8	14,1	0,9	0,8							79,0	76,3 - 81,6
Salinomycin		0,2	27,7	26,1	14,4	31,6									31,6	28,6 - 34,6
Streptomycin							0,2	3,0	43,5	18,2	0,7	0,2	2,2	31,9	35,1	31,9 - 38,1
Gentamicin					6,8	43,0	38,9	8,9	0,2	0,6	0,2	0,3	1,1		2,4	1,4 - 3,4
Chloramphenicol				0,7	9,1	65,7	11,2	12,1	1,2						1,2	0,4 - 1,8
Florfenicol			0,1	7,7	89,9	0,9	0,1		0,9	0,3					1,4	0,6 - 2,1

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. The dashed bars indicate clinical breakpoints.

veal calves, dairy cows and turkeys. Trends over the years are depicted in Figure Ent01.
Data for 2010 and 2011 on *E. faecalis* and *E. faecium* from different meats and from vegetables, fruits and herbs are presented in Table Ent03, trends over the years for enterococci from the various raw meat sources in Figure Ent02.

3.2.2.1 Resistance levels

Compared to other EU member states, resistance levels in enterococci from food producing animals in the Netherlands are among the highest reported in Europe, similar as the situation for *E. coli*.

Tetracyclines

In 2010 and 2011 highest resistance levels among the *Enterococcus* species from animals were detected for tetracyclines, against which 74.2% of all *E. faecalis* (n = 477) and 64.5% of *E. faecium* isolates (n = 952) were resistant (Table Ent01). These levels are lower than those recorded in 2009 (92.8%, n = 194 and 71.1%, n = 298 respectively). Resistance levels for tetracyclines varied among the different animal species. Highest levels were observed in turkey, for which 100% of *E. faecalis* and 89.8% of *E. faecium* tested resistant. Lowest levels in cattle with 19.4% resistance for *E. faecalis* and 1.9% for *E. faecium* in dairy cows and 56.7% *(E. faecalis)* and 55.2% *(E. faecium)* in veal calves.

Erythromycin

Also resistance to erythromycin was high (65.2% for *E. faecalis*, 55.9% for *E. faecium*), although, similar as for

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Table Ent02. Resistance percentages (%) of Enterococcus faecalis and E. faecium isolated from faeces from dairy cows, veal calves, slaughter pigs, broilers and turkey in The Netherlands in 2010 and 2011.

E. faecalis	Slaughter pigs N = 74	Broiler chickens N = 276	Veal calves N = 60	Dairy cows N = 36	Turkey N = 31
Ampicillin	0	0	0	0	0
Linezolid	0	0	1.7	0	0
Tetracyline	86.5	79.0	56.7	19.4	100
Erythromycin	54.1	79.0	43.3	8.3	77.4
Vancomycin	0	0	1.7	0	0
Ciprofloxacin	2.7	3.6	3.3	2.8	0
Quinu/dalfopristin	0	0	0	0	6.5
Salinomycin	0	7.2	0	0	6.5
Streptomycin	23.0	56.2	35.0	0	29.0
Gentamicin	6.8	1.8	3.3	0	6.5
Chloramphenicol	12.2	3.3	31.7	0	22.6
Florfenicol	0	0	1.7	0	0

E. faecium	Slaughter pigs N = 184	Broiler chickens N = 427	Veal calves N = 145	Dairy cows N = 108	Turkey N = 88
Ampicillin	23.4	36.1	12.4	0	44.3
Linezolid	0	0	2.1	0	0
Tetracyline	77.2	72.8	55.2	1.9	89.8
Erythromycin	28.3	78.5	46.9	9.3	76.1
Vancomycin	0.5	0.5	0	0	0
Ciprofloxacin	3.3	10.5	4.1	15.7	13.6
Quinu/dalfopristin	91.8	80.8	65.5	58.3	90.9
Salinomycin	12.0	61.1	0	0	20.5
Streptomycin	13.6	56.0	35.2	1.9	19.3
Gentamicin	0	3.5	4.8	0	1.1
Chloramphenicol	0	0.5	6.2	0	0
Florfenicol	0	0	9.0	0	0

tetracycline, considerable variation in the resistance levels was observed in the different animal species or categories (Table Ent02). Again, highest levels were observed in broiler chickens and turkey (76.1% - 79%), lowest levels were seen in dairy cows (8.3% and 9.3% for *E. faecalis* and *E. faecium* respectively).

Streptomycin

Streptomycin resistance was high in both *E. faecalis* (42.3%) and *E. faecium* (35.1%), ranging from 0% in dairy cattle to 56.2% in broiler chickens for *E. faecalis* and from 1.9% in dairy cattle to 56.0% in broiler chickens.

Vancomycin and linezolid

The overall resistance to vancomycin and linezolid was very low (0.2% in *E. faecalis* and 0.3% in *E. faecium* for both antimicrobials). With respect to vancomycin, in 2010 and 2011 three highly resistant *E. faecium* strains were isolated, two from broiler chickens and one from a pig. In 2010 a vancomycin resistant *E. faecalis* strain was isolated from a calf. Four linezolid resistant isolates were all isolated from calves.

Quinu/dalfopristin

Acquired resistance to the streptogramin combination of quinupristin and dalfopristin (synercid®) was much more common in *E. faecium* than in *E. faecalis* (79.0% and 0.4% respectively). This combination is a last resort drug for the treatment of infections caused by staphylococci and vancomycin-resistant *E. faecium* (VRE). Based on the clinical breakpoint value of >4, 15.9% of the *E. faecium* isolates were resistant. With respect to *E. faecium* strains recovered from meat samples, resistance rates have increased over the years to similar rates as seen in *E. faecium* isolated from live animals.

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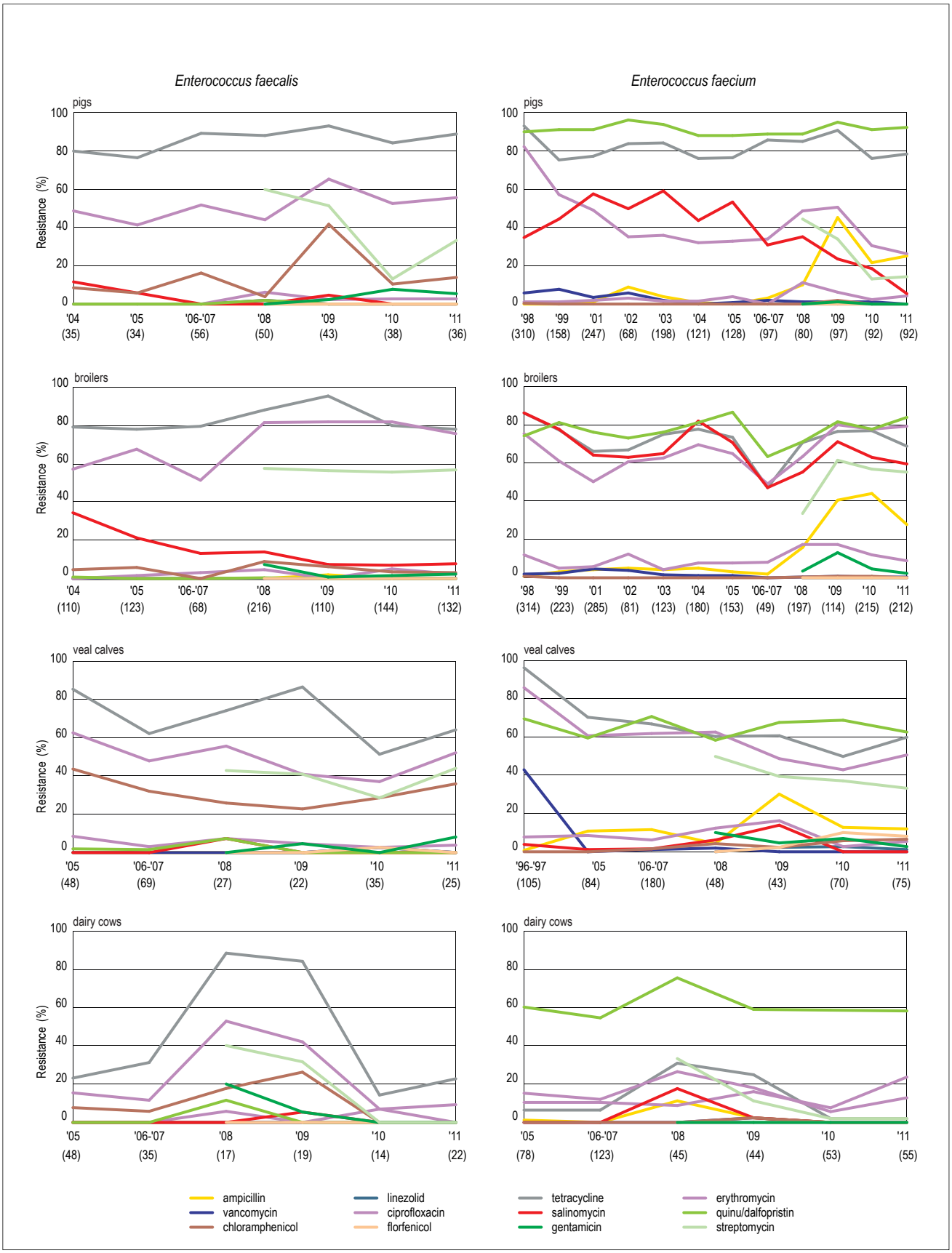

Figure Ent01. Trends in resistance percentages of *Enterococcus faecium* and *E. faecalis* isolated from slaughter pigs, broilers and veal calves in the Netherlands from 1996 – 2011.

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Table Ent03. Resistance % of Enterococcus faecalis and E. faecium strains isolated from raw meat products from pork, poultry, beef, veal, and lamb in the Netherlands in 2010/2011.

E. faecalis	Pork	Poultry	Beef	Veal	Lamb	Turkey	Herbs*	Vegetables and fruits*
	N = 410	N = 330	N = 365	N = 52	N = 52	N = 38	N = 25	N = 96
Ampicillin	0	1.5	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Tetracyline	21.2	70.9	19.7	46.2	38.5	81.6	24.0	16.7
Erythromycin	7.3	62.0	4.7	15.4	17.3	57.9	4.0	6.3
Vancomycin	0	0.6	0.3	0	0	0	0	1.0
Ciprofloxacin	0.7	3.6	0	0	0	7.9	0	0
Salinomycin	1.0	29.1	0	0	1.9	21.1	0	0
Quinu/dalfopristin	0	1.8	0	0	0	2.6	0	0
Gentamicin	0.2	1.8	0.8	1.9	3.8	10.5	4.0	0
Streptomycin	4.4	46.4	5.5	19.2	23.1	21.1	8.0	2.1
Chloramphenicol	1.5	4.8	1.6	7.7	5.8	2.6	0	2.1
Florfenicol	0	0	0	0	0	0	4.0	0

E. faecium	Pork	Poultry	Beef	Veal	Lamb	Turkey	Herbs*	Vegetables and fruits*
	N = 152	N = 86	N = 180	N = 33	N = 11	N = 13	N = 11	N = 66
Ampicillin	3.3	9.3	1.7	0	0	38.5	0	0
Linezolid	0	1.2	0	3.0	0	0	0	0
Tetracyline	14.5	51.2	9.4	27.3	9.1	84.6	27.3	7.6
Erythromycin	19.1	48.8	15.6	45.5	9.1	53.8	18.2	25.8
Vancomycin	0	0	0	0	0	0	0	0
Ciprofloxacin	2.6	20.9	5.6	0	0	0	0	1.5
Salinomycin	4.6	57.0	2.8	0	9.1	46.2	0	0
Quinu/dalfopristin	73.0	76.7	57.8	63.6	54.5	84.6	100	71.2
Gentamicin	0	0	0	3.0	0	0	0	0
Streptomycin	4.6	29.1	4.4	12.1	0	61.5	0	3.0
Chloramphenicol	0	0	0	3.0	0	0	0	0
Florfenicol	0	0	0.6	3.0	0	0	0	0

*Data only from 2011

3.2.2.1.1 Pigs

As in previous years, very high resistance levels were recorded for tetracycline in 2010/2011 in both *E. faecalis* (86.5%) and *E. faecium* (77.2%). Remarkably, resistance levels in pork were much lower (21.2% and 14.5% respectively). Other antimicrobials for which resistance was commonly detected in slaughter pigs included erythromycin in *E. faecalis* (54.1%) and *E. faecium* (28.3%), quinu/dalfopristin in *E. faecium* (91.8%) and streptomycin (23% in *E. faecalis* and 13.6% in *E. faecium*). Streptomycin resistance showed a tendency to decrease from 2008 when it was first included in the test panel to 14.1% in *E. faecium* and 33.3% in *E. faecalis* in 2011.

No vancomycin resistant isolates were present among *E. faecalis*, however a single E. *faecium* isolate with high level resistance was observed in 2010.

In 2009 a remarkable increase (41.9%) to chloramphenicol was reported in *E. faecalis*. However, levels in 2010 (10.5%) and 2011 (13.9%), were again comparable to those in 2008 and before. Also for other antimicrobials fluctuations over subsequent years were noticeable probably due to the limited number of isolates for which MIC information is available.

A strong tendency to decrease was noted for salinomycin resistance levels in *E. faecium* in the previous decade from 59.1% in 2003 to 5.4% in 2011, which is probably associated to the ban on salinomycin as a feed additive in 2006.

3.2.2.1.2 Broilers

Also in broilers, highest resistance levels were observed for tetracycline (79.0% in *E. faecalis* and 72.8% in *E. faecium*), erythromycin (79.0% in *E. faecalis* and

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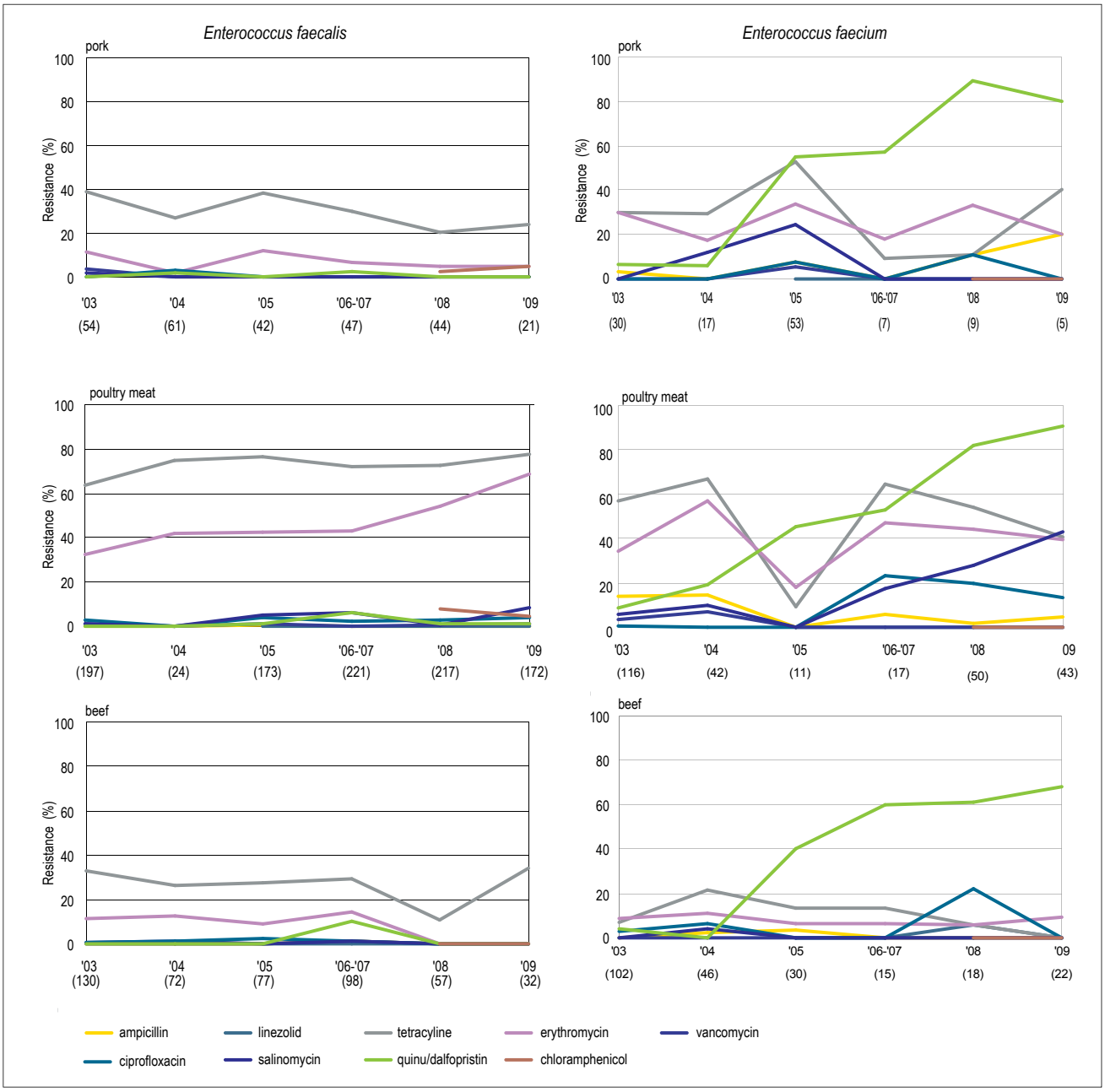

Figure Ent02. Trends in resistance percentages in *Enterococcus faecalis* and *E. faecium* isolated from raw meat products from pork, poultry, and beef in the Netherlands from 2003 to 2011.

78.5% in *E. faecium*), and streptomycin (56.2% in *E. faecalis* and 56.0% in *E. faecium*). In *E. faecalis*, additional high levels of resistance were observed for quinu/dalfopristin (80.8%), salinomycin (61.1%) and to a lesser extent to ampicillin (36.1%).
Over the years, resistance to the tested antimicrobials appears to have remained relatively stable in *E. faecalis*, while in *E. faecium*, more pronounced fluctuations were observed.

3.2.2.1.3 Veal calves
In veal calves, highest resistance levels were observed for tetracycline (56.7% in *E. faecalis*; 55.2% in *E. faecium*), erythromycin (43.3% in *E. faecalis* and 46.9% in *E. faecium*); streptomycin (35.0% in *E. faecalis* and 35.2% in *E. faecium*) and the combination of quinupristin and dalfopristin in *E. faecium* (65.5%). Notably, veal calves were the host species in which florfenicol and chloramphenicol resistance was highest in comparison with other food animals, reflecting the use of florfenicol for respiratory infections. Low levels or no resistance were observed for the other antimicrobials tested. Although for some antimicrobials fluctuations are apparent in resistance levels over the years due to the limited number of isolates, there seems to be a slight decreasing tendency for resistance to tetracycline and

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streptomycin in *E. faecium*.
A single high level vancomycin resistant *E. faecalis* isolate was recorded in a calf in 2010. Also linezolid resistant isolates have been recovered from veal calves, both *E. faecalis* (n = 1) and *E. faecium* (n = 3).

3.2.2.1.4 Dairy cattle
Overall, resistance levels are low in *E. faecalis* and *E. faecium* in dairy cows. In 2010/2011 highest resistance levels in *E. faecalis* were recorded for tetracycline (19.4%) and erythromycin (8.3%). In *E. faecium*, highest level was observed for quinu/dalfopristin (58.3%), ciprofloxacin (15.7%) and to a lesser extent to erythromycin (9.3%). Compared to ciprofloxacin resistance levels in other animals, the relatively high percentage is remarkable, as quinolones are not readily used in dairy cows. Similar to the situation in veal calves, levels show some fluctuations over the years. It should be noted that in dairy cattle a change in sampling strategy was implemented in 2009/2010 as already mentioned for *E. coli* as indicator organism. This might have caused a bias in MIC results and therefore trends should be interpreted with caution.

3.2.2.1.5 Turkey
In 2011, for the first time data on turkey are included. Resistance levels in both E. faecalis and *E. faecium* were very high for tetracycline (100% and 89.8% respectively) and for erythromycin (77.4% and 76.1% respectively). Additionally, resistance was frequently observed for quinu/dalfopristin (90.9%) and ampicillin (44.3%) in *E. faecium* and for streptomycin (29% in *E. faecalis*, 19.3% in *E. faecium*) and chloramphenicol (22.6%) in *E. faecalis*.
Compared to other food animal species, *E. faecium* from turkeys show relatively high resistance levels for salinomycin (20.5%) and ciprofloxacin (13.6%). However, interpretation must be done with caution, as data are based on a limited number samples collected from 20 individual farms.
No resistance has been detected for vancomycin or linezolid in turkeys.

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

Table Ent03 shows resistance percentages of *E. faecalis* and *E. faecium* strains isolated from raw meat products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (NVWA), as well as strains isolated from vegetables, fruits and herbs.
As in previous years, resistance in *E. faecalis* and *E. faecium* isolated from fresh meat was in general lower compared to isolates recovered from fecal samples. Variable resistance levels were observed among *E. faecalis* and *E. faecium* isolated from meat from different host species as shown in Table Ent03. For tetracycline, levels ranged from 19.7% to 81.6% among *E. faecalis* and from 9.1 to 84.6% in *E. faecium*; for erythromycin, levels ranged from 4.7% to 62.0% in *E. faecalis* and from 9.1% to 53.8% in *E. faecium*. For streptomycin, levels ranged from 4.4% to 46.4% in *E. faecalis* and from 0% to 61.5% in *E. faecium*.
No resistance for linezolid was detected in *E. faecalis*, however two resistant *E. faecium* strains are reported, one from poultry meat and one from veal. Vancomycin resistance was observed in *E. faecalis*, isolated from pork (n=1), poultry meat (n=2) and beef (n=1). Noticeably, vancomycin resistance was also observed in an *E. faecalis* strain isolated from vegetables.
Resistance to ampicillin and ciprofloxacin was either low or not detected in enterococci species from meat, with the exception of poultry and turkey meat. Of the 13 *E. faecium* isolates from turkey included in the survey, five expressed MICs ≥8 mg/l for ampicillin (38.5%).
For *E. faecalis*, 3.6% of poultry isolates and 7.9% of turkey isolates were resistant to ciprofloxacin. Also for salinomycin, resistance levels in poultry and turkey meat was higher than in other meats, both in *E. faecalis* and *E. faecium*.
Quinu/dalfopristin resistance was high in *E. faecium*, but low in *E. faecalis* in all sample categories. Gentamicin resistance was low in *E. faecalis*, and absent among *E. faecium* except for a single *E. faecium* strain from veal. Also resistance to chloramphenicol and florfenicol was low or absent in enterococci isolates from the various sample sources.
Trends over time are fairly stable. Noticeable are the increasing tendencies for salinomycin levels in pork, poultry meat and beef as shown in Figure Ent02. With respect to quinu/dalfopristin, after an increase from 2004 onwards, levels seem to have reached a relatively stable, although high, level in all three categories in recent years. In poultry meat, ciprofloxacin and erythromycin resistance levels in *E. faecium* in 2011 show a tendency to increase.
These reported tendencies are not reflected in the temporal resistance data in animals. Together with the overall differences between resistance levels in animals and meat, this might suggest that certain selection pressures could favor the election of certain biotypes in meat. Also imported meat might have biased the results.
For the first time, MIC data on *E. faecalis* and *E. faecium* isolated from herbs, vegetables and fruits are included in this report. Resistance levels are generally low. In *E. faecalis*, highest levels were observed for tetracycline with 24.0% in herbs and 16.7% of the isolates from vegetables and fruits showing resistance. In *E. faecium*, highest levels were observed for the quinu/dalfopristin combination (100% and 71.2% in herbs and vegetables/fruits respectively). In addition relatively high levels of resistance were recorded for erythromycin (up to 27.3%, in herbs) and tetracycline (up to 25.8%, in vegetables and fruit).

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3.2.2.3 Enterococci in other European countries

In 2010, seven member states and one non-member state submitted information on the occurrence of antimicrobial resistance in enterococci isolates from animals and food to the European Commission, the European Food Safety Authority and the European Centre for Disease Prevention and Control. The results are published in the EU Summary Report (The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010 (EFSA Journal 2012; 10(3):2598). Most of the data related to isolates from broilers, pigs and cattle.
In general, wide variation in the level of resistance in different countries was observed. Among the indicator enterococci isolates from animals and food, resistance against tetracyclines and erythromycin was commonly detected in isolates from poultry, pigs and cattle at levels of 13 % to 71 %, the level of resistance being lowest for isolates from cattle. To the same extend as in *E. coli*, the resistance percentages in enterococci from food producing animals in the Netherlands are among the highest reported in Europe.
Since there is cross-resistance between avoparcin and the human antimicrobial vancomycin, the use of avoparcin as an antimicrobial growth promoter was banned in the EU in 1997. Resistance to vancomycin continued to be detected, albeit at low to very low levels, at 0.3 % to 0.9 %, in enterococci isolates from animals.

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4. Appendix I. ESBL and AmpC-producing *E. coli* in food producing animals in the Netherlands

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[3]. These isolates are tested for susceptibility to cefotaxime and proportions of non-wild type isolates are determined based on EUCAST epidemiological cut-off values (non-wild type MIC > 0.25 mg/l).
Figure ESBL01 shows that since 1998 cefotaxime resistance was observed at low levels in all animal species. In broilers after 2001 an apparent increase was observed up to levels that varied from 15 – 20%, followed by a decrease in 2010 which may be attributed to the total ban on the usage of ceftiofur at Dutch hatcheries in March 2010. A prevalence study conducted in 2009 at broiler farms showed that using selective isolation methods, all conventional Dutch broiler farms were positive and within these farms ≥ 80% of the animals shed ESBL-producing *E. coli* in their faeces (MARAN-2009). Although the quantity of ESBL-producing *E. coli* shed by broilers may have been reduced, the ban of ceftiofur has not yet resulted in a reduction

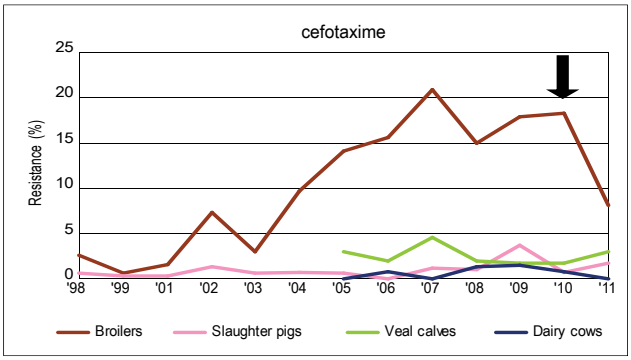

Figure ESBL01. Trends in cefotaxime non-wild type susceptibility in *E. coli* from food –producing animals from 1998 – 2011. The arrow indicates the year 2010, when usage of ceftiofur at broiler hatcheries was stopped.

of the number of farms positive for ESBL-producing *E. coli*. In 2011, prevalence studies of ESBL-producing *E. coli* were initiated in all Dutch food-producing animals in close collaboration between the Dutch Food and Consumer Product Safety Authority (NVWA) and the Central Veterinary Institute (CVI). At Dutch slaughterhouses a

Table ESBL01. Beta-lactamases detected in E. coli from Dutch food-producing animals.

Beta-Lactamase family	Enzyme	Slaughter pigs	Veal calves	Broilers	Dairy cows
			N		
CTX-M-1gr	CTX-M-1	26	25	11	6
	CTX-M-3		2		
	CTX-M-15	1	10		
	CTX-M-32	1	4		2
CTX-M-2gr	CTX-M-2	1	3		
CTX-M-9gr	CTX-M-14	1	9		
TEM	TEM-1a	3	2		
	TEM-1b	4	2		
	TEM-1c		1		
	TEM-20	1			
	TEM-52c	9	4	3	
SHV	SHV-12	3		2	1
pAmpC	CMY-2	1	1	12	1
ampC-promoter mutant	type-3	13	6		4
	type-11	1	1		
	type-18	3			
	type-40			1	
% flocks positive		68%	70%	100%	14%*

* *individual animals*

3 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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Table ESBL02. Beta-lactamases detected in E. coli from wild bird of different species sampled from July 2010 to February 2012.

	Bird species	Alopochen aegyptiaca	Anas platyrhynchos	Anas strepera	Bucephala clangula	Chroicocephalus ridibundus	Columbia livia	Cygnus atratus	Larus argentatus	Larus canus	Larus marinus	Morus bassanus	Philomachus pugnax	Tringa totanus	Total
Beta-Lactamase family	Enzyme							N							
CTX-M-1gr	CTX-M-1		2				1					1			4
	CTX-M-1; TEM-1b			1											1
	CTX-M-1; TEM-1c	1													1
	CTX-M-1; TEM-33'					1									1
	CTX-M-3				1										1
	CTX-M-15		1												1
	CTX-M-15; OXA-1; aac6'-Ib-cr					1									1
	CTX-M-15; TEM-1									1					1
	CTX-M-32							1						1	2
	CTX-M-32; TEM-1b		1												1
CTX-M-9gr	CTX-M-14													2	2
	CTX-M-14;TEM-1b		1												1
TEM	TEM52c + ampC type 3												1		1
pAmpC	CMY-2								1					1	2
	CMY-2; TEM-1c													1	1
	CMY-2; TEM-33										1				1
	Total	1	5	1	1	2	1	1	1	1	1	1	1	5	22

faecal sample was taken from ten (apparently healthy) animals per flock of animals. In total samples were taken from 100 flocks of slaughter pigs, 100 flocks of veal calves and 29 flocks of broiler chickens. Moreover, 100 individual dairy cows were sampled, each representing a different farm.

Each 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. All isolates were sent as pure cultures to CVI for molecular analysis of the ESBL/AmpC-genes. One isolate per flock was screened for beta-lactamase gene families using the ATR0503 Alere 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.

Table ESBL01 shows the ESBL/AmpC genes detected in the different animal species. The highest prevalence was found in broilers, but also in veal calves and slaughter pigs a high proportion of flocks were found to be positive. A wide variation in beta-lactamase genes was identified. $Bla_{CTX-M-1}$, was the dominant variant throughout Dutch food-producing animals. The predominance of other variants varied by animal species. In pigs $bla_{TEM-52c}$ was frequently detected next to individual other variants. In veal calves a surprisingly high proportion of $bla_{CTX-M14}$ and $bla_{CTX-M-15}$, which are considered to be typical 'human' ESBLs, were detected. Promotor mutants of chromosomal *ampC*-genes were detected in all animal species. Because these *ampC*-genes are not horizontally transferable they are not considered to be a source for potential transfer to human bacteria. Also bla_{TEM-1} variants were found as single genes in ESBL-suspected isolates, which may be associated with over-expression resulting in a cephalosporin-resistant phenotype, or a combination with undetected resistance mechanisms since these genes are considered not to be ESBLs.

As part of a continuous surveillance programme of infectious organisms in wild birds in the Netherlands, 165 dead birds (mostly ducks, swans, gulls and waders) were collected from summer 2010 until February 2012 at different locations throughout the Netherlands. All animals were examined for ESBL/AmpC producing *E. coli* in their faeces as described above. Twenty-two (13%) of the birds were positive (Table ESBL02). The beta-lactamase genes found mimic those found in food-producing animals, with $bla_{CTX-M-1}$ being the predominant gene variant detected. However also human associated $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$ were detected. In the latter isolate also bla_{OXA-1} and the

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plasmid mediated quinolone resistance variant *aac(6')-Ib-cr* was detected. This combination of genes is highly typical for the human uropathogenic *E. coli* ST131 with IncF-plasmids harbouring these genes. However, this was not yet confirmed for this isolate. The most frequent bird in which ESBLs were found were Anas platyrhynchos (Wild Duck) and Tringa totanus (Common Redshank). Both birds feed in surface water or meadows, wetlands and mud flats that may be polluted by contaminated manure or faeces of animal or human origin.

It can be concluded that the occurrence of ESBL/AmpC-producing *E. coli* is widespread in Dutch food-producing animals. The potential attribution to infections in humans warrants strict measures to control antibiotic usage and possibilities of transmission of these organisms in animal production chains. The frequent finding of ESBL/AmpCs in *E. coli* from wild birds indicates a widespread contamination of the environment in the Netherlands that may contribute to dissemination of these genes.

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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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