NETHMAP 2011

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

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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 Center for Infectious disease control (CIb) of the RIVM, the National Institute for Public Health and the Environment of the Netherlands. SWAB is fully supported by a structural grant from CIb, on behalf of the Ministry of Health, Welfare and Sports of the Netherlands. The information presented in NethMap is based on data from ongoing surveillance systems on the use of antimicrobial agents in human medicine and on the prevalence of resistance to relevant antimicrobial agents among medically important bacteria isolated from healthy individuals and patients in the community and from hospitalized patients. The document was produced on behalf of the SWAB by the Studio of the RIVM.
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Centres contributing to the surveillance of the use of antimicrobial agents

Alkmaar, MC Alkmaar; Almelo, ziekenhuisgroep Twente; Amersfoort, Meander MC; Amstelveen, ziekenhuis Amstelland; Amsterdam, AMC; Amsterdam, BovenIJ ziekenhuis; Amsterdam, OLVG; Amsterdam, St.Lucas ziekenhuis; Amsterdam, VUMC; Apeldoorn, Gelre ziekenhuizen; Arnhem, Rijnstate ziekenhuis; Arnhem, Zevenaar; Assen, Wilhelmina ziekenhuis; Bergen op Zoom, Lievensberg; Boxmeer, Maasziekenhuis; Capelle a/d Ijsel, Ijselland ziekenhuis; Den Bosch, Jeroen Bosch ziekenhuis; Den Haag, HAGA ziekenhuizen; Den Helder, Gemini ziekenhuis; Doetinchem, Slingerland ziekenhuis; Dokkum, Sionsberg; Dordrecht, Albert Schweizer ziekenhuis; Ede, Gelderse vallei; Eindhoven, Catharina ziekenhuis; Emmen, Scheperziekenhuis; Enschede, Medisch spectrum Twente; Geldrop, St.Anna ziekenhuis; Goes, Scheldezoom; Gouda, Groene hart ziekenhuis; Groningen, Martini ziekenhuis; Groningen, Ommeland ziekenhuis; Groningen, Refaja ziekenhuis; Groningen, UMCG; Haarlem, Kennemergasthuis; Haarlem, Spaarne ziekenhuis; Harderwijk, St.Jansdal; Heerlen, Atrium MC; Hengelo, ziekenhuisgroep Twente; Hoorn, Westfries gasthuis; Leiden, Diaconessenhuis; Leiden, LUMC; Leiderdorp, Rijnland ziekenhuis; Maastricht, AZM; Nieuwegein, St.Antonius ziekenhuis; Nijmegen, CWZ; Nijmegen, UMC St.Radboud; Purmerend, Waterland ziekenhuis; Roermond, Laurentius ziekenhuis; Rotterdam, Erasmus MC; Rotterdam, Ikazia; Rotterdam, Maasstad ziekenhuis; Rotterdam, St.Franciscus gasthuis; Schiedam, Vlietland ziekenhuis; Sittard, Orbis MC; Sneek, Antonius ziekenhuis; Tiel, ziekenhuis Rivierenland; Utrecht, UMCU; Veldhoven, Maxima MC; Venlo, VieCurie; Woerden, Zuwe Hofpoort; Zaandam, Zaans MC; Zoetermeer, Lange Land ziekenhuis; Zutphen, Gelre ziekenhuizen; Zwolle, Isala kliniek.

Centres contributing to the surveillance of resistance to antimicrobial agents

Province	Town	Name and type of centre	COM	IUP	ISIS	Men	GRAS
Groningen	Delfzijl	Delfzicht Hospital				0	
	Groningen	Academic Medical Centre				0	
		Regional Laboratory for Public Health		0	0	0	0
		Municipal Health Service Groningen					0
	Stadskanaal	Refaja Hospital				0	
	Winschoten	St Lucas Hospital				0	
	t Zandt	General practice	0				
Friesland	Leeuwarden	Regional Laboratory for Public Health Izore		0	0	0	0
		Municipal Health Service Fryslan					0
Drente	Assen	General practice	0				
		Municipal Health Service Drenthe					0
	Emmen	Scheper Hospital				0	
Overijssel	Deventer	Deventer Hospital			0		
		Regional Laboratory for Public Health				0	
	Enschede	Regional Laboratory for Public Health		0	0	0	
		Municipal Health Service Twente					
	Hardenberg	Regional Laboratory for Public Health				0	
	Zwolle	Isala Clinics			0		
		Hanze laboratory				0	
		Regional Laboratory for Public Health		0			
Gelderland	Apeldoorn	Medical Laboraties ZCA				0	
		Gelre Hospitals			0		
	Arnhem	Regional Laboratory for Public Health			0	0	
		Alysis Centre			0		
		Hulpverlening Gelderland Midden					
	Barneveld	General practice	0				
	Dieren	General practice	0				
	Doetinchem	Slingeland Hospital				0	
	Ede	Gelderse Vallei Hospital				0	
	Harderwijk	St Jansdal Hospital				0	
	Heerde	General practice	0				
	Nijmegen	University Medical Centre St Radboud		0	0	0	
		Regional Laboratory for Public Health CWZ			0	0	
		Municipal Health Service Nijmegen					
	Zelhem	General practice	0				
Utrecht	Amersfoort	Meander Medical Centre				0	
		General practice	0				
	Bilthoven	National Institute for Public Health and the Environment			0		
	Nieuwegein	Sint Antonius Hospital		0	0	0	
	Utrecht	Diakonessenhuis			0	0	
		General practice	0				
		Neth Institute for Health Services Research NIVEL	0				
		Mesos Medical centre				0	
		SALTRO			0		
		University Medical Centre			0	0	0
		Municipal Health Service Utrecht					0
	Zeist	Diakonessenhuis				0	
Noord Holland	Alkmaar	General practice	0				
		Medical Centre Alkmaar			0	0	
	Amsterdam	Academic Medical Centre				0	
		Academic Hospital VU				0	
		General practice	0				
		Onze Lieve Vrouwe Gasthuis		0		0	
		Regional Laboratory for Public Health					0
		Slotervaart Hospital				0	
		St Lucas Andreas Hospital				0	
		Municipal Health Service Amsterdam					0
	Baarn	Medical Centre I				0	
	Haarlem	General practice	0				
		Regional Laboratory for Public Health		0	0		0
	Hilversum	Central Bacteriological Laboratory			0	0	
	Hoorn	Westfries Gasthuis				0	
	Huizen	General practice	0				
	Zaandam	Zaans Medical Centre				0	

Centres contributing to the surveillance of resistance to antimicrobial agents (continued)

Province	Town	Name and type of centre	COM	IUP	ISIS	Men	GRAS
Zuid Holland	Capelle a/d IJssel	IJsselland Hospital				0	
	Delft	Diagnostic Center SSDZ			0	0	
	's-Gravenhage	Bronovo Hospital		0		0	
		General practice	0				
		Leyenburg Hospital				0	
		Regional Laboratory for Public Health				0	
		Haga Hospital			0	0	
		Medical Centre Haaglanden			0	0	0
		Municipal Health Service Den Haag					0
	Dordrecht	Regional Laboratory for Public Health				0	0
	Gorkum	Regional Laboratory for Public Health				0	
	Gouda	Groene Hart Hospital				0	
	Leiden	Diakonessenhuis		0		0	
		KML Laboratory				0	
		University Medical Centre			0		
	Leiderdorp	Rijnland Hospital				0	
	Rotterdam	General practice	0				
		Erasmus University Medical Centre				0	0
		Ikazia Hospital					
		Maasstadziekenhuis		0		0	
		Sophia Children's Hospital				0	
		St Franciscus Gasthuis				0	
		Municipal Health Service Rotterdam - Rijnmond					0
	Schiedam	Vlietland Hospital				0	
	Spijkenisse	Ruwaard vd Putten Hospital			0	0	
	Voorhout	General practice	0				
	Woerden	Zuwe Hofpoort Hospital				0	
Noord Brabant	Bergen op Zoom	Lievensberg Hospital			0	0	
	Breda	Amphia Hospital			0		0
		Municipal Health Service West-Brabant					0
	Eindhoven	Municipal Health Service Zuidoost Brabant					0
	Helmond	Jeroen Bosch Medical Centre			0		0
	's Hertogenbosch	Regional Laboratory for Public Health				0	
		General practice	0				
	Ravenstein	Franciscus Hospital			0	0	
	Roosendaal	General practice	0				
	Rosmalen	Regional Laboratory for Public Health		0	0	0	0
	Tilburg	Municipal Health Service Hart voor Brabant					0
		General practice	0				
	Uden	Laboratory for Medical Microbiology				0	0
Limburg	Geleen	Municipal Health Service Zuid Limburg	0				0
	Heerlen	Regional Laboratory for Public Health			0	0	0
		Atrium Medical Centre				0	0
	Maastricht	General practice	0				
		Nursing home Vivre location KLevarie	0				
		Nursing home De Zeven Bronnen	0				
		Academic Medical Centre		0		0	0
		Municipal Health Service Zuid-Limburg					0
	Roermond	Laurentius Hospital			0	0	0
	Sittard	Maasland Hospital				0	
	Venlo	VieCuri Medical Centre		0		0	0
		Municipal Health Service Limburg Noord					0
	Weert	St Jansgasthuis			0	0	
Zeeland	Goes	Regional Laboratory for Public Health		0	0	0	0
		Municipal Health Service Zeeland					0
	Middelburg	General practice	0				
	Terneuzen	General practice	0				
		Regional Laboratory for Public Health			0	0	

COM=Community, IUP=Intensive Cares/Urology Services/Pulmonology Services, ISIS= Former ISIS (until 2007) and ISIS-AR laboratories, Men=Meningitis Surveillance, GRAS=Gonococcal Resistance Surveillance.

Preface

This is NethMap 2011, the ninth SWAB/RIVM report on the use of antibiotics and trends in antimicrobial resistance in The Netherlands in 2009 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 (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, that 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), 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 2011 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). Recently MARAN 2009 has been published. 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. 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 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 (www.ecdc.europa.eu/en/activities/surveillance/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.

The editors:
Prof dr Mieke Hoogkamp-Korstanje
Dr Johan W. Mouton
Dr Nienke van de Sande-Bruinsma

Content

1. Summary

NethMap is the annual report of SWAB on the use of antimicrobial agents and the prevalence of resistance to these agents among common human pathogens isolated in the Netherlands. Until 2009 this information was restricted to antibacterial agents and bacterial species, but from that year on NethMap contains data on use and 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.

The overall use of antimicrobial agents in primary health care remained below 10 defined daily dosages (DDD) per 1000 inhabitants per day until 2005. In 2005 there was a slight increase in use to 10.5 DDD/1000 inhabitant days, and since then there was a further increase to 11 DDD/1000 inhabitant days in 2008 that stabilised in 2009 and 2010. 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. Of the antibiotics used in general practice 24% are tetracyclines, whereas these drugs are seldom prescribed in hospitals. Nitrofurantoin use has been on the rise in recent years, most probably because of the increased resistance to trimethoprim in *Escherichia coli* in urinary tract infection. This was reported by the SWAB surveillance and resulted in subsequent changes in treatment guidelines. Consequently a decrease was noticed in the use of trimethoprim and sulphonamide, but this has now stabilized. Trimethoprim is nowadays a second choice antibiotic for treatment of urinary tract infections. Fluoroquinolone use has also stabilized although there were some minor changes within the class. NethMap 2011 reports a further substitution of amoxicillin by co-amoxiclav and an increase in macrolide use, in particular azithromycin. The background of some of these changes needs further study since this is often not supported by evidence of less effectiveness of the current guidelines or proven efficacy of specific drugs. The use of antimicrobials against tuberculosis and other mycobacterial infections is in line with the specialist guidelines. In the Netherlands resistance problems are limited in this field.

Since 2003 the number of hospital admissions as well as the antibiotic use has increased with 22%. Total use and clinical activities are obviously running in parallel. Different trends within the given groups of antibiotics are recognisable when usage per bed day and usage per admission are compared.

In 2009, the total antibiotic use increased with reference to the year before when expressed in DDD per 100 patient-days, but decreased when expressed in DDD per 100 admissions. 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. The use per individual patient has remained constant, but overall an increase in the exposition of antibiotics is observed in the hospitals which increases the risk of resistance development. The increase in DDD per 100 patient-days is seen for almost all groups of antibiotics except for combinations of sulphonamides and trimethoprim (including derivatives) and nitrofuran derivatives. A decrease in DDD per 100 admissions is also seen in most of the antibiotic classes. However, the use of penicillins with extended spectrum, the beta-lactamase sensitive penicillins and the other quinolones has increased compared to 2008. Nonetheless, these were just minor increases. Over the past few years the use of these compounds remained constant. Amoxicillin, co-amoxiclav, other penicillins and cephalosporins still account for almost half of all antibiotics used in Dutch hospitals.
The use of systemic antimycotic drugs in university medical centres is still increasing compared to that in general hospitals and has now surpassed four times the use. This is a clear indication of the difference in patient populations between these two types of hospitals, the former harbouring a large group of severely immune compromised patients.
NethMap 2011 again shows a difference in the use of antiviral drugs between university hospitals and general hospitals, The use of systemic antivirals in 2009 has been divided in use of antivirals for acute and for chronic infections. When expressed in DDD per 100 patient-days, use of antivirals for acute infections was similar in both university and general hospitals. However, the use of antivirals for chronic infections was more than six times higher in university hospitals compared to general hospitals.

Like before NethMap 2011 presents data on antimicrobial resistance in the community and in hospitals. SWAB resistance surveillance data are derived from the so called 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 2011 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. Thus resistance data are presented for *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Proteus mirabilis* and *Pseudomonas aeruginosa* and the results for patients visiting the general practitioner, patients in

outpatient departments, hospital departments and patients in the intensive care are compared. 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 169 unselected strains of *E. coli*, derived from uncomplicated urinary tract infections in general practice in men (>11 years), collected through the NIVEL Sentinel Stations Network, and compared to those in men and women in 2009 as well as by selecting patients in the ISIS-AR database. Overall, the resistant rates for most other antibiotics stabilized and for trimethoprim decreased slightly. Resistance rate were 34% for amoxicillin, 12% for co-amoxiclav, 1% for nitrofurantoin and 3.5% for quinolones, respectively. The resistance rates as reported from the ISIS-AR database in selected patients from general practice are all higher. However, these include complicated urinary tract infections as well and therefore include patients that have been treated more often. This is particularly true for quinolones with resistance rates just above 10%. Amoxicillin resistance was 43% and co-amoxiclav resistance 20%.
Resistance against ciprofloxacin of *N. gonorrhoeae* has not further increased and was 47% in 2010 (52 % in 2009). In 2010 the development of resistance against third generation cephalosporins has further increased and is now 9%. Incidental therapy failure of ceftriaxone has been reported. In the so-called GRAS project of the RIVM the development of resistance of *N. gonorrhoeae* is closely monitored.
Resistance in *M. tuberculosis* strains appears to be maintained at the same low level as before. A slow rise in rifampicin resistance over previous years has stabilized. The level of multiresistance in 2010 was at 0.4%.
For many antimicrobials, resistance is steadily increasing. Ciprofloxacin resistance among *E. coli* from Unselected Hospital Departments is now over 12%, and resistance to co-amoxiclav 24%. Increasing *E. coli* resistance was found in all study populations for almost all antibiotic groups. In intensive care units, data are reported from SIRIN. In ICUs, *E. coli* multiresistance was not yet reported before 1998 but increased to 9% in 2008 and did not increase further. ESBL producing strains, stable over the last years at 6%, now has increased to 9%. Carbapenems and the (toxic) colistin are then often the only remaining effective drugs when infections with such strains can be treated. Similar conclusions can be drawn for the *Klebsiella*, *Enterobacter* and *Proteus* strains studied. Carbapenem resistance was found for the first time. Ceftazidime resistance increased further to 12% for *P. aeruginosa* in ICUs.
The results for *Staphylococcus aureus* were not much different from previous years, although methicillin resistant *S. aureus*, MRSA, increased slightly and is now 1.6 % in Unselected Hospital Departments. Vancomycin resistance in *S. aureus* is rarely encountered in the Netherlands. Vancomycin is still the rescue drug for methicillin resistant *S. aureus* infection. Rifampicin resistance was lower than 1%, and mupirocin resistance 1% in *S. aureus*.
Animal husbandry related MRSA isolates (CC398 strains) were at approximately the same level over the last year, 38% 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 amoxicillin resistance to 17 % as well as co-amoxiclav resistance to 4.5% was 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 in 2010 still at a critical height of 10% and tetracycline resistance parallels this. Resistance to penicillin, the most important antibiotic prescribed for serious pneumococcal disease, remains at the low level of 1.8 %.
Studies in *Aspergillus* spp. indicate that resistance to azoles is increasing significantly. A large retrospective study in the Radboud UNMC showed that azole resistance emerged in 2000. Since then, resistance has slowly crept upwards and is now over 5%. At the same time, the resistance mechanisms have been elucidated, and a second resistance mechanism has now been described. It is expected that azole resistance will continue to rise in the near future. This will limit treatment option significantly.
Finally, data are presented from surveillance studies of influenza viruses in The Netherlands are indicating treatment limitations due to emerging resistance against anti -influenza specific drugs such as oseltamivir, but this is strain dependent.
NethMap 2011 furthermore provides the reader with an overview of more than 40 published studies performed in The Netherlands since 1990 on antibiotic resistance.

We can conclude that, in general and on the basis of these and many more data presented in NethMap 2011, we can, again, not be too optimistic about the situation of the emergence of antibiotic resistance in the Netherlands, while at the same time we are still better off than many countries surrounding us in Europe, according to data of the European Antimicrobial Resistance Surveillance System.

2. Samenvatting

NethMap is het jaarlijkse rapport van de SWAB over het gebruik van antimicrobiële middelen en resistentie in de meest voorkomende, voor de mens pathogene, micro-organismen in Nederland. Tot 2009 beperkte deze informatie zich tot antibiotica en verschillende voor de geneeskunde relevante bacteriesoorten. Vanaf 2009 wordt de informatie in NethMap aangevuld met trends in het gebruik van middelen tegen diepe schimmelinfecties, antivirale middelen (bij influenzavirussen) en resistentie bij schimmels. De data in NethMap zijn gebaseerd op sinds 1996 systematisch verzamelde en bewerkte gegevens over antimicrobiële middelen en de trends in resistentie daartegen.

Het gebruik van antibiotica in de Nederlandse eerstelijns gezondheidszorg is tot 2005 steeds onder de 10 standaard dagdoseringen (DDDs) per 1000 inwoners per dag gebleven. In 2005 was het gebruik iets hoger, 10,5 DDD/1000 inwoner-dagen, en het is sindsdien licht verder gestegen tot 11. DDD/1000 inwoner-dagen in 2008. In 2009 lijkt er een stabilisatie tot stand te zijn gekomen en ook dit jaar is dit niet verder gestegen. Het antibioticagebruik in Nederland is nog steeds laag vergeleken met andere landen in Europa.
De verdeling van het gebruik van antibiotica bij de verschillende patiëntpopulaties is duidelijk heel verschillend. Zo is te zien dat tetracyclinen 24 % uitmaken van het gebruik buiten het ziekenhuis, terwijl deze middelen intramuraal slechts zelden worden toegepast. Het gebruik van nitrofurantoïne was al langere tijd aan het stijgen. Waarschijnlijk kwam dit door de toegenomen resistentie tegen trimethoprim bij urineweginfecties en de hierop volgende aanpassingen in de richtlijnen voor huisartsen, mede ten gevolge van de resultaten van de SWAB surveillance. We zagen dan ook een gelijktijdige daling van het trimethoprim en sulfonamide gebruik optreden, maar er heeft in 2010 geen verdere daling plaats gevonden. Trimethoprim is nu een tweede keus middel geworden bij de behandeling van ongecompliceerde urineweginfecties. Het gebruik van fluorochinolonen lijkt ook gestabiliseerd, hoewel er wel verschuivingen binnen deze klasse optrad. Wat in 2010 weer opvalt, is de toenemende vervanging van amoxicilline door de combinatie van amoxicilline met de beta-lactamase remmer clavulaanzuur (co-amoxiclav). Ook zien we een verder toenemend gebruik van macroliden, in het bijzonder azithromycine.
Het gebruik van middelen tegen tuberculose en tegen infecties veroorzaakt door andere mycobacteriën is overeenkomstig de specialistische richtlijnen. Resistentie tegen deze middelen komt in Nederland beperkt voor.

Vanaf 2003 is zowel het aantal ziekenhuisopnames als het antibioticagebruik in DDD's gestegen met 22%. Het totale gebruik en de klinische activiteiten houden klaarblijkelijk gelijke pas. Tussen de verschillende groepen antibiotica zijn echter over de laatste jaren verschillende trends zichtbaar als gebruik per opname en gebruik per beddag in ogenschouw worden genomen. Het gebruik nam toe als het gemeten wordt in DDD per 100 patiëntendagen maar nam juist af als het uitgedrukt wordt in het aantal ziekenhuisopnames. Het gebruik per patiënt blijft daarmee gelijk, maar over het geheel genomen is er een toename, wat een hogere expositie in het ziekenhuis ten gevolge heeft en daardoor meer risico op resistentieontwikkeling geeft. In NethMap 2011 zien we deze ontwikkeling voor bijna alle groepen antibiotica met uitzondering van de combinatie daarvan met sulfamethoxazol en nitrofurantoïne.
Bijna de helft van het antibioticagebruik in ziekenhuizen bestaat uit amoxicilline, al of niet in combinatie met de beta-lactamaseremmer clavulaanzuur, en andere middelen uit de penicillinegroep.
Het verschil in gebruik van systemische antimycotica in universitaire centra ten opzichte van algemene ziekenhuizen is verder gestegen naar een factor 4, hetgeen het verschil in patiëntenpopulaties weergeeft. In universitaire centra worden meer oncologische en transplantatie patiënten behandeld die extra vatbaar zijn voor infecties.
NethMap 2011 toont ook de verschillen van het hogere gebruik van antivirale middelen bij universitaire centra vergeleken met andere ziekenhuizen. Deze zijn opvallend. Ook dit is een reflectie van de verschillende patiëntenpopulaties in ziekenhuizen.

De SWAB surveillance gegevens over resistentie in specifieke patiëntenpopulaties worden ontleend aan het ISIS-AR (Infectieziekten Surveillance Informatie Systeem – Antibiotica Resistentie), de SERIN- en de SIRIN (Surveillance van Extra-/Intramurale Resistentie in Nederland) projecten. In NethMap2011 worden de data, die door deze drie activiteiten zijn verkregen, met elkaar vergeleken en besproken met inachtneming van de verschillende methodes die zijn toegepast om de data te kunnen verzamelen. Gegevens worden gepresenteerd voor o.a. *E. coli*, *Klebsiella*- en *Enterobacter* soorten, *Proteus mirabilis* en *Pseudomonas aeruginosa*. De resultaten van de resistentiemetingen van isolaten van patiënten uit de huisartsenpopulatie, poliklinieken, algemene en specifieke afdelingen (urologie, intensive care, longafdeling) van ziekenhuizen worden met elkaar vergeleken. Het onderzoek bij de bevolking richt zich op het dragerschap van resistente, potentieel pathogene bacteriesoorten bij gezonde personen, resistente bacteriën gevonden in materialen afkomstig van patiënten die de huisarts bezoeken en resistentie in bacteriesoorten die een bedreiging vormen voor de publieke gezondheid zoals mycobacteriën (tuberculose), meningokokken en gonokokken.

Bij 169 stammen van *E. coli*, geïsoleerd bij ongeselecteerde manlijke patiënten (>11 jaar) met ongecompliceerde urineweginfecties in huisarts-praktijken, aangesloten bij het NIVEL, werden in 2009 de volgende resistentiepercentages gevonden voor: amoxicilline 34% (20% in 2000), co-amoxiclav 12% (stabiel sinds 2004), trimethoprim 19% (23% in 2004) en nitrofurantoïne 1% (stabiel). Voorts werd voor ciprofloxacine een stabiele 3,5% resistentie gevonden. ESBL producerende stammen worden incidenteel waargenomen, 5 stammen (1%) in 2009. Drie procent van de *E. coli* stammen vertoonden multiresistentie tegen 3 groepen antibiotica waardoor de empirische keuze van het juiste middel door de huisarts ernstig wordt belemmerd.
De resistentie bij *Neisseria gonorrhoeae* is op een verontrustend hoog niveau. Voor ciprofloxacine werd in 2010 een resistentie percentage van 47% (52% in 2009) gevonden. De resistentie tegen derde generatie cefalosporinen is inmiddels 9%. Casuïstiek van therapiefalen met ceftriaxon is gepubliceerd.
In het zogenaamde GRAS project wordt de resistentieontwikkeling van gonokokken nauwlettend in de gaten gehouden.
Resistentie van *Mycobacterium tuberculosis* stammen blijkt zich op hetzelfde niveau te handhaven als in vorige jaren. Er is een lichte stijging van rifampicineresistentie waarneembaar. Multiresistentie wordt slechts in 0,4 % van de isolaten gevonden.
Bij *E. coli* is de voordurende stijging van ciprofloxacine-resistentie in ziekenhuizen opmerkelijk (12%). In alle studiepopulaties wordt bij *E. coli* een stijging van de resistentie tegen vrijwel alle verschillende antibioticagroepen gevonden. In ICUs gerapporteerd door SIRIN, werd van deze soort vóór 1998 nog geen multiresistentie gerapporteerd. In 2008 werd een niveau van 9% multiresistentie waargenomen, maar dit steeg daarna niet verder. ESBL vormende stammen vormen sinds 2000 een bedreiging en bereikten een percentage van 9%. Reserve antibiotica uit de carbapenem groep en het toxische colistine zijn nu de enige optie wanneer infecties met deze stammen moeten worden bestreden. Ongeveer hetzelfde kan worden gezegd voor *Klebsiella*-, *Enterobacter*- en *Proteus* soorten, die eveneens het ESBL resistentiemechanisme kunnen herbergen.
Resistentie tegen carbapenems wordt nu voor het eerst in NethMap 2011 gerapporteerd en geeft een zorgwekkende ontwikkeling weer. In een aantal Europese landen vormt dit nu al een ernstig probleem en met verdere import moet rekening worden gehouden.
De resistentie van *P. aeruginosa* tegen het derde generatie cefalosporine ceftazidime steeg verder en bereikt 12% op intensive cares.
Staphylococcus aureus gedraagt zich weinig anders dan in voorgaande jaren, hoewel het percentage meticilline-resistente stammen (MRSA) iets steeg naar 1,6% bij ongeselecteerde ziekenhuisafdelingen. Vancomycine vormt het ultieme reservemiddel bij MRSA infecties. Resistentie tegen vancomycine is uiterst zeldzaam. MRSA stammen (CC398) die geassocieerd zijn met contact met nutsdieren (varkens, mestkalveren) vormden in 2009 38 % van de isolaten. Rifampicine resistentie was iets onder de 1%, en mupirocine resistentie 1% bij *S. aureus*.

In de ziekenhuizen zijn gegevens verzameld van resistentie onder pneumokokken en *Haemophilus influenzae*. Deze bacteriesoorten zullen in het overgrote deel community acquired zijn en hun resistentieprofielen zullen daarom waarschijnlijk ook een redelijke afspiegeling vormen van die van stammen buiten het ziekenhuis. Opmerkelijk is de toename van resistentie bij *Haemophilus* tegen zowel amoxicilline (17%) als amoxicilline met clavulaanzuur (4.5%) op ongeselecteerde afdelingen. Dit is een aanwijzing voor de verspreiding van zogenaamde Beta-Lactamase Negatieve Amoxicilline Resistente (BLNAR) stammen. Doxycycline is nog een redelijk alternatief bij dit type resistente *H. influenzae* infecties. Resistentie tegen derde generatie cefalosporinen is zeldzaam (<1%).
Bij pneumokokken blijft de macrolideresistentie op 10%, en gaat ongeveer gelijk op met resistentie tegen tetracyclinen. Resistentie tegen penicilline, het belangrijkste middel tegen ernstige pneumokokken-infecties, blijft in Nederland op een laag niveau van 1.8%.
Diepe schimmelinfecties met *Aspergillus* soorten vormen een ernstige bedreiging voor immuundeficiënte patiënten in het ziekenhuis. Azolen zijn belangrijke middelen om deze infecties te bestrijden. NethMap 2010 presenteerde voor het eerst resistentieontwikkeling tegen azolen. Dit jaar laat een verdere resistentieontwikkeling zien, waarbij ook nu voor het eerst een tweede resistentiemechanisme wordt beschreven. Een grote retrospectieve studie in het UMC St Radboud, Nijmegen laat zien dat er een aanzienlijke resistentieontwikkeling is sinds 2000 tot meer dan 5%. Verwacht wordt dat deze ontwikkeling zich voortzet, waardoor de behandelopties voor dit type levensbedreigende infecties aanzienlijk worden beperkt.
Tenslotte worden de resultaten van surveillance studies naar influenzavirus in Nederland weergegeven. Resistentie tegen antivirale middelen zoals oseltamivir stijgt, maar dit is wel sterk afhankelijk van de stam.
NethMap 2011 biedt voorts een overzicht van de belangrijkste in Nederland bewerkte wetenschappelijke publicaties op het gebied van resistentieontwikkeling, meer dan 40 studies sinds 1990.

Helaas kan NethMap ook nu geen optimistisch beeld geven van de zich ontwikkelende resistentieproblematiek in Nederland, al is de situatie in vergelijking met vele andere ons omringende landen nog vrij gunstig.
Zie voor deze vergelijking de websites van ISIS-AR (www.ISIS-web.nl) en European Antimicrobial Resistance Surveillance network (EARSS-net).

3. Use of antimicrobials

This part of the report considers the use of antimicrobial agents in human medicine only. Data on the use of such agents in animal husbandry and veterinary medicine are reported elsewhere (1). Human consumption is presented in two sections. The first section describes the prescription and use of antibiotics in the community, also termed as "Primary Health Care". About 85% of antibiotic use in primary health care is prescribed by general practitioners (2). The second section presents surveillance data on the total hospital consumption of antimicrobial agents in acute care hospitals in the Netherlands. Details on the structural acquisition and analysis of these consumption data are presented in the Materials and Methods section.

3.1 Primary health care

3.1.1 Use of antibiotics

From 1998-2004, the total antibiotic use was 10 DDD/1000 inhabitant-days. Over the past five years, the use gradually increased to 11 DDD/1000 inhabitant-days. In 2010, the use of antibiotics remained stable compared to 2009. The distribution of antibiotics by class in 2010 is presented in figure 1. Tetracyclines (mainly doxycycline) represented 24% of total antibiotic use in primary health care. Other frequently use antibiotics were penicillins with extended spectrum (mainly amoxicillin), combinations of penicillins with beta-lactamase inhibitors (essentially co-amoxiclav) and macrolides, each representing 16%, 16% and 13% of the total use, respectively.

From 2005 to 2009, the use of amoxicillin remained stable at about 1.9 DDD/1000 inhabitant-days, but it decreased in 2010. Meanwhile, the use of co-amoxiclav further increased and equalled the use of amoxicillin at 1.8 DDD/1000 inhabitant-days in 2010 (table 1; figure 2). The use of macrolides remained stable at 1.31 DDD/1000 inhabitant-days in 2010 (table 1). The use of the different macrolides is depicted in figure 3. Clarithromycin was still the most commonly used macrolide. However, the use of azithromycin was rapidly increasing. The use of erythromycin remained stable at 0.10 DDD/1000 inhabitant-days in 2010.

Regarding the fluoroquinolones, the use of levofloxacin remained stable at 0.06 DDD/1000 inhabitant-days, whereas the use of ciprofloxacin slightly increased to 0.50 DDD/1000 inhabitant-days. On the contrary, the use of ofloxacin and norfloxacin decreased slightly towards 0.05 and 0.20 DDD/1000 inhabitant-days respectively (table 1; figure 4). After the decrease in use of

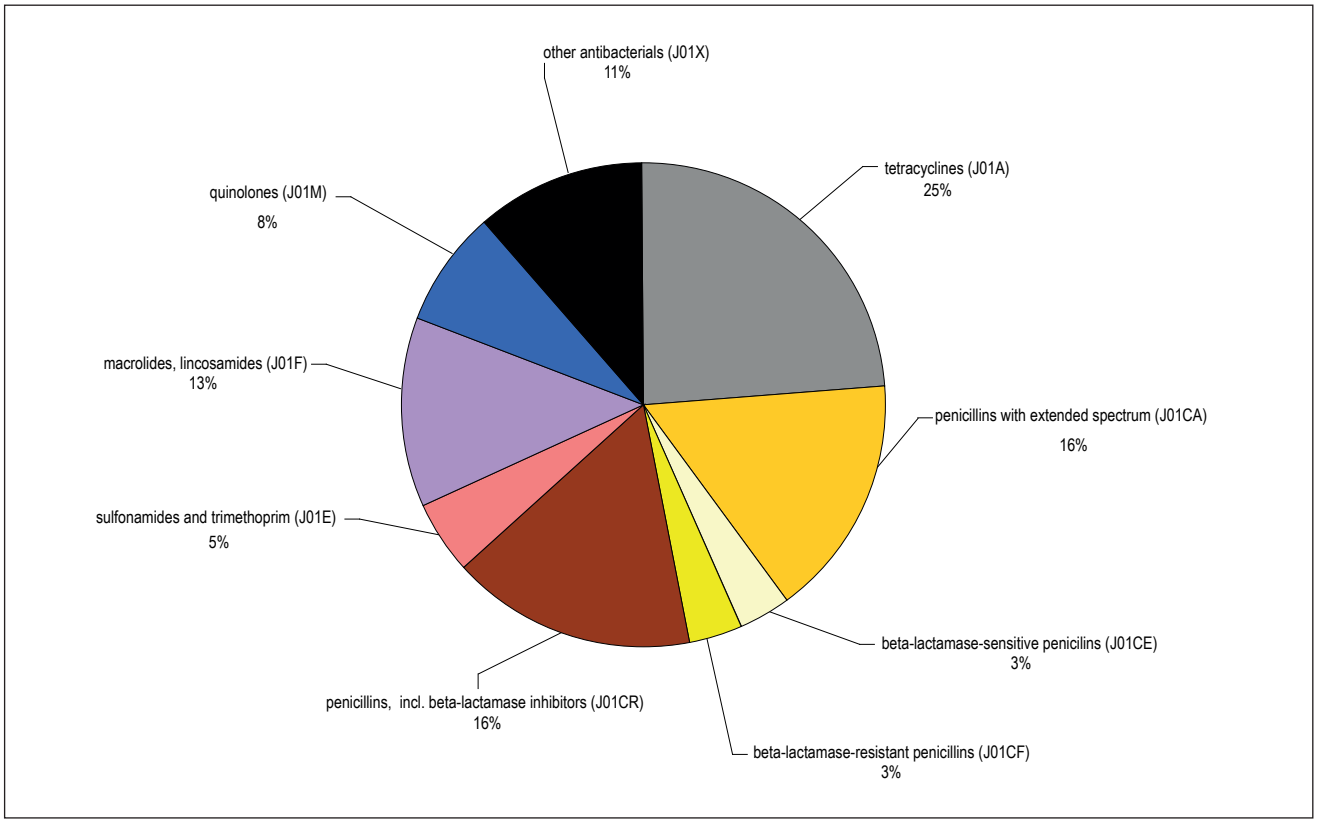

Figure 1. Distribution of the use of antibiotics for systemic use (J01) in primary health care, 2010 (SFK).

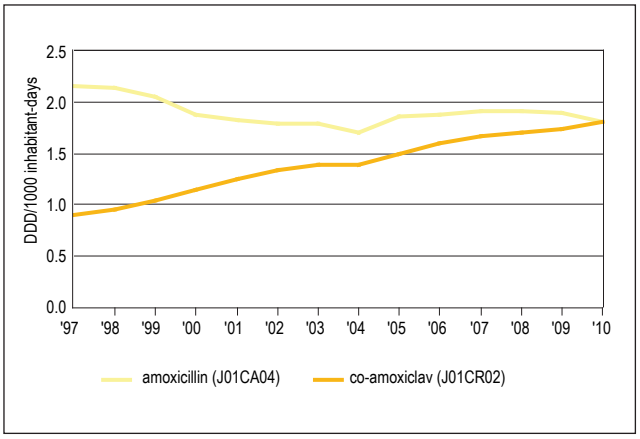

Figure 2. Use of amoxicillin and co-amoxiclav for systemic use in primary health care, 1997-2010 (SFK).

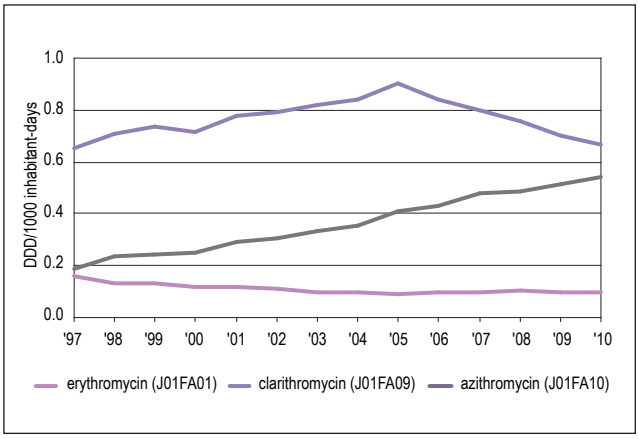

Figure 3. Use of macrolides for systemic use in primary health care, 1997-2010 (SFK).

moxifloxacin in 2008, it remained stable in the following years at 0.04 DDD/1000 inhabitant-days.
The use of nitrofurantoin was still increasing to 1.23 DDD/1000 inhabitant-days in 2010 compared to 1.17 DDD/1000 inhabitant-days in 2009 (table 1; figure 5).
Over the past four years, the use of sulphonamides and trimethoprim (J01EA and J01EE combined) remained rather stable at 0.55 DDD/1000 inhabitant-days (table 1; figure 5).

3.1.2 Use of antimycobacterials

Between 1998 and 2010 the use of antimycobacterials in primary health care remained rather constant (table 2). Isoniazid, rifampicin and dapsone were the most frequently prescribed antimycobacterials in 2010. The use of ethambutol equalled the use of pyrazinamide.

3.1.3 Use of antibiotics and chemotherapeutics for dermatological use

The use of fusidic acid increased from 1.31 DDD/1000 inhabitant-days in 1998 to 2.67 DDD/1000 inhabitant-days in 2010 (table 3; figure 6). The use of silver sulfadiazine initially slightly decreased but remained rather constant in the last four years. This also accounted for mupirocin. The data on topical use of acyclovir were not reliable as most of its use concerned unregistered 'over the counter sales'. The use of metronidazole increased from 0.38 in 1998 to 0.83 DDD/1000 inhabitant-days in 2010.

Discussion

Antibiotic consumption in primary health care remained stable at 10 DDD/1000 inhabitant-days until 2004.

Table 1. 12-years data on the use of antibiotics for systemic use (J01) in primary care (DDD/1000 inhabitant-days), 1999-2010 (Source: SFK).

ATC Group*	Therapeutic group	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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

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

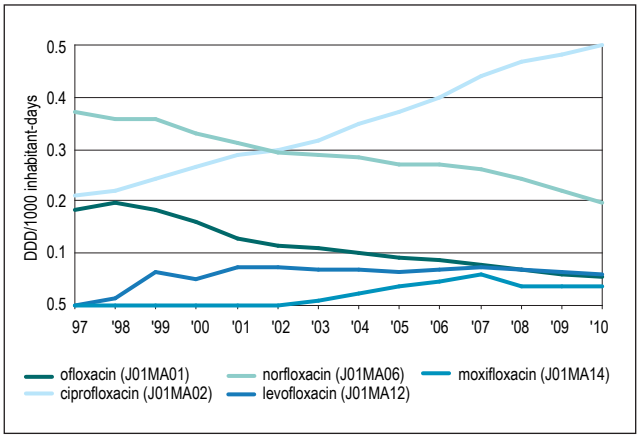

Figure 4. Use of fluoroquinolones for systemic use in primary health care, 1997-2010 (SFK).

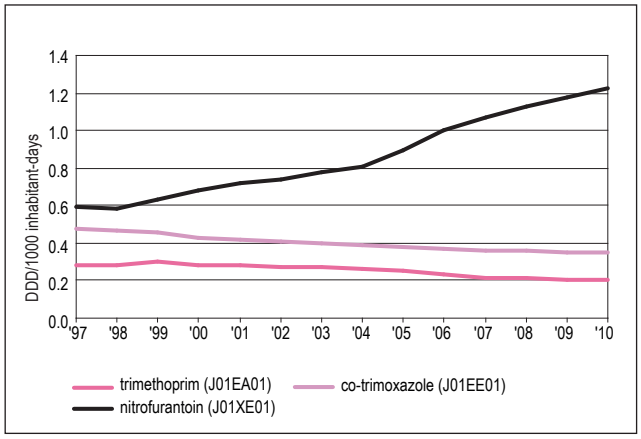

Figure 5. Use of nitrofurantoin, trimethoprim and co-trimoxazole for systemic use in primary health care, 1997-2010 (SFK).

From 2005 to 2009, consumption gradually increased to 11 DDD/1000 inhabitant-days. In 2010 consumption remained stable at 11.2 DDD/1000 inhabitant-days. The use of antibiotics in the Netherlands is still low compared to other European countries (3).

The use of nitrofurantoin is still increasing. This is probably explained by the national guideline of the Dutch College of General practitioners (NHG)(4) regarding the prescription of nitrofurantoin for the treatment of uncomplicated urinary tract infections. In 2005 this guideline was revised and because of lower resistance levels to nitrofurantoin this drug was classified as the first choice treatment (for 5 days). Trimethoprim is nowadays ranked as the antibiotic of second choice in treatment of uncomplicated urinary tract infections.

Furthermore, 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 2010. Also within the class of macrolides, a shift was observed from erythromycin to newer macrolides such as clarithromycin and azithromycin. The use of azithromycin further increased in 2010. These trends may be relevant from the perspective of growing rates of resistance among common pathogens and therewith the rate of treatment failures.

Table 2. 12-years data on antimycobacterial drugs in primary care (DDD/1000 inhabitant-days), 1998-2010 (Source: SFK).

ATC Group*	Antimycobacterials	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J04AB02	Rifampicin	0.05	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.07
J04AC01	Isoniazid	0.11	0.12	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.07
J04AK01	Pyrazinamide	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02
J04AK02	Ethambutol	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J04AM02	Rifampicin and isoniazid	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J04BA02	Dapsone	0.10	0.10	0.09	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08

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

Table 3. 12-years data on antibiotics and chemotherapeutics for dermatological use in primary care (DDD/1000 inhabitant-days), 1998-2010 (Source: SFK)

ATC Group*	Antibiotics and chemotherapeutics	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
D06AA04	Tetracycline	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.02
D06AX01	Fusidic acid	1.31	1.55	1.72	1.91	2.08	2.29	2.29	2.26	2.65	2.46	2.45	2.55	2.67
D06AX09	Mupirocin	0.48	0.43	0.40	0.39	0.38	0.40	0.38	0.37	0.20	0.29	0.27	0.26	0.25
D06BA01	Silver sulfadiazine	1.24	1.32	1.25	1.25	1.23	1.27	1.17	1.11	1.15	1.15	1.17	1.18	1.16
D06BB03	Acyclovir	0.18	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
D06BB04	Podophyllotoxin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D06BX01	Metronidazole	0.38	0.44	0.50	0.56	0.60	0.61	0.64	0.67	0.68	0.75	0.78	0.80	0.83

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

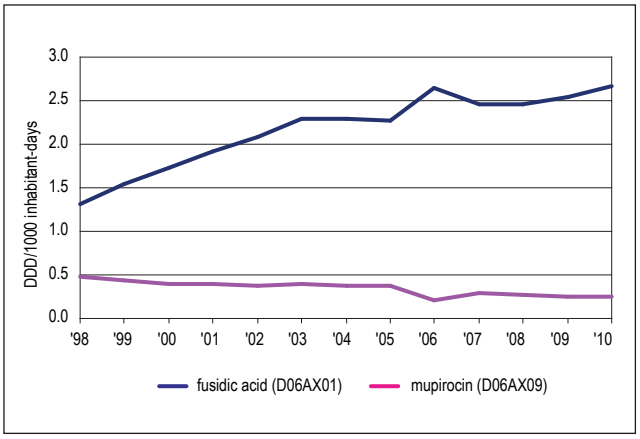

Figure 6. Use of fusidic acid and mupirocin in primary health care, 1998-2010 (SFK)

The use of antimycobacterials seems to be in line with the general principles of the treatment and prophylaxis of tuberculosis. The constant use of these drugs over the years is suggestive for limited resistance problems over the past years.
To better understand the topical use of fusidic acid and mupirocin, an in depth analysis of the indications of use is warranted. Since topical acyclovir is nowadays freely available as an 'over the counter' drug, no use has been registered by the community pharmacies.

3.2 Hospitals

3.2.1 Hospital use of antibiotics

The data on hospital use of antibiotics (J01 class) for the years 2007 and 2008 have been recalculated due to some discrepancies. In this current NethMap report, the corrected data of these years are presented.
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 both units of measurement do not always correlate (tables 4 and 5).
In 2009, the total systemic use of antibiotics in our cohort of hospitals increased to 70.88 DDD per 100 patient-days (+6.2% compared to 2008). The total number of DDD per 100 admissions decreased by 6.8% from 345 DDD in 2008 to 321 DDD in 2009 (tables 4 and 5). This decrease was seen for almost all groups of antibiotics. The distribution of antibiotics per class in 2009 is depicted in figure 7.
The relative use of different subclasses of antibiotics remained more or less constant over the past years,

Table 4. Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2000-2009 (Source: SWAB).*

ATC group*	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	1.6	1.6	1.7	1.4	1.5	1.6	1.6	1.4	1.4	1.6
J01CA	Penicillins with extended spectrum	5.8	6.0	6.1	6.0	6.0	6.7	7.6	7.3	5.5	7.6
J01CE	Beta-lactamase sensitive penicillins	1.1	1.3	1.2	1.2	1.4	1.4	1.4	1.2	1.1	1.6
J01CF	Beta-lactamase resistant penicillins	4.3	4.3	4.4	5.4	5.7	5.8	5.9	5.6	5.4	6.6
J01CR	Combinations of penicillins. incl. beta-lactamase-inhibitors	8.9	9.9	12.2	12.1	12.8	13.9	15.1	14.0	13.5	16.5
J01DB -DE	Cephalosporins	5.6	6.1	6.3	6.5	7.0	7.4	8.4	8.4	7.4	10.1
J01DF	Monobactams	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.0
J01DH	Carbapenems	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.8	0.85	1.1
J01EA	Trimethoprim and derivatives	0.3	0.5	0.5	0.5	0.4	0.6	0.8	0.5	0.3	0.4
J01EC	Intermediate-acting sulfonamides	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.05	0.0
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	2.3	2.3	2.4	2.3	2.1	2.3	2.1	2.3	2.0	2.0
J01FA	Macrolides	2.1	2.3	2.7	2.4	2.3	2.8	2.5	2.7	2.3	2.6
J01FF	Lincosamides	1.2	1.3	1.5	1.6	1.8	1.9	2.0	2.1	1.8	2.4
J01GB	Aminoglycosides	2.1	2.0	2.1	2.5	2.2	2.6	2.5	2.5	3.3	4.2
J01MA	Fluoroquinolones	4.7	5.5	5.7	6.4	6.5	7.3	8.0	7.6	9.6	9.3
J01MB	Other quinolones	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.05	0.1
J01XA	Glycopeptides	0.5	0.5	0.5	0.5	0.6	0.8	0.7	1.0	1.0	1.3
J01XB	Polymyxins	0.3	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2
J01XC	Steroid antibacterials (fusidic acid)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.06	0.1
J01XD	Imidazole derivatives	1.1	1.3	1.5	1.6	1.7	1.5	1.7	1.8	1.4	1.8
J01XE	Nitrofuran derivatives	0.5	0.5	0.5	0.7	0.9	1.0	1.0	1.1	1.0	1.1
J01XX05	Methenamine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.1
J01	Antibiotics for systemic use (total)	43	46.5	50.2	51.9	53.8	58.3	62.2	60.9	58.1	70.9

* from the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

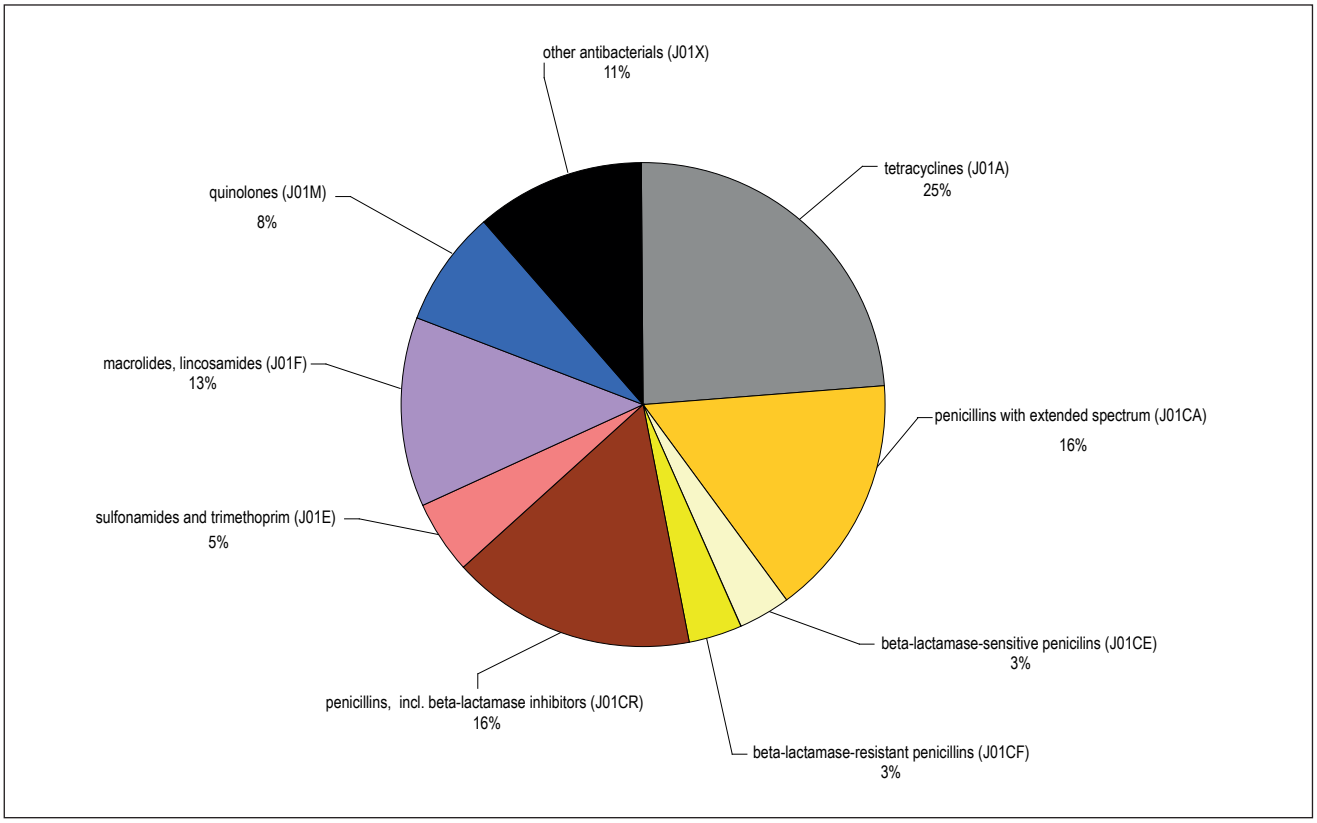

Figure 7. Distribution of the use of antibiotics for systemic use (J01) in hospitals, 2009 (SWAB).

although we saw an increase in the use of cephalosporins (6.3 to 10.1 DDD per 100 patient-days), aminoglycosides (2.1 to 4.2 DDD per 100 patient-days), fluoroquinolones (5.7 to 9.3 DDD per 100 patient-days) and glycopeptides (0.5 to 1.3 DDD per 100 patient-days) (table 4). The use of carbapenems has increased particularly over the last three years, whereas the use of trimethoprim and its derivatives has decreased in the same period. The use of fusidic acid has increased in 2007 as well as in 2008 but tends to be stabilized in 2009. Finally, use of beta-lactamase sensitive and beta-lactamase resistant penicillins, combinations of penicillins and the use of glycopeptides has gradually increased over the past few years (table 4).

All categories of beta-lactam antibiotics showed an increase in use compared to 2008 when measured in DDD per 100 patient-days (figure 8A). When combined, they showed an increase from 30.4 to 32.3 DDD/100 patient-days. However, when measured in DDD per 100 admissions a decrease was seen in total penicillin use (156.9 to 146.2 DDD/100 admissions). This decrease is mainly caused by a decrease in the use of flucloxacillin (33.2 to 30.1 DDD per 100 admissions) and amoxicillin/clavulanic acid (79.9 to 71.3 DDD per 100 admissions) (figure 8B).

The total use of cephalosporins increased from 8.8 to 10.1 DDD per 100 patient-days and remained constant when expressed in DDD per 100 admissions (45.5 in 2008 compared to 45.9 in 2009) (figure 9).

The use of carbapenems increased in 2009 to 1.14 DDD per 100 patient-days compared to 0.99 DDD per 100 patient-days in 2008. Per 100 admissions, use remained stable in 2009 compared to 2008 at 5.1 DDD per 100 admissions (figure 10).

The use of macrolides is stable over the past years. Use of azithromycin is decreasing over the last two years from 0.70 in 2007 to 0.52 DDD per 100 patient-days in 2009, and from 3.86 in 2007 to 2.36 DDD per 100 admissions in 2009 (figure 11).

In figure 12 the use of aminoglycosides is depicted. Use increased from 3.9 to 4.2 DDD per 100 patient-days and decreased from 20.2 to 18.9 DDD per 100 admissions. This increase was due to an increase in use of gentamicin from 2.9 to 3.5 DDD per 100 patient-days. The decrease in total DDD per 100 admissions was due to a decrease in the use of tobramycin from 5.0 to 3.1 DDD per 100 admissions (figure 12).

The use of fluoroquinolones increased from 8.8 to 9.3 DDD per 100 patient-days and decreased from 45.7 to 42.2 DDD per 100 admissions. These changes are both caused by the use of ciprofloxacin (figure 13), which is the most commonly used fluoroquinolone.

The use of vancomycin further increased from 1.0 to 1.1 DDD per 100 patient-days and from 5.1 to 5.2 DDD per 100 admissions. The use of teicoplanin remained constantly low at 0.1 DDD per 100 patient-days and 0.6 DDD per 100 admissions (figure 14).

Table 5. Use of antibiotics for systemic use (J01) in hospitals (DDD/100 admissions) 2000-2009 (Source: SWAB).*

ATC group*	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	12.1	11.3	11.2	8.8	8.4	8.8	8.7	7.8	8.5	7.4
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
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
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
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
J01DB-DE	Cephalosporins	42.8	42.3	42.0	42.0	39.4	39.8	45.3	46.0	45.5	45.9
J01DF	Monobactams	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
J01DH	Carbapenems	3.3	2.4	3.2	3.3	2.8	3.2	3.0	4.6	5.1	5.1
J01EA	Trimethoprim and derivatives	2.5	3.6	3.3	3.1	2.3	3.0	4.2	2.7	2.0	1.7
J01EC	Intermediate-acting sulfonamides	0.5	0.1	0.2	0.8	0.3	0.3	0.1	0.4	0.3	0.2
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	17.3	15.6	16.0	14.4	12.1	12.2	11.5	12.7	12.2	9.3
J01FA	Macrolides	15.4	15.7	17.3	15.4	13.4	15.1	13.4	15.1	13.9	11.9
J01FF	Lincosamides	9.0	9.2	10.0	10.2	10.2	10.5	10.8	11.4	11.1	10.8
J01GB	Aminoglycosides	16.2	14.0	14.2	15.8	12.5	13.9	13.7	14.5	20.4	18.9
J01MA	Fluoroquinolones	35.9	38.0	38.2	41.0	37.2	39.7	43.3	41.9	45.6	42.2
J01MB	Other quinolones	0.4	0.5	0.5	0.6	0.8	0.5	0.3	0.2	0.3	0.5
J01XA	Glycopeptides	3.8	3.2	3.4	3.4	3.5	4.1	3.9	5.4	5.9	5.7
J01XB	Polymyxins	2.3	0.8	0.4	0.5	0.6	1.1	0.9	0.7	1.2	1.0
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
J01XD	Imidazole derivatives	8.5	9.0	9.7	10.1	9.6	7.9	9.0	10.1	8.8	8.3
J01XE	Nitrofuran derivatives	2.8	3.3	3.6	4.7	4.9	5.6	5.2	6.1	6.2	5.0
J01XX05	Methenamine	0.3	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.1	0.1
J01XX08	Linezolid	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.2	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

* from the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

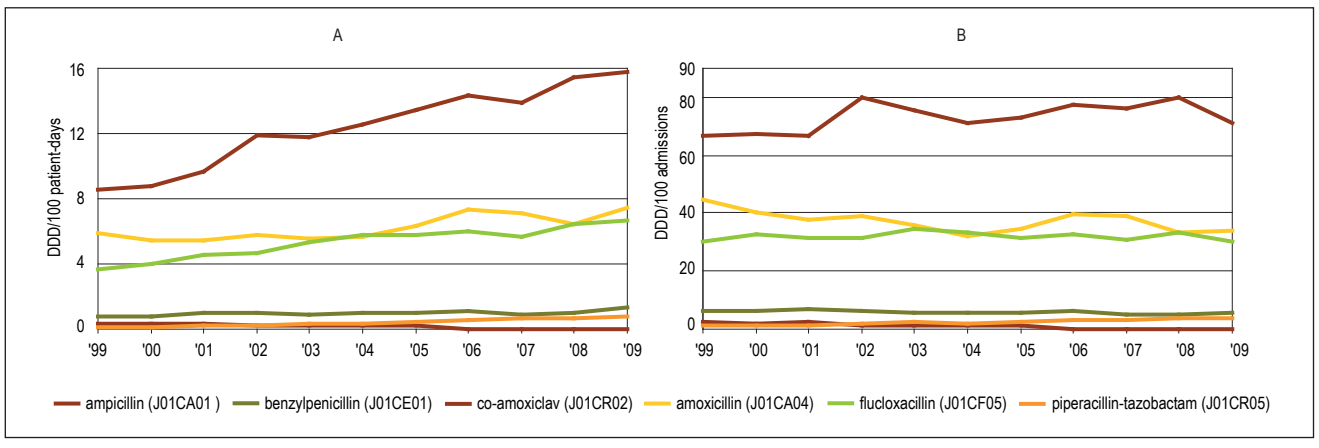

Figure 8. Use of penicillins in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

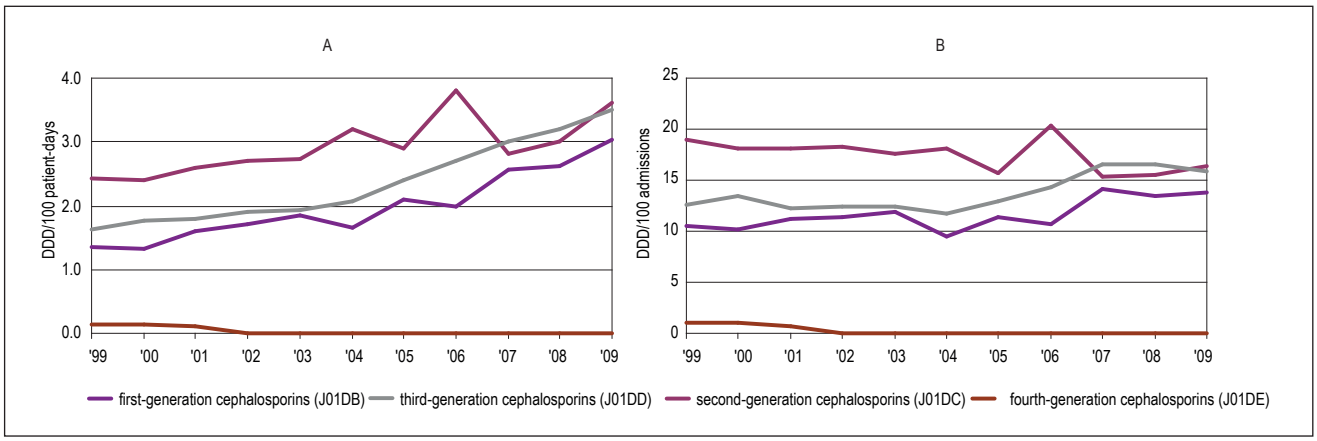

Figure 9. Use of cephalosporins in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

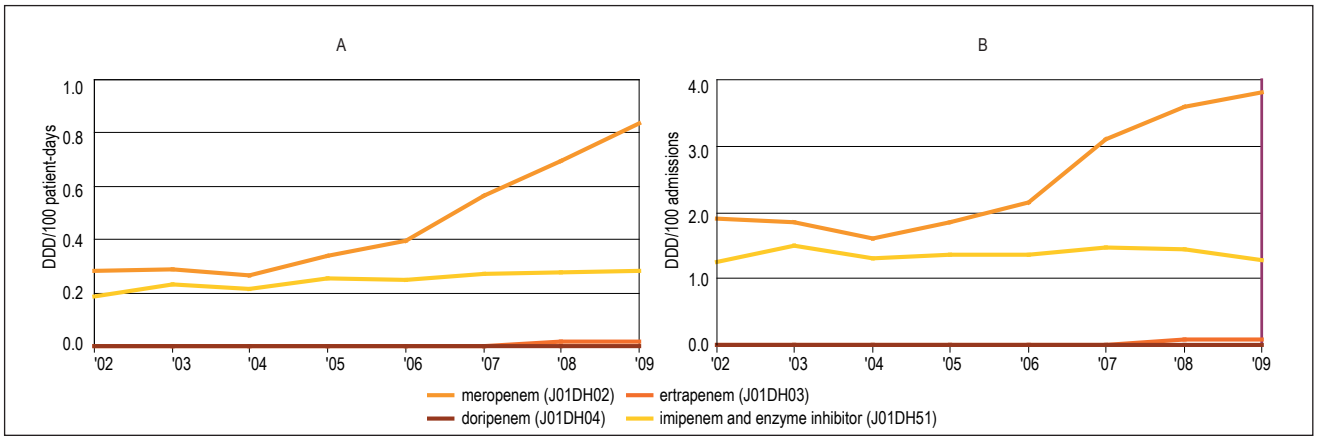

Figure 10. Use of carbapenems in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2002-2009 (SWAB).

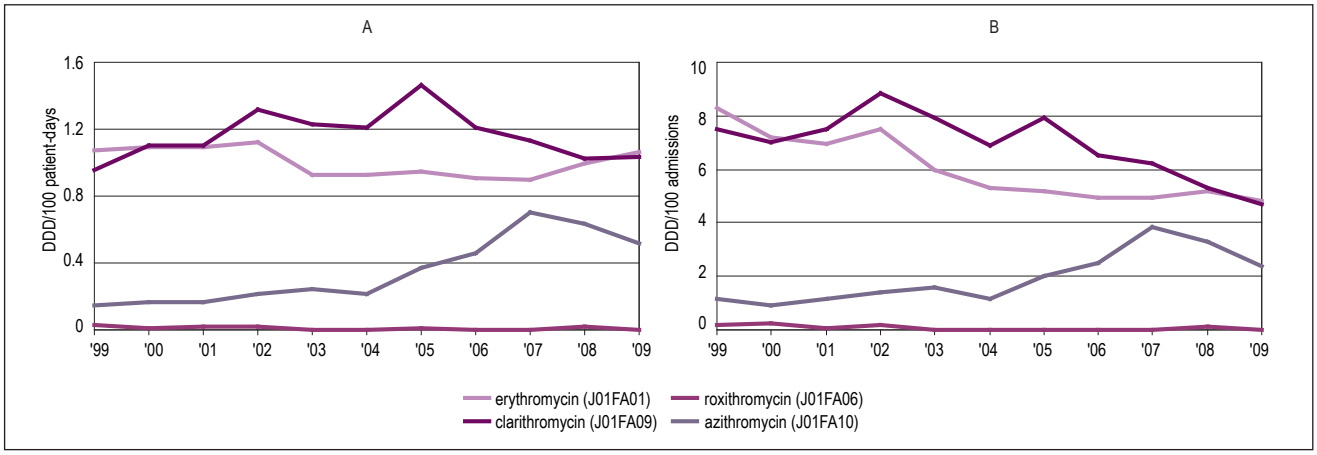

Figure 11. Use of macrolides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

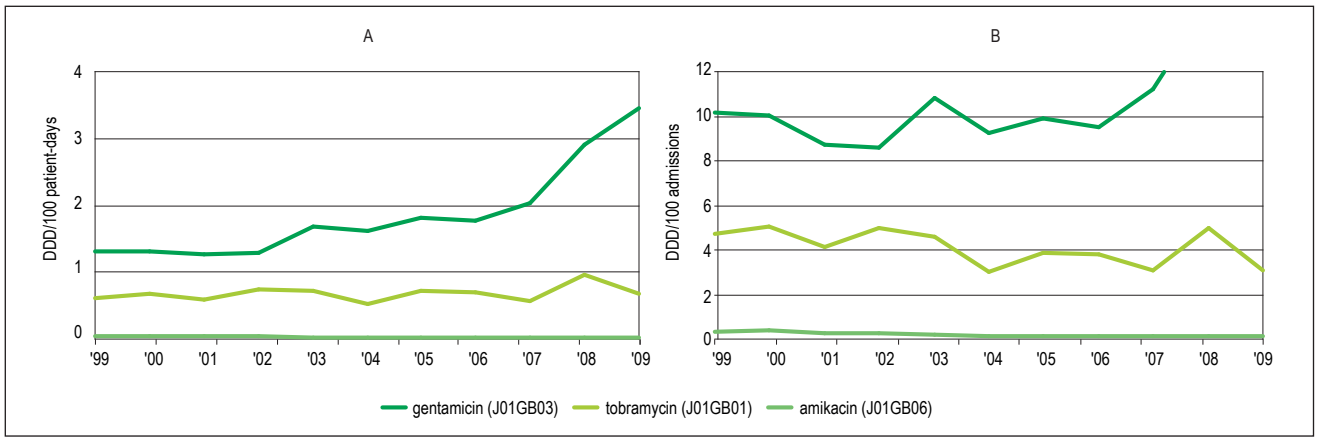

Figure 12. Use of aminoglycosides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

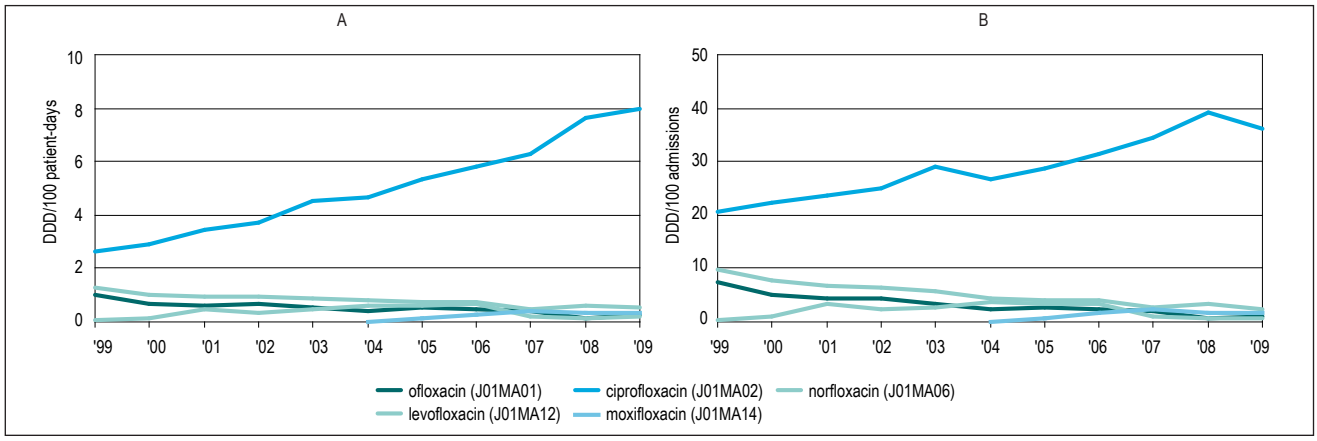

Figure 13. Use of fluoroquinolones in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

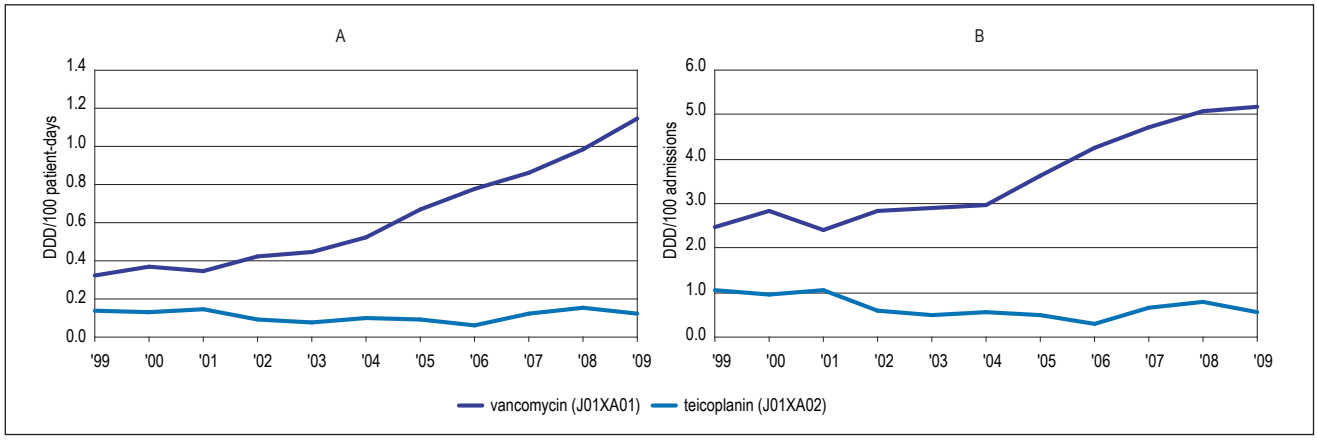

Figure 14. Use of glycopeptides in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 1999-2009 (SWAB).

Table 6. Use of antimycotics for systemic use (J02) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2009 (Source: SWAB).

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

* from the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 7. Use of antimycobacterials for systemic use (J04) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2009 (Source: SWAB).

ATC group*	Therapeutic group	2007			2008			2008		
		total 37	general 30	academic 7	total 49	general 41	academic 8	total 63	general 55	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
J04AB	Antibiotics (mainly rifampicin)	0.83	0.52	1.44	0.92	0.75	1.34	0.86	0.74	1.27
J04AC	Hydrazides (mainly isoniazide)	0.28	0.22	0.39	0.21	0.18	0.29	0.21	0.14	0.40
J04AD	Thiocarbamide derivatives	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
J04AM	Combinations of drugs for tuberculosis	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
J04	Antimycobacterials for systemic use (total)	1.63	1.06	2.74	1.46	1.11	2.33	1.43	1.15	2.35

* from the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

3.2.2 Hospital use of systemic antimycotics

Total use of antimycotics for systemic use in 2009 was 3.7 DDD per 100 patient-days (table 6; figure 15). In university hospitals, the use of systemic antimycotics was 8.8 DDD per 100 patient-days compared to 2.1 DDD per 100 patient-days in general hospitals. Compared to 2008, the total use of amphotericin B formulations remained stable. Total use of other antimycotics for systemic use has increased from 0.16 DDD/100 patient-days in 2008 to 0.29 DDD/100 patient-days in 2009. This increase was seen in general hospitals as well as in university hospitals.

3.2.3 Hospital use of systemic antimycobacterials

The total use of antimycobacterials for systemic use in 2009 was 1.4 DDD per 100 patient-days (table 7). The distribution of the different groups of drugs was more or less similar in university hospitals and in general hospitals (table 7 and figures 16A, B and C). The proportion of use of rifampicin, also used for Staphylococci infections, has slightly decreased, mainly due to a decrease in use in university hospitals (from 67 to 54%).

3.2.4 Hospital use of systemic antivirals

The use of systemic antivirals in 2009 is divided into two categories: antivirals mainly used for acute infections (J05AB-AD) and antivirals mainly used for chronic infections (J05 AE-AG, AR, AX). Use of neuraminidase inhibitors (J05AH) is excluded from this report because of alternative distribution during the pandemic. Nucleosides and nucleotides – reverse transcriptase inhibitors excluded – were the mainly used antivirals in both general and university hospitals (figures 17B and C), accounting for almost half of the use of antivirals. Total use of antivirals mainly used for chronic infections (J05 AE-AG, AR and AX combined) remained stable in 2009 compared to 2008 at 1 DDD/100 patient-days (table 8; figure 17 and 18). Use increased in general hospital, whereas a decrease was seen in university hospitals from 0.48 to 0.60 and 2.54 to 2.24 DDD/100 patient-days, respectively (table 8; figure 18). In 2009, total use of

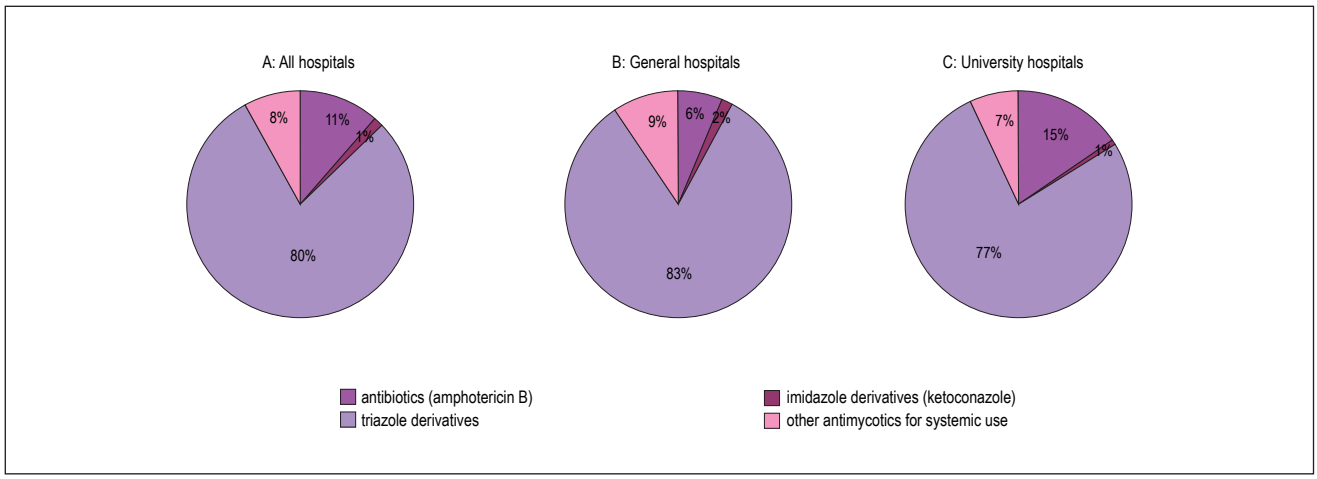

Figure 15. Distribution of the use of antimycotic drugs in all hospitals (A), General Hospitals (B) and University Hospitals (C) in 2009 (SWAB).

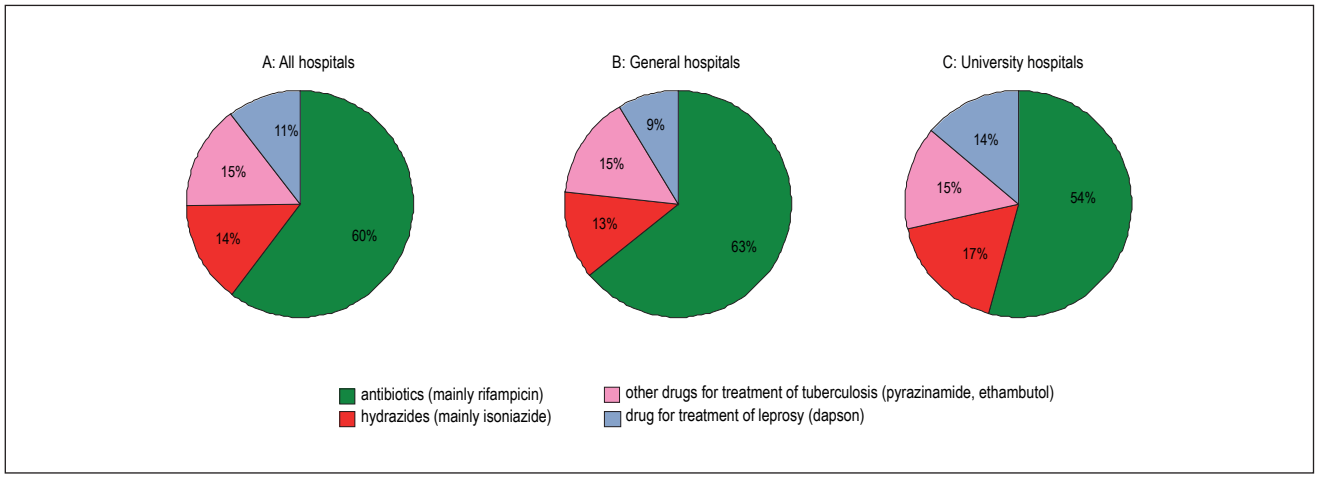

Figure 16. Distribution of the use of antimycobacterial drugs in all hospitals (A), General Hospitals (B) and University Hospitals (C) in 2009 (SWAB).

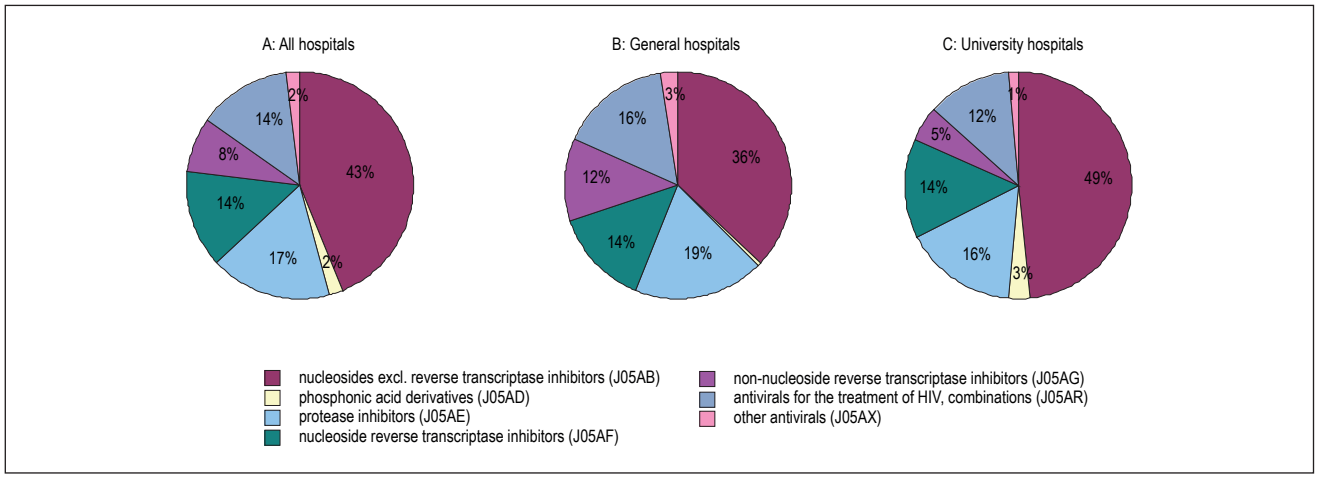

Figure 17. Distribution of the use of antiviral drugs (neuraminidase inhibitors excluded) in all hospitals (A), General Hospitals (B) and University Hospitals (C) in 2009 (SWAB).

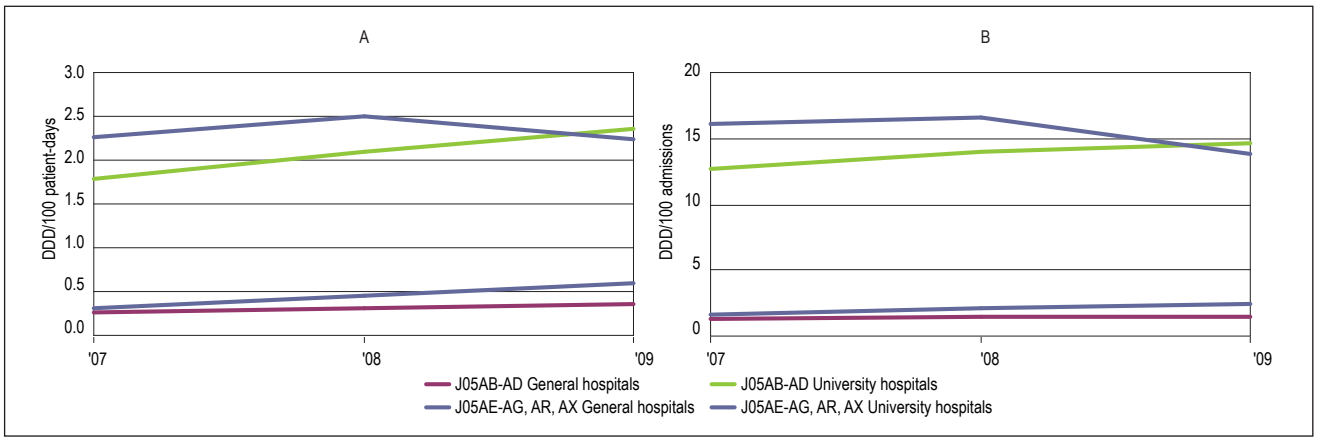

Figure 18. Use of antiviral drugs for chronic (J05 AE-AG, AR, AX) vs acute (J05 AB-AD) infections in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B), 2007-2009 (SWAB).

antivirals mainly used for acute infections (J05 AB-AD combined) slightly increased in both general and university hospitals compared to 2008, from 0.30 to 0.35 and from 2.11 to 2.35 DDD/100 patient-days respectively (table 8; figure 18).
In university hospitals, use of antivirals for acute infections was more than six times as high compared to general hospitals, and more than three times as high in case of antivirals for chronic infections (2.35 vs. 0.35 and 2.24 vs. 0.60 DDD per 100 patient-days, respectively; table 8).

Discussion

The unit in which antibiotic usage is expressed matters (5). This is important when hospital resource indicators change over a study period. In relation to antibiotic resistance development, the measure of antibiotic use should be a reflection of 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 (6). 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.
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 the number of DDD per 100 patient-days and the number per 100 admissions is worrisome. No increase in either unit is not worrisome with respect to development of resistance. When a constant use per patient is seen in combination with an increase in the number of admissions, this would indicate an increase in selection pressure exerted by antibiotics in hospitals over the years. An intensification of antibiotic therapy per 100 patient-days, however, may in part be due to an increase in the number of admitted patients, and possibly a shortening of the duration of antibiotic treatment. Such shortening of the duration of therapy may lead to less selection of resistant micro-organisms(7).

In 2009, the total antibiotic use increased with reference to the year before when expressed in DDD per 100 patient-days, but decreased when expressed in DDD per 100 admissions. 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. The use per individual patient has remained constant. Moreover, 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?
The increase in DDD per 100 patient-days is seen for almost all groups of antibiotics except for combinations of sulfonamides and trimethoprim (including derivatives) and nitrofuran derivatives. A decrease in DDD per 100 admissions is also seen in most of the antibiotic classes. However, the use of penicillins with extended spectrum, the beta-lactamase sensitive penicillins and the other quinolones (J01MB) has increased compared to 2008. Nonetheless, these were just minor increases. Over the past few years the use of these compounds remained constant.
In university hospitals, the use of systemic antimycotics is more than four times higher compared to general hospitals. This can be explained by the larger patient population of haematology- and oncology-patients in university hospitals.
Although university hospitals use twice as much antimycobacterials, the distribution of the different groups is rather similar in both hospital settings. The higher use in university hospitals might be explained by the fact that rifampicin, besides its use for tuberculosis, is also being used as an adjuvant in certain infections with staphylococci.
Treatment of tuberculosis in the Netherlands consists of a limited combination of antimycobacterials; therefore there is not much room for variation (8). The use of

Table 8. Use of antivirals for systemic use (J05) in general hospitals, university hospitals and all hospitals (DDD/100 patient-days), 2007-2009 (Source: SWAB).

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

* from the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system
# Total use not to be assesed because of alternative distribution during the pandemic

dapsone is explained by its role in the prophylaxis and treatment of *Pneumocystis carinii* and toxoplasmic encephalitis.
The largest group of antivirals used are the nucleosides (reverse transcriptase inhibitors excluded), like (val) acyclovir and (val)ganciclovir (J05AB). The use of systemic antivirals in 2009 has been divided in use of antivirals for acute and for chronic infections. When expressed in DDD per 100 patient-days, use of antivirals for acute infections was similar in both university and general hospitals. However, the use of antivirals for chronic infections was more than six times higher in university hospitals compared to general hospitals.
In the Netherlands, all the university hospitals and also a few general hospitals are specialised in the treatment of HIV patients.

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3.3 Gentamicin-impregnated beads and cement

HM Kwint, MMB Roukens and S Natsch
on behalf of the SWAB's working group on surveillance of antibiotic use

Since 2007, an increase in the use of the aminoglycoside gentamicin is seen in hospitals when measured in DDD/100 patient-days as well as in DDD/100 admissions (figure 19a). The SWAB's working group on surveillance of antibiotic use further investigated this increase, trying to reveal what might be causing it.
Further investigation revealed that the increase in total use

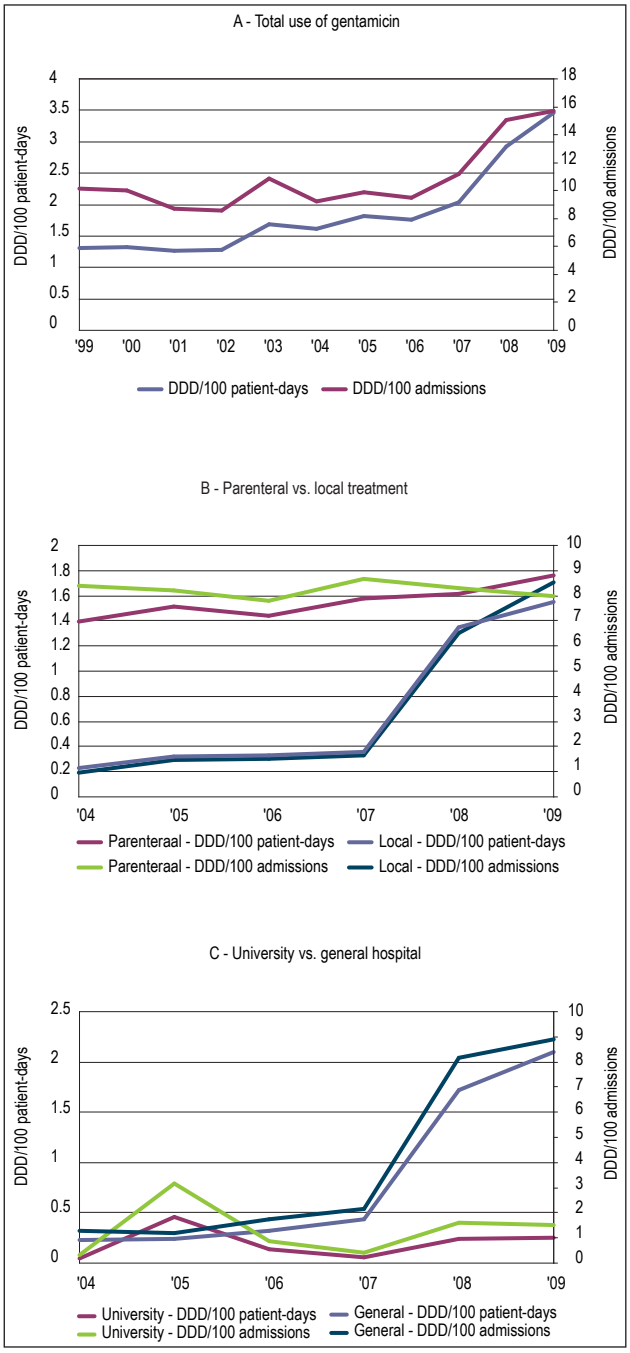

Figure 19. Use of gentamicin in Dutch hospitals, expressed as DDD/100 patient-days and DDD/100 admissions, 1999-2009. a) Total use of gentamicin; b) Parental versus local treatment with gentamicin; and c) Local treatment with gentamicin in university versus general hospitals.

of gentamicin is caused by an increase in its use for local treatment (beads and cement) (figure 19b). In addition, the increase in local treatment is caused by an increase in the use of gentamicin in general hospitals (figure 19c).
What can be found in literature is that the highest concentration of gentamicin will be achieved by placing as many beads as possible in the infected area (1,2). However, as far as we know, an elevated use of gentamicin for local treatment has not been implemented in any national guidelines. Perhaps it has only been implemented in local orthopedic guidelines since 2007?
There is a remarkable negative aspect to this increase in the use of gentamicin for local treatment. Very recently an observational cohort study (3) showed that measurable gentamicin serum concentrations (>0.4 mg/L) can be expected after implantation of gentamicin-PMMA beads in certain patients with infected hip joints. Furthermore, these serum concentrations are associated with nephrotoxicity. These are aspects that need further research but should certainly be taken into account in daily practice.
From these observations it may be clear that university and general hospitals might use different guidelines or recommendations concerning the use of antibiotics. Also this evaluation suggests that in specific cases it is useful to look at the product level instead of the ATC level to explain the observed changes. It might be useful to split future surveys in both university and general hospitals because of different patterns of use and in context to that, the development of different local policies to contain the resistance problem.

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4. Resistance among commmon bacterial Pathogens

4.1 Surveillance of resistance in the community

The studies on resistance levels in the community focus on three different goals, including estimation of resistance in:
(1) micro-organisms isolated from the indigenous flora of healthy persons in various circumstances and of various ages, providing information on the basic level of resistance in human reservoirs and
(2) pathogens isolated from patients visiting their general practitioner (GP) and
(3) special pathogens such as meningococci, gonococci and mycobacteria.
Several longitudinal multicentre studies within the national project Surveillance of Extramural Resistance in The Netherlands (SERIN) were carried out or are ongoing in various parts of The Netherlands 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) to that purpose.
In 2006 the RIVM started a surveillance of resistance of *Neisseria gonorrhoeae* among patients from outpatient-STI centres, the so-called GRAS project.
Since 1993 The Netherlands Reference Laboratory for Bacterial Meningitis has each year determinedthe resistance level of *Neisseria meningitidis* from patients admitted to the hospital for meningococcal disease.
The first isolate of *Mycobacterium tuberculosis* of each patient with tuberculosis in The Netherlands is routinely sent to the RIVM for susceptibility testing and confirmation of identification.
Results of all these studies are presented here.

4.1.1 *Escherichia coli*

The prevalence of antibiotic resistance among bacteria causing community acquired urinary tract infections (UTI) was determined for strains collected from patients visiting their general practitioner (GP). From January 2009 to December 2010, GPs from 42 general practices from the NIVEL Sentinel Stations Network participated in the study for the recruitment of patients. The Network is nationally representative for age, gender, regional distribution and population density. The GPs collected urinary samples from male patients (>11 years) with symptoms indicative for a UTI in the absence of fever. Patients were excluded when they were catheterized, had urological or renal problems, diabetes mellitus or other immune compromising diseases. See material and methods section for details regarding the acquisition and testing of isolates.

In total 545 urine samples were collected in 2009/2010, of which 351 (64%) were positive; 169 revealed *E. coli*. The age of the patients ranged from 20-91 years with a median of 64.8 years. The data were compared with the results found in 2004 for a comparable group of patients. Then, 422 male patients were included, age ranged from 18-104 years and a median of 62 years from whom 236 (56%) positive samples were obtained and 103 *E. coli* strains were collected.
Furthermore, these data were compared with results from two cohorts of women in 2004 and 2009 respectively, who visited the GP for an uncomplicated UTI for the first time. The cohorts of 2009/2010 were also analyzed with respect to age group. It appeared that age distribution of men and women with UTI differed. A total of 12% of women was younger than 20 years of age and 31% were between 20-50 years, totalling 43% below 51 years of age. In contrast, of the male patients only 15% was younger than 51 years of age and confirmed that UTI in men at younger age is relatively uncommon.

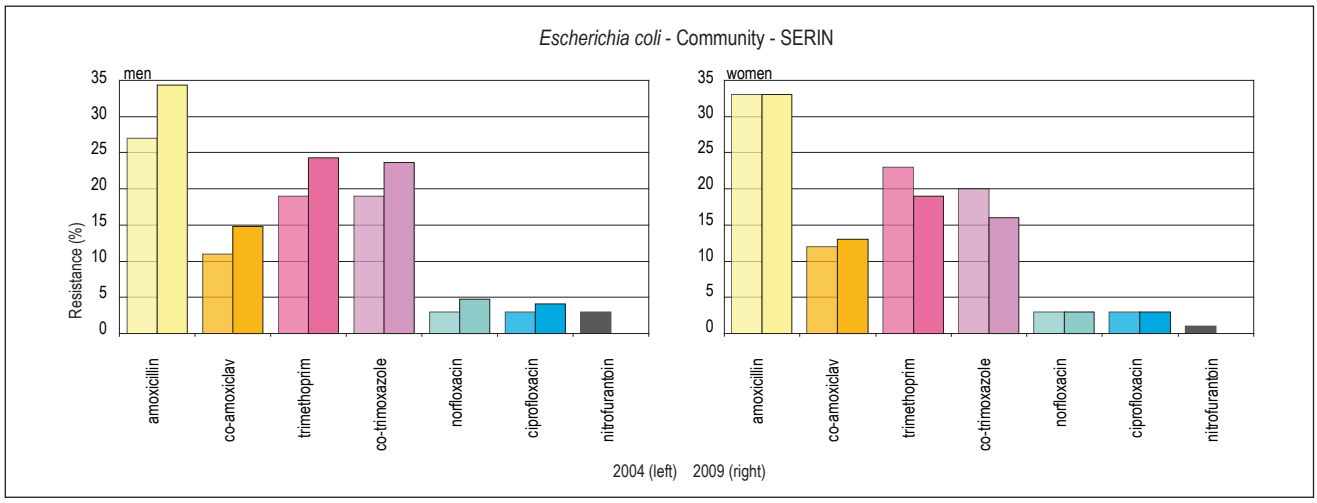

Figure 1. Resistance to antibiotics among *Escherichia coli* from male patients in 2004 (N=103) and in 2009/2010 (N=169) and female patients in 2004 (N=1724) and in 2009 (N=489) in the community.

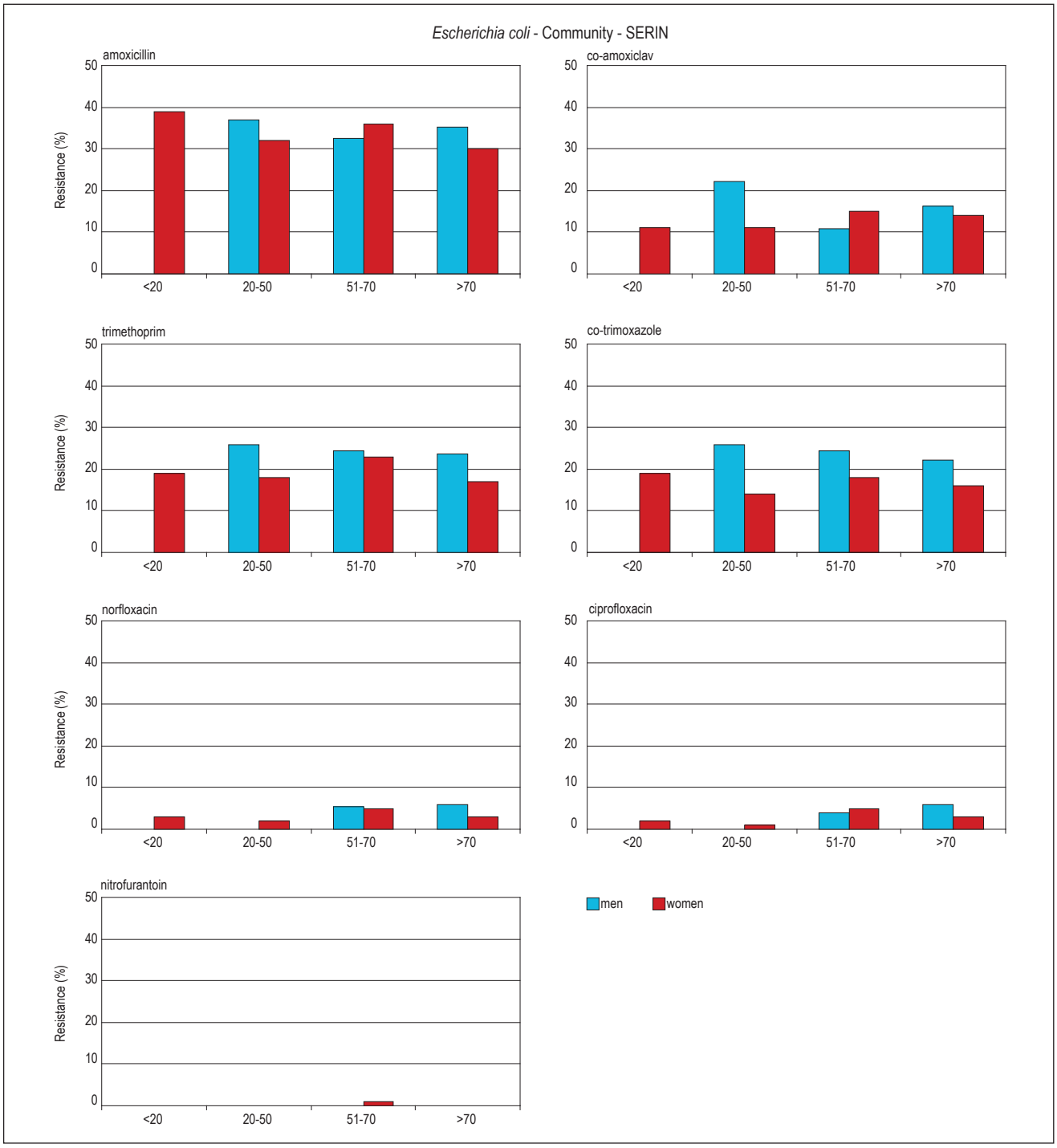

Figure 2. Resistance to antibiotics among *Escherichia coli* from male patients in 2004 (N=103) and in 2009/2010 (N=169) and female patients in 2004 (N=1724) and in 2009 (N=489).

Overall amoxicillin resistance among *E. coli* in men increased from 27% in 2004 to 34% in 2009 (figure 1), similar to that reached in women already in 2004 (figure 1). These differences were statistically not significant. The highest resistance level for amoxicillin in men in 2009/2010 was in the age group of 20-50 years, whereas in women this was less than 20 years of age (Figure 2). The MIC distribution of amoxicillin was bimodal with one subpopulation with MICs ranging from 1-8 mg/l and another with MICs > 32 mg/l (figure 3). MIC distributions for strains from men and women were identical. Co-amoxiclav resistance in men increased from 11% in 2004 to 15% in 2009/2010, which was in the range found for women (12-13%). Similar to amoxicillin, the highest resistance level for co-amoxiclav in men was found in the age group 20-50 years. However, that for women was at older age instead of younger age. The MIC distributions in 2009 were similar for men and women, showing a unimodal shape with MICs over a broad range from 1-> 32 mg/l (figure 3).

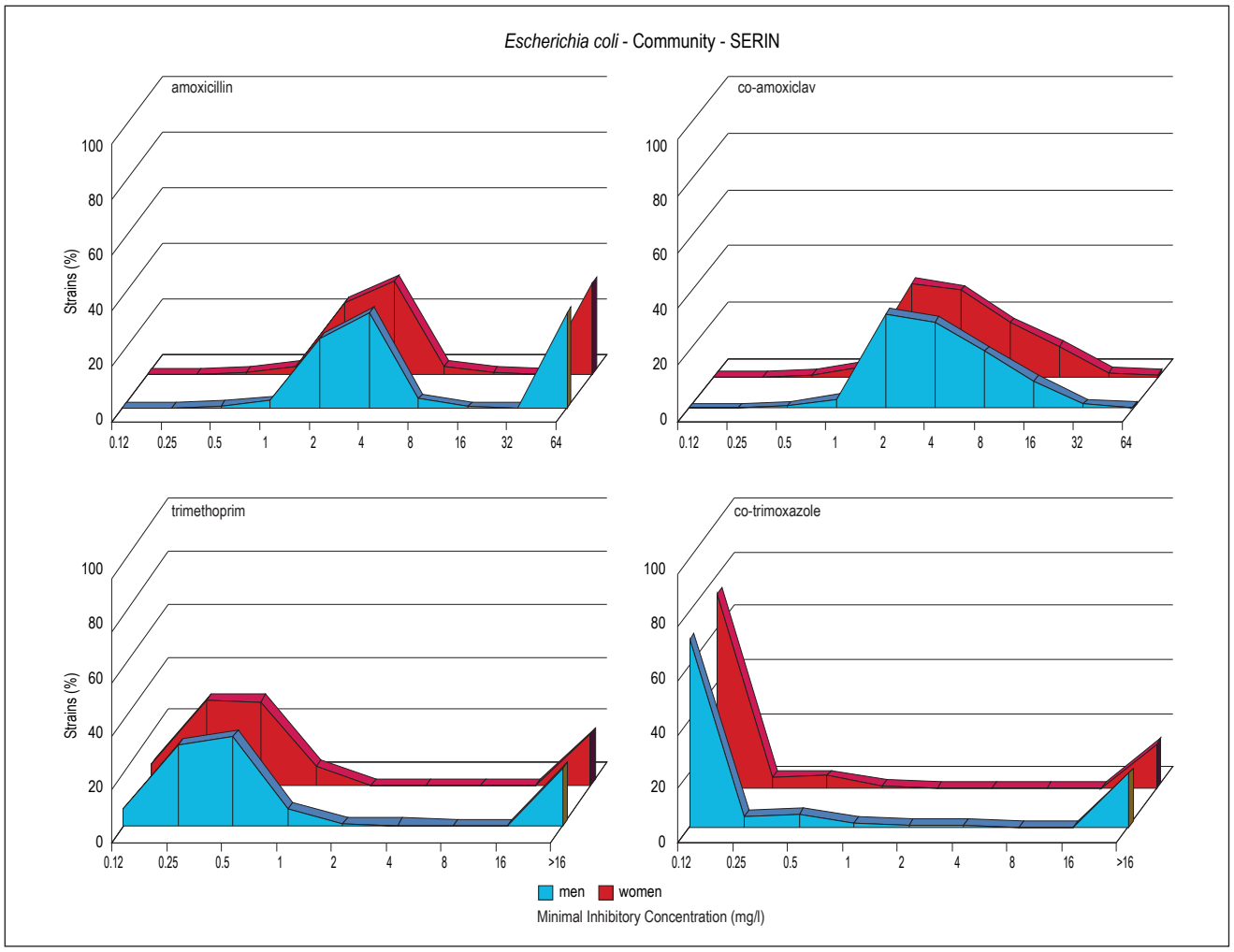

Figure 3. MIC distributions of amoxicillin, co-amoxiclav, trimethoprim and co-trimoxazole for *Escherichia coli* from men and women from the community in 2009/2010.

Trimethoprim resistance in male increased from 19% in 2004 to 24% in 2009/2010; this difference was not significant. This level seems higher than the percentages found in women, in whom a decrease in resistance level was observed in 2009 compared to 2004. The same pattern was found for co-trimoxazole. We found higher resistance levels of trimethoprim and co-trimoxazole at younger age for men and at higher age for women. The difference in resistance level of co-trimoxazole in male and female in 2009/2010 was significant ($p < 0.05$). This suggests more frequent use of co-trimoxazole in men with subsequent development of resistance. It was observed in the study in 2004 that 25% of male patients were treated with co-trimoxazole initially. This is not conform the Dutch Guidelines for uncomplicated UTI in men. Probably GPs feel more "comfortable" with prescription of an antibiotic which is known to achieve tissue concentrations, as uncomplicated UTI in men, 50 years of age is rare.
The decrease in trimethoprim resistance in women may be the result of the change in the Dutch Guidelines for treatment of urinary tract infections in general practice (NHG) in 2005. It was already indicated in NethMap 2003 and 2004 that resistance to amoxicillin and trimethoprim among *E. coli* causing community acquired urinary tract infection had emerged above an acceptable level in the community, and thus not useful anymore as empiric therapy for these infections. The NHG changed its standard accordingly in 2005 and replaced trimethoprim by nitrofurantoin as the first choice for the empiric treatment of uncomplicated urinary tract infection. Since then the prescription rate of trimethoprim has significantly decreased which may have contributed to a decrease in resistance prevalence. MIC distributions (figure 3) for strains from men and women were identical, showing a bimodal shape with one subpopulation with MIC 0.12-1 mg/l for trimethoprim and < 0.12 mg/l for co-trimoxazole and one subpopulation with MIC > 32 mg/l.
Norfloxacin- and ciprofloxacin resistance were stable at 3-5% in men and 3% in women in both study periods. The highest resistance levels in men and women were found at older age. It was shown in the 2004 study that quinolones were prescribed in 35% of older men. This behaviour may be contributed to development of resistance in this age group.

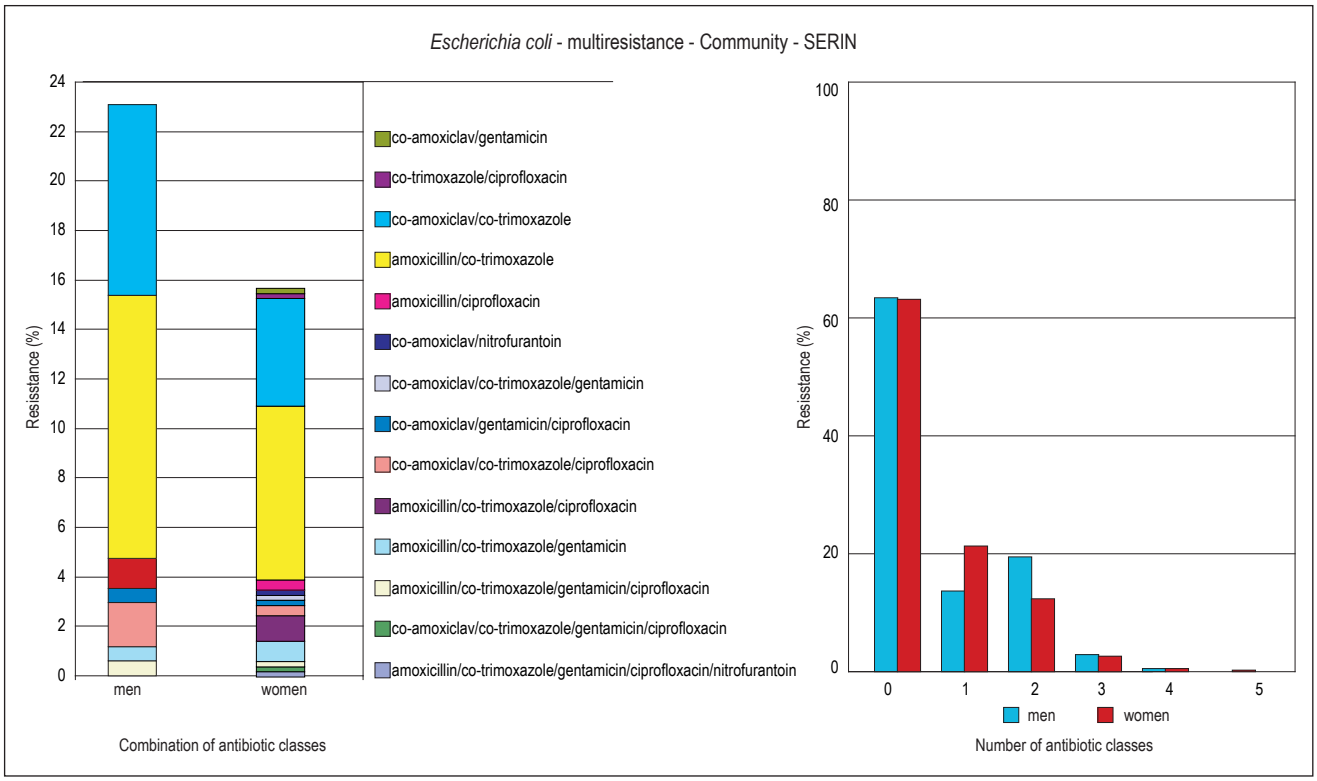

Figure 4. Multiresistance among *Escherichia coli* from male and female patients in the community in 2009/2010.

Nitrofurantoin resistance was 3% in men in 2004 and not found in 2009/2010; similar observations were made for women before.
Fosfomycin resistance was not found in the community isolates
Gentamicin resistance was 2% in men and 2.5% in women in 2009/2010.

ESBL production
Escherichia coli isolates resistant to co-amoxiclav were assessed for the presence of ESBL production. One strain from men in 2009/2010 appeared ESBL positive (0.6%), compared to five strains from women (1%), altogether less than 1%.

Multiresistance
To calculate resistance to combinations of the various classes of antibiotics, co-trimoxazole was taken as representative for both trimethoprim and co-trimoxazole. A total of 63% of all strains from men and women in 2009 were susceptible to all classes of antibiotics tested (figure 4); 21.3% of "female" strains was resistant to one class of antibiotics, most frequently to amoxicillin (11.5%) or co-amoxiclav (7%) compared to 14% of "male" strains, most frequently to amoxicillin (8% or co-amoxiclav (2%). A total of 12% of "female" strains was resistant two classes of antibiotics tested, most frequently to the combination amoxicillin/co-trimoxazole (7%) and co-amoxiclav/co-trimoxazole (4%); for male strains these figures were: 19.5% resistance to two classes, most frequently to the combination amoxicillin/co-trimoxazole (11%) and co-amoxiclav/co-trimoxazole 8%). A total of 3% of "female" and "male" strains was resistant to three classes of antibiotics (multiresistant) and 0.6% was even resistant to four or five classes of antibiotics.

Summary – *Escherichia coli*
1. Overall resistance levels in men and women were similar
2. The resistance to amoxillicin (34%), co-amoxiclav (12%), nitrofurantoin (1%) and quinolones (3.5%) was stable since 2004 in both men and women.
3. Trimethoprim and co-trimoxazole resistance in men was stable since 2004, but decreased in women. Co-trimoxazole resistance in men was higher than in women in 2009 ($p < 0.05$).
4. Fosfomycin resistance was not found
5. The prevalence ESBL producing strains was 1% among the isolates in 2009/2010
6. Multiresistance (resistant to three or more classes of antibiotics) in the community was 3% in 2009/2010

4.1.2 *Neisseria meningitidis*
From 1994-2010 a total of 4620 strains from cerebrospinal fluid (CSF) and 2805 strains from blood were included in the surveillance project of The Netherlands Reference

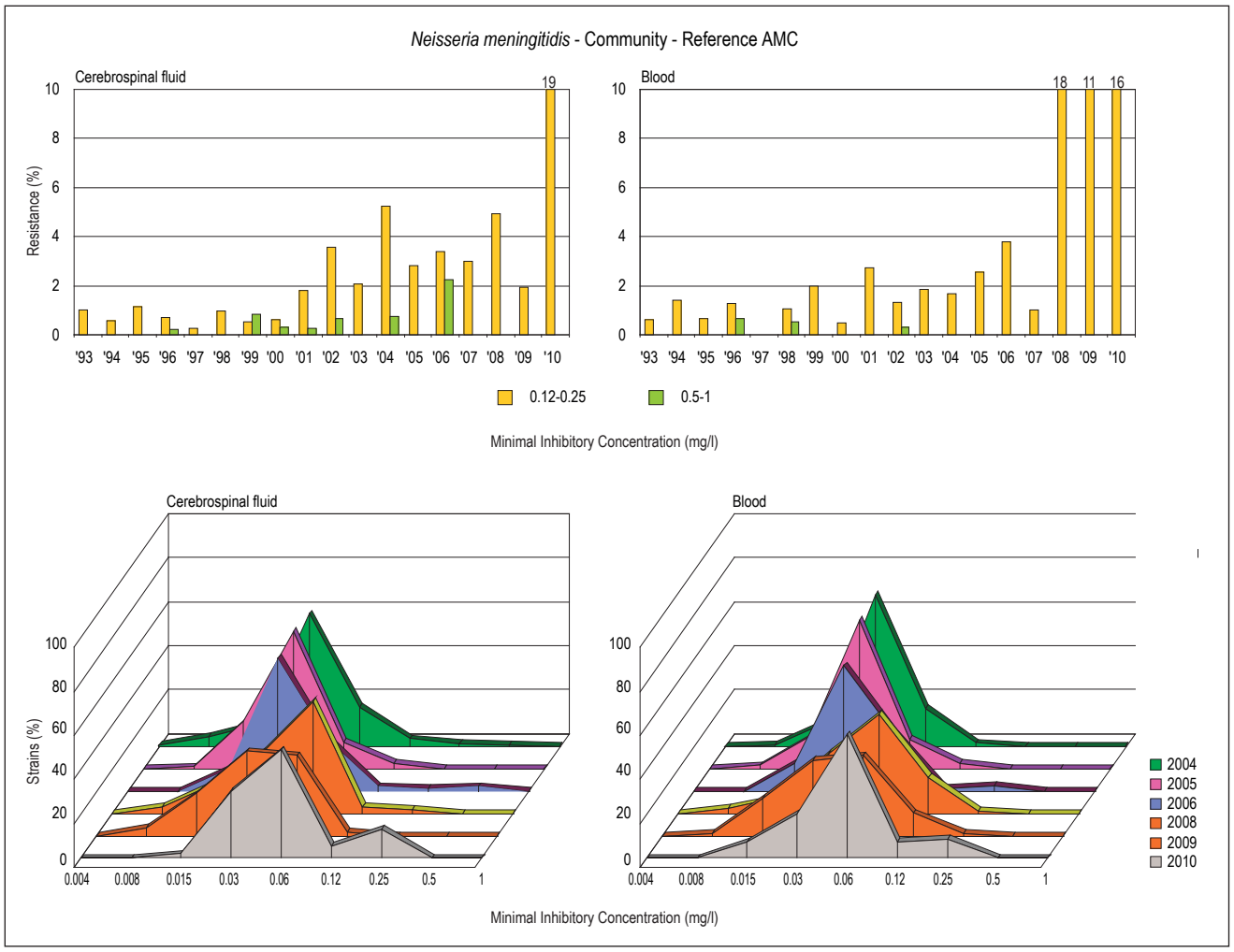

Figure 5. Trends in penicillin resistance and MIC distributions of penicillin for *Neisseria meningitidis* from CSF (N= 4.620) and blood (N=2.805).

Laboratory for Bacterial Meningitis of the Academic Medical Centre, Amsterdam. Strains moderately susceptible to penicillin (MIC 0.125-0.25 mg/l) occurred in less than 2% of the strains before 2002. From 2002-2007, 2-5% of strains from CSF and blood appeared moderately susceptible, but this increased from 2008 on to 19% of strains for CSF and 16% for blood isolates in 2010 (figure 5). 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 (figure 5). The MIC distributions for penicillin showed a slow movement of the peak to the right from 0.03 to 0.06 mg/l from 2008 on with appearance of a shoulder at 0.25 mg/l in 2010.
Fourteen of the 23 strains isolated in 2010 moderately susceptible belonged to serogroup B, four to serogroup W135, three to Y and two were not groupable.
Resistance to rifampicin and ceftriaxone was not found.

Summary – *Neisseria meningitidis*
1. Penicillin resistance was not found since 2006
2. 16% of strains from blood and 19% from CSF were moderately susceptible to penicillin in 2010.
3. Resistance to ceftriaxone and rifampicin was not found.

4.1.3 *Neisseria gonorrhoeae*

In 1999, the nationwide surveillance of antibiotic resistance in gonococci was discontinued and since then insight into the susceptibility patterns of gonococci has been limited.
In 2003, data of increasing quinolone resistance resulted in a revision of the guidelines from the Netherlands Dermatological and Venereological Society (Nederlandse Vereniging voor Dermatologie en Venereologie, NVDV), making cefotaxime the first-choice therapy for gonorrhea infections. At the end of 2006, ceftriaxone was selected as primary therapy. Also, the NHG revised their guidelines in 2004, making cefotaxime their first choice, although ciprofloxacin remained second-choice therapy for gonorrhea.

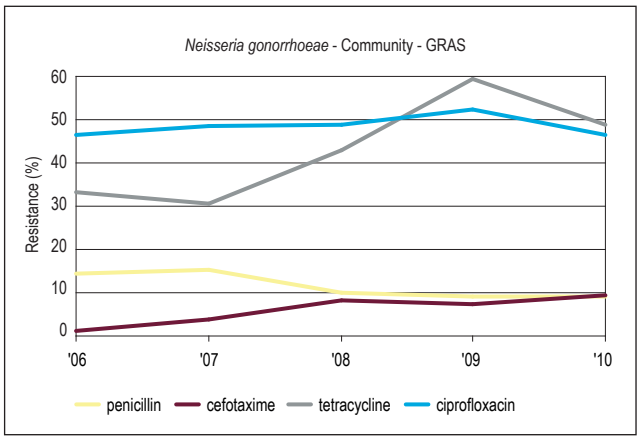

Figure 6. Trends in antibiotic resistance among *Neisseria gonorrhoeae* (N=4.362).

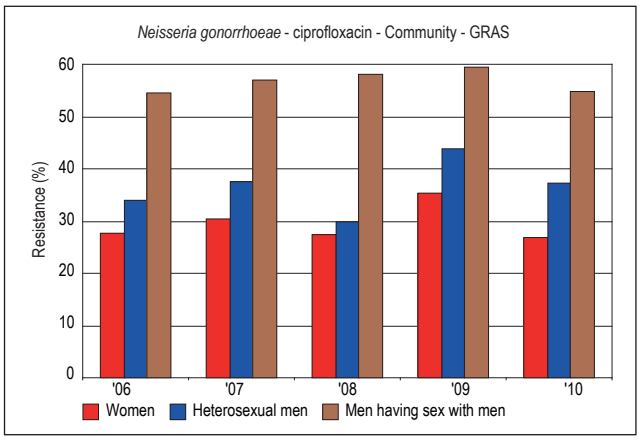

Figure 7. Trends in ciprofloxacin among *Neisseria gonorrhoeae* (2006-2010) in different study groups.

Concerns about the increasing resistance to ciprofloxacin resulted in the implementation of the national project Gonococcal Resistance to Antimicrobials Surveillance (GRAS) in 2006. This surveillance consists of systematically collected data on gonorrhea from Sexually Transmitted Infections (STI) centres and standardized measurement of resistance patterns by using E-test (for penicillin, tetracycline, ciprofloxacin and cefotaxime), linked with epidemiological data. Isolates with unusual resistance patterns are forwarded to the RIVM for confirmation. STI centres and associated laboratories that identify the majority of STI in high risk populations participate in this surveillance.
In July 2006, GRAS was implemented in the first STI centre. Throughout the years, GRAS was further expanded and now includes most STI centres in the Netherlands, representing approximately 80% of the total population of patients visiting an STI centre. From July 2006 through December 2010, the susceptibility of *N. gonorrhoeae* from 4362 patients was tested. Resistance levels were calculated using the breakpoints for resistance according to the EUCAST guidelines. Prevalences of resistance are shown in figure 6.
Overall penicillin resistance decreased significantly from 15% in 2006 to 9% in 2010 (figure 6). Tetracycline resistance increased from 33% in 2006 to 60% in 2009 and decreased in 2010 to 49%.
Ciprofloxacin resistance increased from 46% in 2006 to 52% in 2009 and decreased slightly in 2010 to 47%. This increase was mostly due to increase in resistance among men having sex with men (MSM), who had the highest level of resistance (up to 60%, figure 7).
At the same time, a survey among GPs found that ciprofloxacin was still prescribed in approximately 40% of the cases in 2008. GP guidelines will be updated in 2011, no longer recommending ciprofloxacin as second-choice therapy.
Cefotaxime resistance (MIC > 0.12 mg/l) increased from, 1% in 2006 up to 9% in 2010 (figure 6). The MIC distribution of cefotaxime (figure 8) showed a unimodal shape over a broad range (< 0.002 – 0.5 mg/l). The shape of the curve is changing in 2009 and 2010 with broadening of the range and a tendency to dissociate: in 2006 the peak of the distribution was at 0,008 mg/l with MIC 90 at 0.064 mg/l, in 2010 one peak at 0.002 mg/l can be observed presenting highly susceptible strains and smaller peaks at 0.032 and 0.015 mg/l. MIC90 in 2010 is at the breakpoint (0.12 mg/l). This MIC creep has been shown in other countries as well and predicts upcoming resistance.
Cefotaxime resistance was higher in isolates obtained from men who have sex with men (MSM) (13% in 2010), compared to heterosexual men and women (both 3% in 2010).
The changing antibiotic resistance pattern of gonococci underlines the need for a continuous standardized surveillance of antimicrobial susceptibility to detect changes in resistance patters which might necessitate modification of treatment guidelines, to explore risk factors for infection with such strains, and to understand high risk transmission patterns.

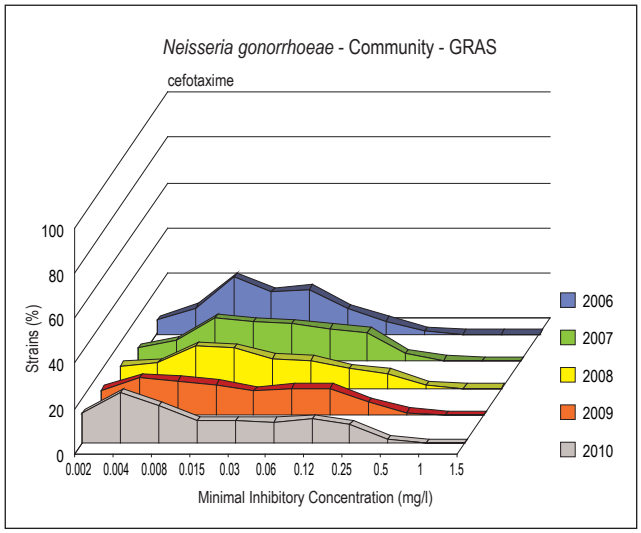

Figure 8. MIC distributions of cefotaxime for *Neisseria gonorrhoeae*.

Summary- *Neisseria gonorrhoeae*
1. Penicillin resistance has decreased to 9%
2. Resistance to tetracycline (49%) and ciprofloxacin (47%) was stable and high
3. Cefotaxime resistance increased to 9% for the whole study group in 2010; the highest resistance (13%) was found among isolates from men havinge sex with men compared to 3% in isolates from heterosexual men and women.

4.1.4 *Mycobacterium tuberculosis*

A total of 11705 strains of *M. tuberculosis* complex were obtained during 1998-2010. In the period of 2001 till 2006, the number of *M. tuberculosis* complex strains isolated per year has gradually decreased, from 1080 in 2001 to 727 in 2006 (33% decline). However, in the last four years (2007-2010) this number is increasing again, from 729 in 2007 to 789 in 2010 (8% increase).
The two most important first line drugs against *M. tuberculosis* complex are rifampicin and isoniazid (INH). INH resistance fluctuated between 6.5 and 8.7% from 1998 to 2003, decreased to 6.3% in 2007 and increased thereafter to 9% in 2010 (figure 9).
Rifampicin resistance increased to 2.1% in 2008 and 2.8% in 2009, but dropped to 1.6% in 2010.

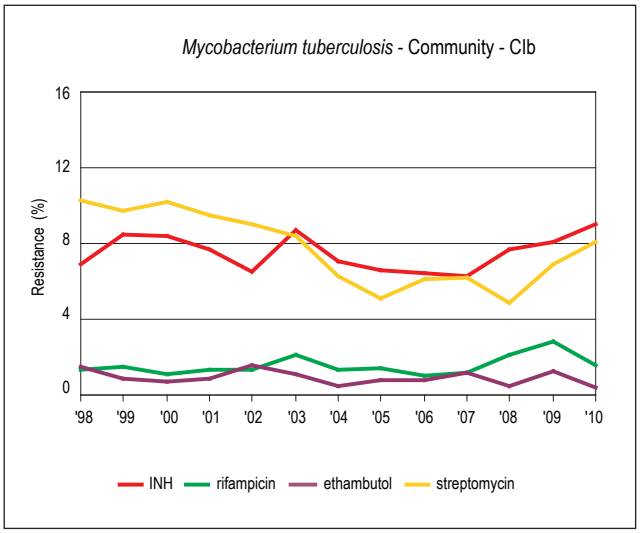

Figure 9. Trends in antibiotic resistance among *Mycobacterium tuberculosis* (N= 11.705).

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 0.4% in 2010.
Streptomycin has been the first and major drug to combat TB in the era anti-TB drugs were introduced, but it 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 to 8.1% in 2010.

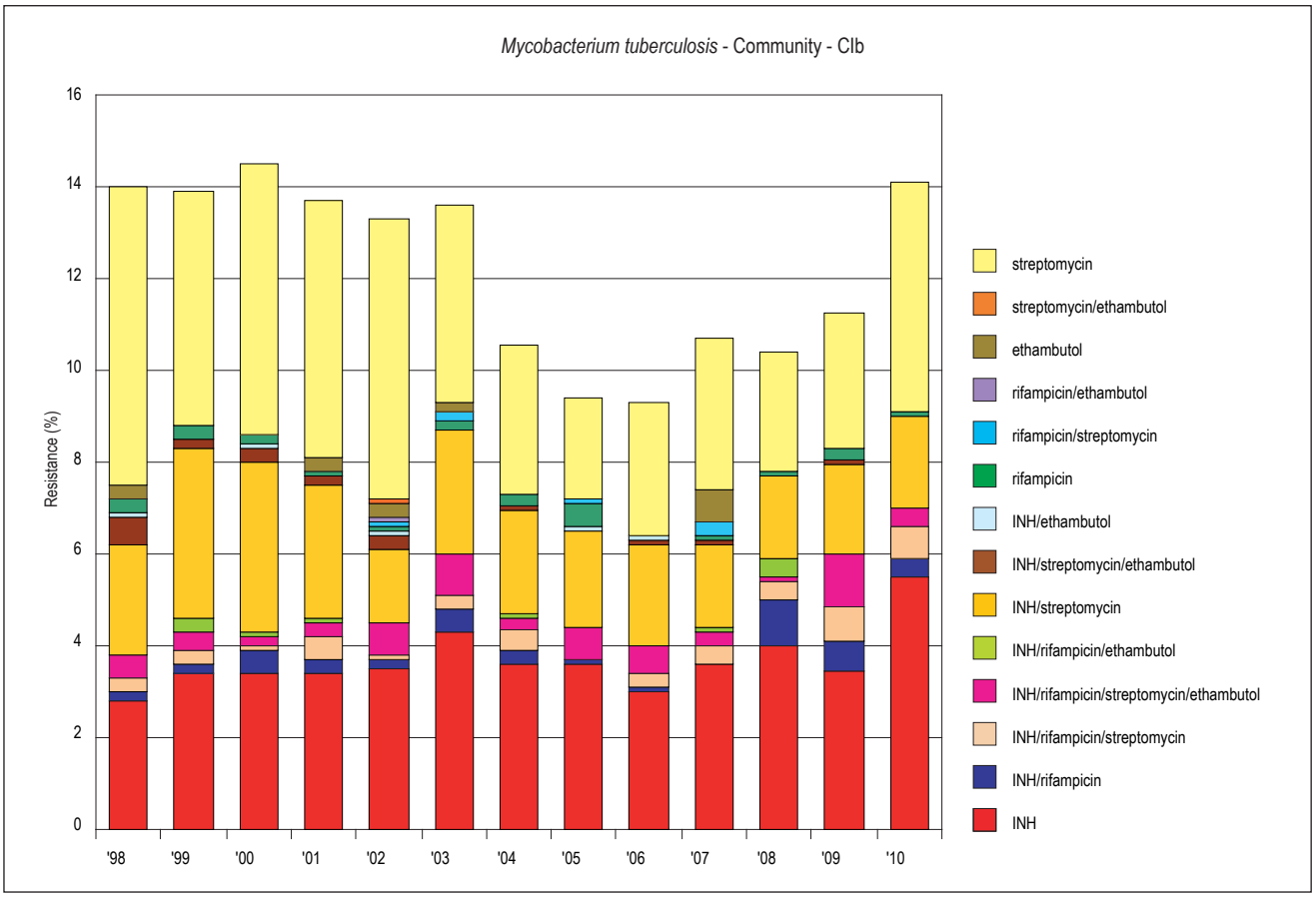

Figure 10. Trends in combined resistance among *Mycobacterium tuberculosis*.

Combined resistance to more than one drug in 2010 was observed in 3.5% of all isolates (figure 10); combined resistance to rifampicin and INH was recorded in 1.5%, whereas resistance to all four had decreased from 1.2% in 2009 to 0.4% in 2010.

Summary – *Mycobacterium tuberculosis*

1. The prevalence of new isolates has increased since 2007
2. Increased resistance was found for INH (9%) and streptomycin (8%)
3. Low resistance was found for rifampicin (1.6%) and ethambutol (0.4%) in 2010.
4. Combined resistance to INH and rifampicin was 1.5%; multiresistance to the four drugs tested was 0.4% in 2010.

4.2 Surveillance of resistance in specific patient populations

ISIS-AR

In 2007, the new surveillance system ISIS-AR replaced the old ISIS system that started in 1998 with collecting data from Dutch medical laboratories. The new ISIS-AR collects, apart from antibiotic resistance data, all epidemiological data present in the laboratory information systems connected to ISIS-AR. Furthermore, there is strong focus on the quality of data by national standardisation, structural quality control, and confirmation of unusual resistance data. The change to the new system also resulted in a change of the participating laboratories. In 2010, 21 laboratories reported results to ISIS-AR, two laboratories in academic hospitals and 19 laboratories serving non-academic hospitals and public health institutions. These laboratories provided ISIS-AR with data from 2008 onwards, which was used for the trend analysis.
The susceptibility of the isolates reported to ISIS-AR was routinely determined according to the standard techniques used in the individual laboratories. The majority of participating laboratories used automated systems for susceptibility testing, and still used CLSI breakpoints, except for two laboratories using CRG (Dutch) breakpoints and two laboratories using EUCAST breakpoints. The S-I-R interpretation as reported by the local laboratory was used for calculating resistance percentages. In reference to NethMap 2010, we took the number of intermediate and resistant isolates for the species tested as these are identical to the R breakpoint of EUCAST for most antibiotics.
The susceptibility results are presented as graphics where the change between ISIS and ISIS-AR (2007 to 2008) is displayed by a break in the trend line. See Materials and Methods (chapter 8) for details.

4.2.1 Selected patients from General Practice - ISIS-AR

4.2.1.1 Escherichia coli

From 2008 to 2010, susceptibility data on average 40.000 *E. coli* strains from urine were reported (except for fosfomycin for which about 8.000 isolates were tested) from selected GP patients to ISIS-AR. Significant trends in resistance were found for several of the antibiotics tested during the study period (2008-2010) (figure 11). A slight, but significant increase was observed for co-amoxiclav (18.7% to 20.3%, $p \leq 0.05$), ciprofloxacin (9.4% to 10.1%, $p \leq 0.05$) and norfloxacin (9.8% to 11.9% $p \leq 0.05$) and a decrease in resistance for trimethoprim (30.6% to 29.3%, $p \leq 0.05$ and co-trimoxazole (28.7% to 27.5%, $p \leq 0.05$).
In 2010 amoxicillin resistance was 43%, and co-amoxiclav resistance was 20%; both levels were higher than the levels found in the community for patients visiting the GP for an uncomplicated urinary tract infection in the same period (34% and 15% respectively. Trimethoprim resistance was 29% and co-trimoxazole resistance 27%. These levels were in the range of resistance found in hospitalized patients (see below). Resistance levels to quinolones (norfloxacin (12%) and ciprofloxacin (10%) and nitrofurantoin (5%) were comparable with the levels found in hospitalized patients, but were significantly higher than the levels found in the community (3%), but lower than the resistance levels found for patients visiting the outpatients departments (17%). Fosfomycin resistance was less than 1% during the study period (2008-2010).

4.2.1.2 Klebsiella pneumoniae

Resistance data from strains isolated from urine samples sent in by GPs were evaluated for the period 2008 -2010. The average number of strains tested was 4000 per year, except for fosfomycin, for which around 800 isolates were tested yearly. Co-amoxiclav resistance was 12% in 2010 (figure 12). Trimethoprim resistance increased from 27% in 2008 to 33% in 2010 ($p \leq 0.05$) and co-trimoxazole followed this pattern at a lower level, from 21% to 24%. Norfloxacin resistance increased from 6% to 10% ($p \leq 0.05$), whereas that of ciprofloxacin remained stable at 6%. Nitrofurantoin resistance decreased from 80% to 78% ($p \leq 0.05$); fosfomycin resistance increased from 7% in 2008 to 9% in 2010 ($p \leq 0.05$).

4.2.1.3 Klebsiella oxytoca

For *K. oxytoca* data of an average of 1200 urinary strains were reported yearly, except for fosfomycin, for which only 300 isolates were tested yearly. In 2010, co-amoxiclav resistance was similar to the levels among *K. pneumoniae* (11%), whereas the resistance percentages to the fluoroquinolones (1-2%), co-trimoxazole (7%), trimethoprim (8%), and nitrofurantoin (31%) were lower than those for *K. pneumoniae*.

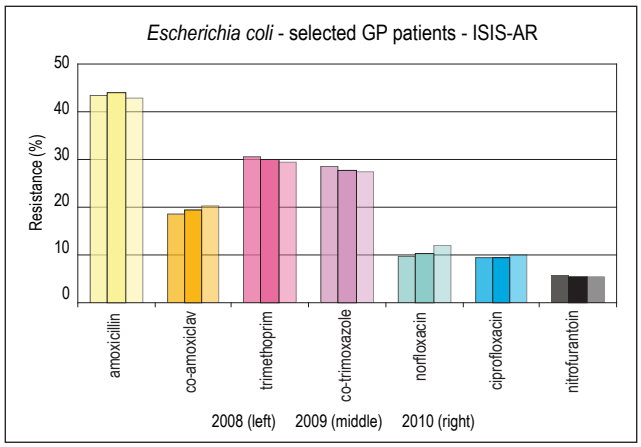

Figure 11. Trends in antibiotic resistance (2008-2010) among urinary strains of *Escherichia coli* from selected patients of general practice (N=40.000 per year), reported to ISIS-AR.

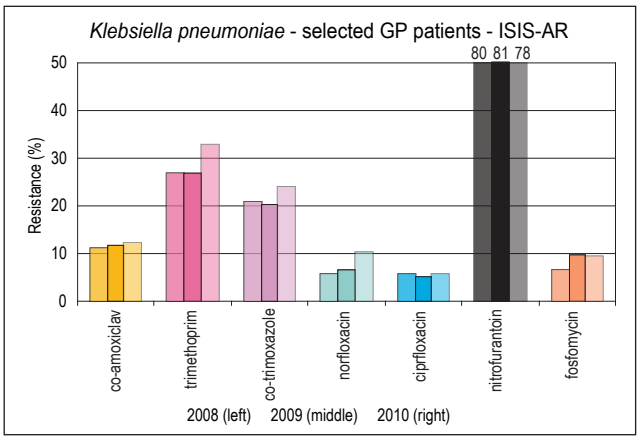

Figure 12. Trends in antibiotic resistance (2008-2010) among urinary strains of *Klebsiella pneumoniae* from selected patients of general practice (N=4.000 per year), reported to ISIS-AR.

Fosfomycin resistance was higher in *K. oxytoca* (12%) compared to *K. pneumoniae* (10%) (figure 13). Most resistance levels were relatively stable over 2008-2010, except for fosfomycin that increased from 4% to 12% ($p \leq 0.05$), whereas ciprofloxacin- (2% to 0.9%, $p=0.02$) and nitrofurantoin resistance (35.2% to 31.1%, $p \leq 0.05$) decreased significantly (figure 13).

4.2.1.4 Proteus mirabilis

The number of strains of *P. mirabilis* tested from 2008 to 2010 was around 6000, depending on the antibiotic tested (for fosfomycin 700 isolates were tested). Resistance to amoxicillin among selected patients from general practice in 2010 was 27% and seemed stable (figure 14); co-amoxiclav resistance increased from 9% to 11% ($p \leq 0.05$). These levels were comparable with those found in Outpatients Departments (10%) and Unselected Hospital Departments (12%). Trimethoprim and co-trimoxazole resistance was higher (39% and 36% respectively), than resistance found in Outpatients Departments (37% and 33% respectively) and Unselected Hospital Departments (34% and 30% respectively),

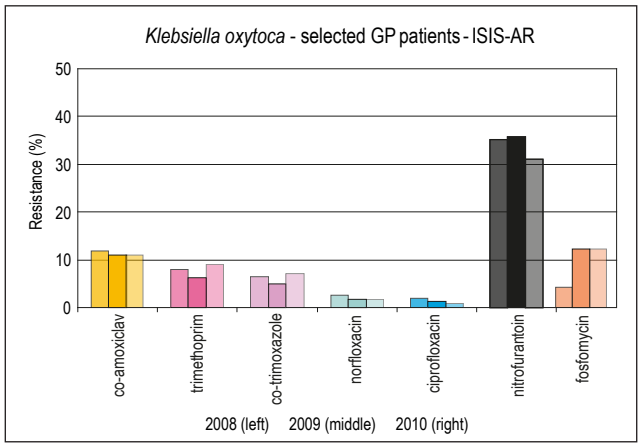

Figure 13. Trends in antibiotic resistance (2008-2010) among urinary strains of *Klebsiella oxytoca* from selected patients of general practice (N=1.200 per year), reported to ISIS-AR

suggesting that these drugs are commonly and frequently used in this group of patients. Quinolone resistance was increasing from 5 to 7%. Keeping in mind the lower number of isolates tested for fosfomycin, a matter of concern is the significant ($p \leq 0.05$) increase of fosfomycin resistance, from 5% in 2008 to 9% in 2010.

4.2.1.5 Pseudomonas aeruginosa

Data of resistance of among *P. aeruginosa* from urine of selected GP patients were available since 2008; on average 1500 isolates were reported per year. In 2010, resistance to ciprofloxacin was 10% and for tobramycin less than 1% to (figure 15).

Conclusion (see also table 1)

1. Co-amoxiclav resistance was stable at 11-12%.
2. Trimethoprim and co-trimoxazole resistance was high, except for *K. oxytoca* and increased among *K. pneumoniae*. Therefore these compounds are not usable for empirical therapy.
3. Quinolone resistance was stable, and highest among *E. coli* and *P. aeruginosa* (10%).
4. Fosfomycin resistance increased in *Klebsiella* spp and *P. mirabilis* to levels which may make the drug unusable for empiric therapy.

4.2.2 Outpatient Departments - ISIS-AR

4.2.2.1 Escherichia coli

Resistance data from outpatients departments could be evaluated for the period 2008-2010 (Figure 16). The numbers of *E. coli* strains, derived from urine were around 15.000 yearly.

In 2010 amoxicillin resistance was 48%, which is higher ($p<0.05$) than the levels found for selected GP patients (43%) and in the same range as for patients hospitalized in Unselected Hospital Departments (47%). Co-amoxicillin resistance increased from 21% in 2008 to 23% in 2010 ($p \leq 0.05$). Trimethoprim resistance was

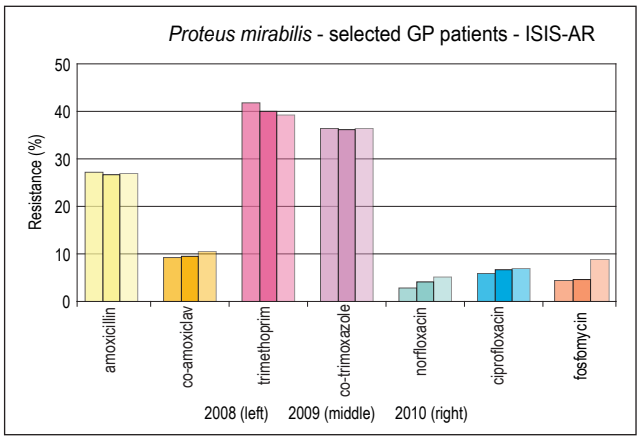

Figure 14. Trends in antibiotic resistance (2008-2010) among urinary strains of *Proteus mirabilis* from selected patients of general practice (N=6.000 per year), reported to ISIS-AR.

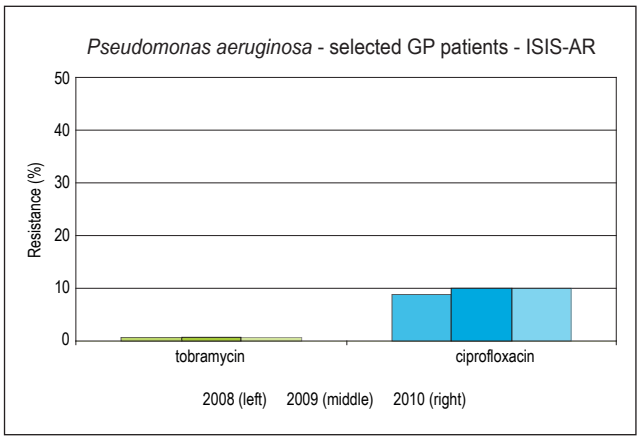

Figure 15. Trends in antibiotic resistance (2008-2010) among urinary strains of *Pseudomonas aeruginosa* from selected patients of general practice (N=1.500), reported to ISIS-AR.

33%, which was significantly higher (p≤0.05) than the levels found in selected GP patients (29%) and those in Unselected Hospital Departments (29%). Co-trimoxazole resistance was 31%. Ciprofloxacin resistance (17%) was significantly higher (p≤0.05) in outpatients compared to selected patients from GP (10%) and hospitalized patients (13%). This was also observed for nitrofurantoin resistance, which was 8% in outpatients compared to 6% in other patients groups. Fosfomycin resistance was less than 1%.Patients visiting outpatient departments are mostly referred by GPs or are controlled by specialists after a stay in the hospital and it is obvious that they have been exposed to antibiotic treatment before.

4.2.2.2 Klebsiella pneumoniae

Resistance data from urinary strains of *K. pneumoniae* (N=2000 yearly) reported to ISIS-AR by the Outpatients Departments were evaluated for the period 2008-2010. In general, resistance levels and trends were comparable with those found in samples from selected GP patients (figure 17). Co-amoxiclav resistance was stable at 12%; trimethoprim resistance increased from 23% in 2008 to 31% in 2010 (p≤0.05), co-trimoxazole resistance increased from 19% to 24% (p≤0.05). Norfloxacin resistance increased from 6% to 11% (p≤0.05) and

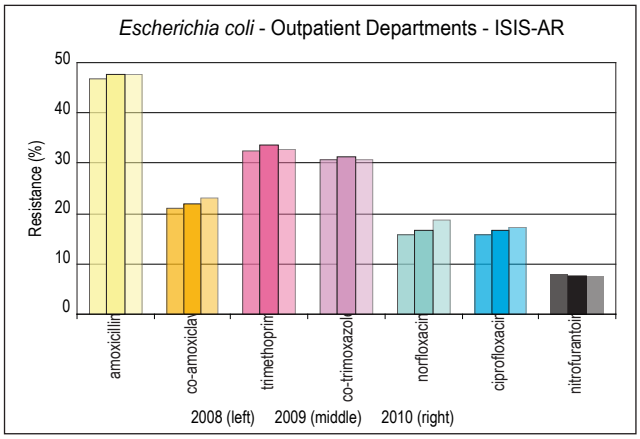

Figure 16. Trends in antibiotic resistance (2008-2010) among urinary strains of *Escherichia coli* from patients of outpatients departments (N=15.000), reported to ISIS-AR.

ciprofloxacin remained stable at 7%. Nitrofurantoin resistance was around 78%; fosfomycin resistance increased from 6% in 2008 to 12% in 2010 (p≤0.05).

4.2.2.3 Klebsiella oxytoca

Resistance data from urinary strains of *K. oxytoca* (N=850 yearly) from patients visiting the Outpatients

Table 1. Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa from selected patients of general practice in 2010.

Antibiotic	*E.coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*	*P. aeruginosa*
amoxicilin	43			27	
co-amoxiclav	19	12	11	11	
trimethoprim	29	33	9	39	
co-trimoxazole	28	24	7	36	
norfloxacin	12	10	2	5	
ciprofloxacin	10	6	1	7	10
nitrofurantoin	5	78	31		
fosfomycin	0.4	9	12	9	
tobramycin					0.6

increasing (p<=0.05)
stable
decreasing (p<=0.05)

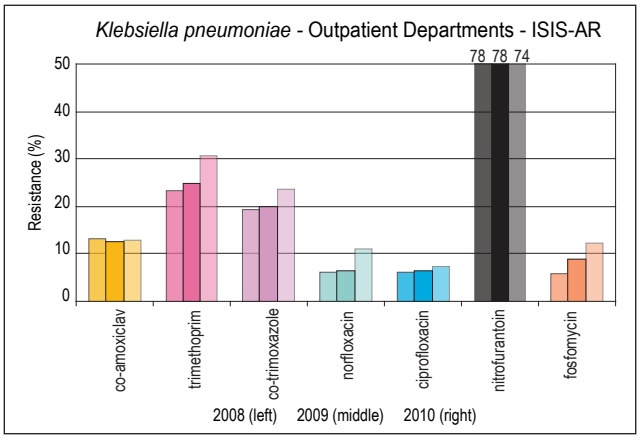

Figure 17. Trends in antibiotic resistance (2008-2010) among urinary strains of *Klebsiella pneumoniae* from patients of outpatients departments (N=2.000 per year), reported to ISIS-AR.

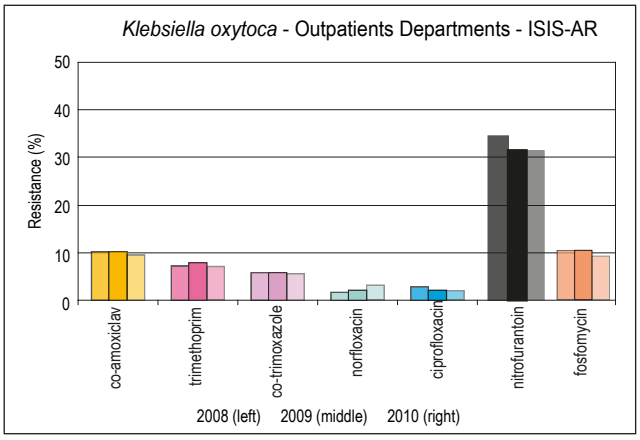

Figure 18. Trends in antibiotic resistance (2008-2010) among urinary strains of *Klebsiella oxytoca* from patients of Outpatients Departments (N= 850 per year), reported to ISIS-AR.

Departments were evaluated for the period 2008-2010. Resistance levels were stable and trends (figure 18) were comparable with those found in the samples from selected GP patients (except for fosfomycin and nitrofurantoin) (figure 13).

4.2.2.4 Proteus mirabilis

About 1800 strains of *P. mirabilis* from urine of patients visiting the Outpatients Departments were yearly tested from 2008 to 2010. Amoxicillin- and co-amoxiclav resistance was comparable to those found in Selected GP patients (figure 19). Trimethoprim and co-trimoxazole resistance were lower (37% and 33% respectively) compared to the selected GP patients. Trimethoprim resistance was 40% in 2008, but had decreased significantly ($p \leq 0.05$) to 37%. Ciprofloxacin resistance appeared stable (10%); in contrast fosfomycin resistance increased from 4.5% in 2008 to 13% in 2010 ($p \leq 0.05$). These findings suggest replacement of trimethoprim and co-trimoxazole by fosfomycin, since it is introduced in the NHG standard as an alternative for nitrofurantoin and trimethoprim.

4.2.2.5 Pseudomonas aeruginosa

Data of resistance of among *P. aeruginosa* from urine of patients from Outpatients Departments were available since 2008 (on average a 1000 isolates per year were reported). It was observed that 13% of all strains were resistant to ciprofloxacin, which is higher than that found in selected GP patients and patients from Unselected Hospital departments. Tobramycin resistance was 1% (figure 20).

Conclusion (see also table 2)

1. Co-amoxiclav resistance was stable and highest in *E. coli* (22%) compared to the other pathogens (9-13%), which makes co-amoxiclav unusable for empiric therapy.
2. Trimethoprim and co-trimoxazole resistance was high, except in *K. oxytoca* and increased in *K. pneumoniae*. Therefore thes compounds are not usable for empiric therapy.
3. Quinolone resistance was stable and highest among *E. coli* and *P. aeruginosa*, and increased in *Klebsiella* spp and *P. mirabilis*. Therefore these compounds are not usable for empiric therapy
4. Fosfomycin resistance was low in *E. coli*, but increased in *K. pneumoniae* and *P. mirabilis* to levels which may make the drug unusable for empiric therapy.

Table 2. Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa from patients of Outpatients Departments in 2010.

Antibiotic	*E.coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*	*P. aeruginosa*
amoxicilin	48			26	
co-amoxiclav	23	13	10	10	
trimethoprim	33	31	7	37	
co-trimoxazole	31	24	6	33	
norfloxacin	19	11	3	7	
ciprofloxacin	17	7	2	10	13
nitrofurantoin	8	74	31		
fosfomycin	0.6	12	9	13	
tobramycin					1

increasing (p<=0.05)
stable
decreasing (p<=0.05)

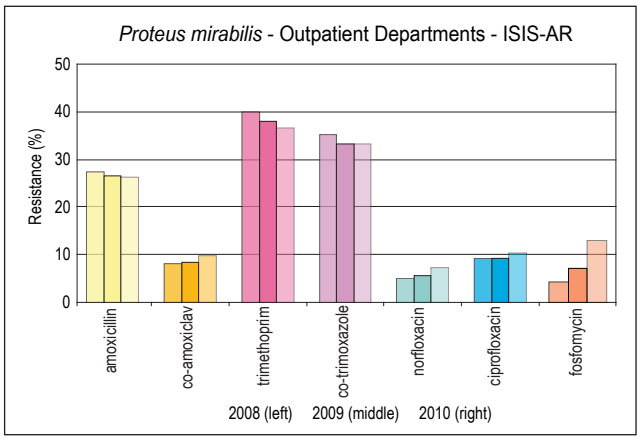

Figure 19. Trends in antibiotic resistance (2008-2010) among urinary strains of *Proteus mirabilis* from patients of Outpatients Departments (N=1.800 per year), reported to ISIS-AR.

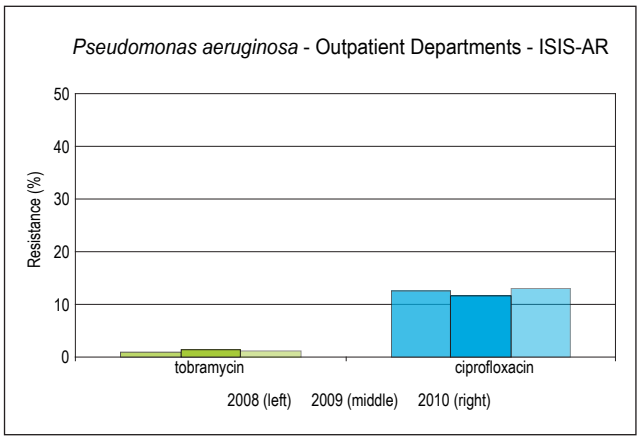

Figure 20. Trends in antibiotic resistance (2008-2010) among urinary strains of *Pseudomonas aeruginosa* from patients of Outpatients Departments (N=1.000 per year), reported to ISIS-AR.

4.2.3 Unselected Hospital Departments - ISIS-AR

4.2.3.1 Escherichia coli

The number of *E. coli* strains from Unselected Hospital Departments participating in ISIS-AR, was around 20,000 in the last 3 years; not all strains were tested for all antibiotics. Amoxicillin resistance increased from 36% in 1998 to 47% in 2010 (figure 21) ($p \leq 0.05$).

Co-amoxiclav resistance was fluctuating around 20% (range 16-25%) from 1998 to 2007, but seems to increase steadily since 2008 from 21% to 24% in 2010 ($p \leq 0.05$) (figure 21).

Piperacillin resistance was reported from 2008 on (figure 21), and increased from 32% in 2008 to 44% in 2010 ($p \leq 0.05$). At the same time piperacillin-tazobactam resistance increased significantly ($p \leq 0.05$) from 3 to 8%

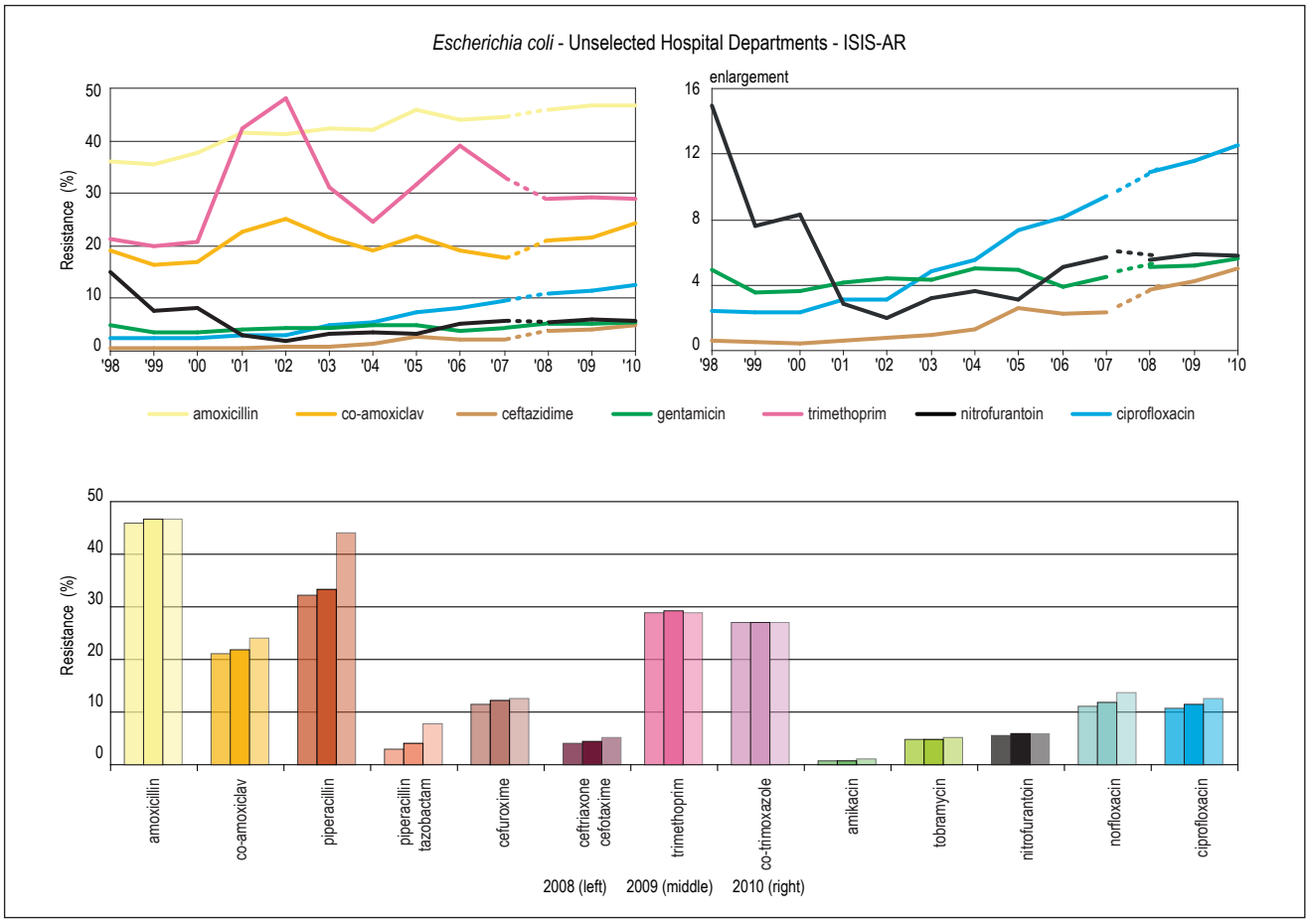

Figure 21. Trends in antibiotic resistance (1998-2010) among clinical strains of *Escherichia coli* from Unselected Hospital Departments (N =20.000 per year), reported to ISIS-AR.

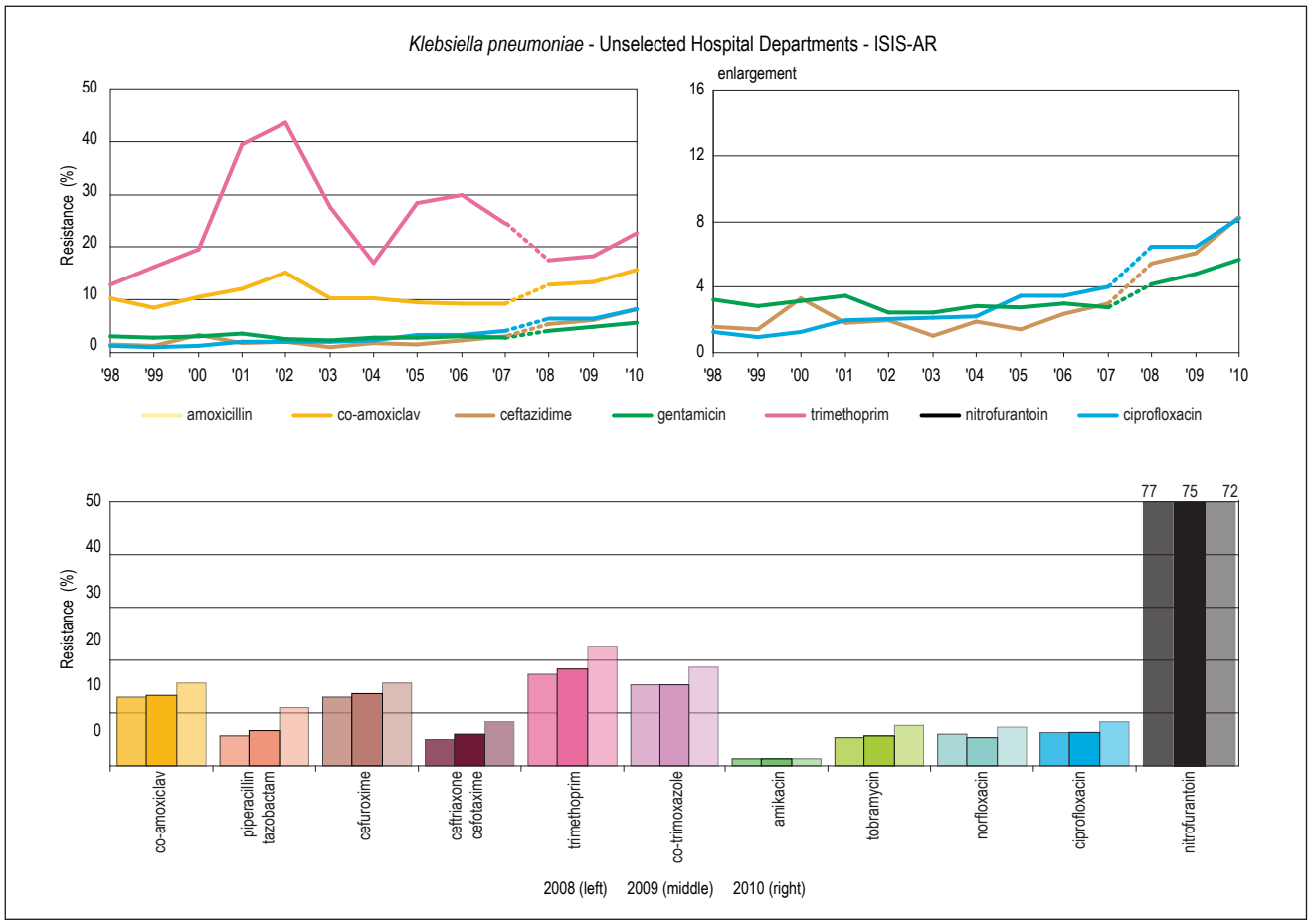

Figure 22. Trends in antibiotic resistance (1998-2010) among clinical strains of *Klebsiella pneumoniae* from Unselected Hospital Departments (N =3.800 per year), reported to ISIS-AR.

(figure 21) Imipenem- and meropenem resistance was sporadic reported in 2009 and was 0.03% in 2010. Cefuroxime resistance was around 12-13% from 2008 to 2010 (figure 21). For ceftazidime resistance, a longer surveillance period was available showing a significant increase from 0.6% in 1998 to 5% in 2010 ($p \leq 0.05$) (figure 21). In 2010, resistance levels of ceftriaxone and cefotaxime equalled those of ceftazidime.
The levels of trimethoprim (figure 21) resistance reported fluctuated strongly until 2007; this may be explained by differences in interpretation by use of various breakpoints in the past and the existence of many strains with MIC around the breakpoints. Since 2008 the resistance level reported is 29% and seems stable (figure 21). Co-trimoxazole resistance was reported since 2008 and it was stable at 27% from 2008 to 2010 (figure 21) Gentamicin resistance increased from 3% in 1998 to 6% in 2010 (figure 21). Nitrofurantoin resistance reported has slowly increased from 3% in 2001-2005 to 5-6% in 2010. The very high resistance levels reported before 2001 were most probably the result of interpretation based on different breakpoints (figure 21).
Ciprofloxacin resistance increased significantly from 2-3% in 1998 to 12.5% in 2010 (figure 21) ($p \leq 0.05$), norfloxacin resistance increased from 11% in 2008 to 14% in 2010 ($p \leq 0.05$).

4.2.3.2 Klebsiella pneumoniae

In 2010 susceptibility data of around 3800 *K. pneumoniae* isolates were reported to ISIS-AR.Co-amoxiclav resistance fluctuated between 10-15% until 2002, then decreased to 10% in 2003, remained stable until 2007. From 2008 the resistance level increased significantly from 13% to 16% in 2010 ($p \leq 0.05$) (figure 22) Piperacillin-tazobactam resistance was determined from 2008 on and increased from 6% to 11% in 2010 ($p \leq 0.05$) (figure 23). In 2010, imipenem- and meropenem resistance was low at 0.2%).
Cefuroxime resistance increased from 13% in 2008 to 16% in 2010 ($p \leq 0.05$) (figure 23). Resistance to ceftazidime fluctuated around 1-3% until 2007, but increased from 5% in 2008 to 8% in 2010 ($p \leq 0.05$) (figure 22). The same trend was reported for ceftriaxone and cefotaxime.
Trimethoprim resistance increased gradually from 11% in 1998 to 23% in 2010 ($p \leq 0.05$), with large fluctuations over the years ranging from 13% in 1998 to 44% in 2002 (figure 22). This might be explained by the presence of many strains with MICs around the breakpoint. The resistance rate to co-trimoxazole increased from15% in 2008 to 19% in 2010 ($p \leq 0.05$).
Gentamicin resistance was stable around 3-4% until 2007. From 2008, resistance increased from 4% to 6%

in 2010 (p≤0.05). Resistance to tobramycin showed the same trend and increased from 5% in 2008 to 8% in 2010; amikacin resistance remained stable at 1.5% during 2008-2010 (figure 22). Nitrofurantoin resistance reported fluctuated strongly between 38-77% until 2007 (not shown), which was probably caused by use of various and/or changing breakpoints. From 2008 on the resistance levels decreased from 77% to 72% in 2010. Ciprofloxacin resistance increased gradually from 1% in 1998 to 8% in 2010 (figure 22) (p≤0.05).

4.2.3.3 Enterobacter cloacae

Resistance data of *E. cloacae* for a limited number of antibiotics were reported from 2008 on (on average 2200 isolates per year). Resistance to piperacillin-tazobactam increased from 19% in 2008 to 29% in 2010 (p≤0.05) (figure 23). Imipenem- and meropenem resistance was occasionally found, and was 0.2% in 2010. Trimethoprim and co-trimoxazole resistance increased to 9% in 2010 (figure 23).
Gentamicin resistance increased from 5% in 2008 to 7% in 2010 (p≤0.05), amikacin- and tobramycin resistance was stable at 3% and 7% respectively in 2010. Ciprofloxacin resistance reported was stable at 6%.

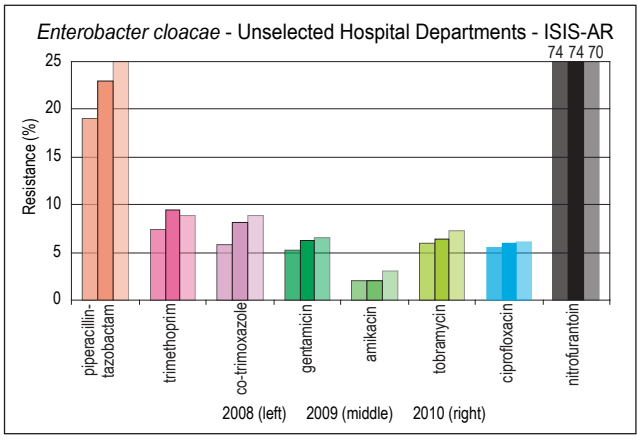

Figure 23. Trends in antibiotic resistance (2008-2010) among clinical strains of *Enterobacter cloacae* from Unselected Hospital Departments (N =2.200 per year), reported to ISIS-AR.

4.2.3.4 Proteus mirabilis

In 2010, the total number of strains of *P. mirabilis* tested was 3.000. Not all antibiotics were tested with all strains. Amoxicillin resistance among *P. mirabilis* showed a continuous increase from 13% in 1998 to 25% in 2006 and remained stable thereafter and remained stable at 27% from 2008 to 2010 (figure 24). Co-amoxiclav

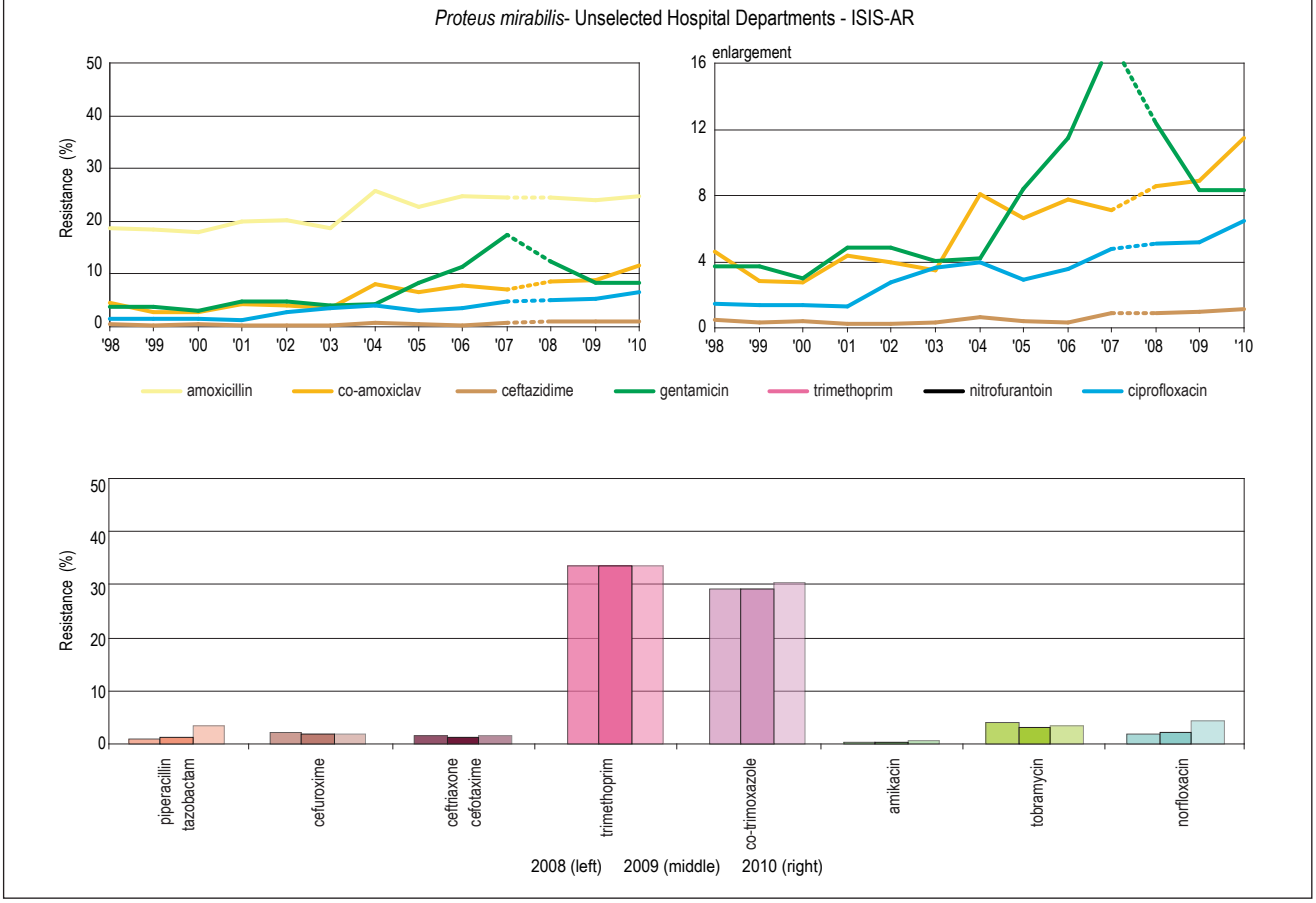

Figure 24. Trends in antibiotic resistance (1998-2010) among clinical strains of *Proteus mirabilis* from Unselected Hospital Departments (N =3.000 per year), reported to ISIS-AR.

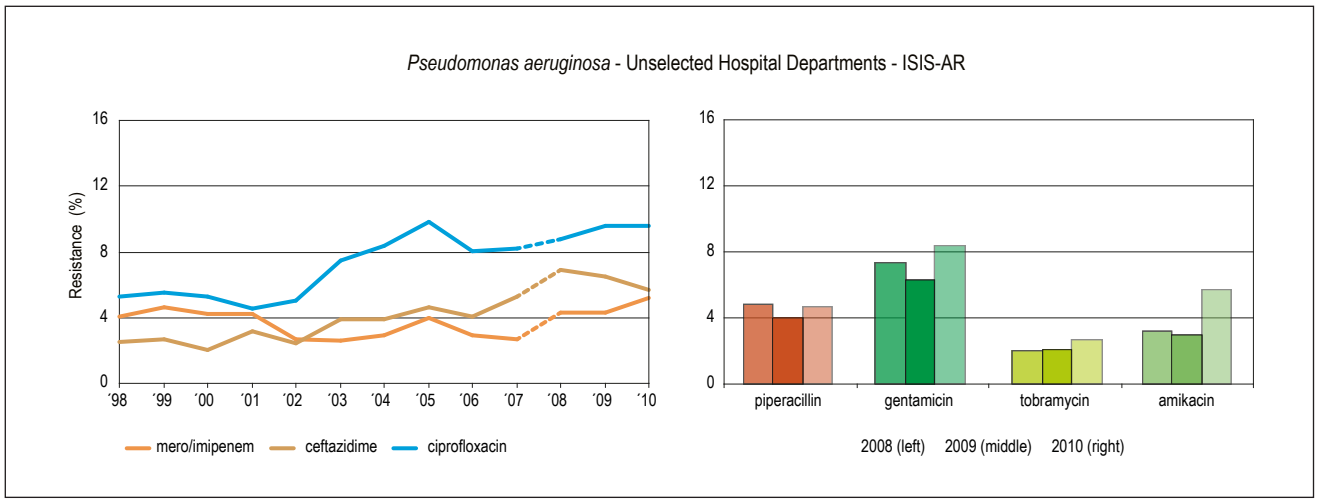

Figure 25. Trends in antibiotic resistance among clinical strains of *Pseudomonas aeruginosa* from patients of Unselected Hospital Departments (N=5.000 per year), reported to ISIS-AR.

resistance increased from 5% in 1998 to 12% in 2010. Cefuroxime-, cefotaxime-, ceftriaxone- and ceftazidime resistances were 2% or less and showed no trends over time (figure 24).
Trimethoprim resistance in *P. mirabilis* was higher than 50% until 2002; it decreased thereafter to 34% in 2008, and remained stable thereafter. The high resistance level before 2002 is not well-understood. Co-trimoxazole resistance followed the pattern of trimethoprim at a lower level with 30% resistance in 2010. Both levels were lower (but not significantly) than those found for selected patients from GP and Outpatients Departments.
Gentamicin resistance increased slowly with fluctuations from 4% in 1998 to 8% in 2010. This level is comparable with the level in Intensive Care Units (see below).
Tobramycin- and amikacin resistance remained less than 3% and 1% respectively.
Ciprofloxacin resistance increased from 2% in 1998 to 7% in 2010. In 2010, this level was similar to that of selected GP patients and significantly lower ($p \leq 0.05$) than the resistance levels found in Outpatients Departments (10%).

4.2.3.5 Pseudomonas aeruginosa
In 2010, resistance data of about 5.000 strains were reported. Piperacillin resistance among *P. aeruginosa* isolated in Unselected Hospitals was routinely recorded from 2008 on. The resistance level from 2008 to 2010 was 4-5% (figure 25).
Meropenem resistance fluctuated around 3-4% during the whole study period, but has now increased 5% in 2010 ($p \leq 0.05$) (figure 25).
Ceftazidime resistance increased slowly from 2-3% in 1998 to 4-5% in 2003-2007 and 6-7% in 2008-2010 (figure 25). Gentamicin resistance reported was 7-8% from 2008 to 2010. Before 2008 unusual high amounts of gentamicin-resistant strains were reported by some laboratories, which could not be confirmed (data not shown). Tobramycin resistance was low and stable 2-3% from 2008 to 2010; in contrast an increase in resistance to amikacin was reported from 3% in 2008 to 6% in 2010 ($p \leq 0.05$).
Ciprofloxacin resistance was stable around 5% until 2002, then increased slowly with some fluctuations to 10% in 2010 (figure 25).

4.2.3.6 Staphylococcus aureus
Resistance data of in total 65.000 strains of *S. aureus* for methicillin, erythromycin, gentamicin, ciprofloxacin and vancomycin were collected since 1998. From 2008 also resistance data for other antimicrobial agents were reported (N=10.000 per year). The overall percentage of MRSA increased slowly from less then 1% until 2002 to almost 2.8% in 2007. Due to the exclusion of screening isolates, the %MRSA reported by the new surveillance system was lower at 1.2% in 2008, but increased to 1.6% in 2010 ($p \leq 0.05$) (figure 26). In 2010, a total number of 3262 MRSA isolates were forwarded to the Centre for Infectious Disease Control in the Netherlands at the National Institute for Public Health and the Environment (RIVM) for typing, 292 isolates more than the number received in 2009 (figure 26). The number of the livestock-related CC398 strains remained constant (1249 in 2010, 38% of the total number of strains).
Erythromycin resistance increased from 5% in 1998 to 11% in 2010 (figure 26); clarithromycin resistance was reported since 2008 and was 9% (figure 26). The same level was reported for clindamycin since 2008.
Doxycycline resistance was 3.5% in 2010 compared to 5% resistance reported to tetracycline. It is known that doxycycline has a higher intrinsic activity than tetracycline towards some staphylococci. Co-trimoxazole resistance decreased from 5% in 2008 to 4% in 2010.
Gentamicin- and tobramycin resistance remained low (1%), though a slight increase of tobramycin resistance to 1.4% was observed in 2010.
Ciprofloxacin resistance rose from 3% in 1998 to 11% in 2010 (figure 26). Resistance to rifampicin was less than 1%. Vancomycin resistance was reported rarely during

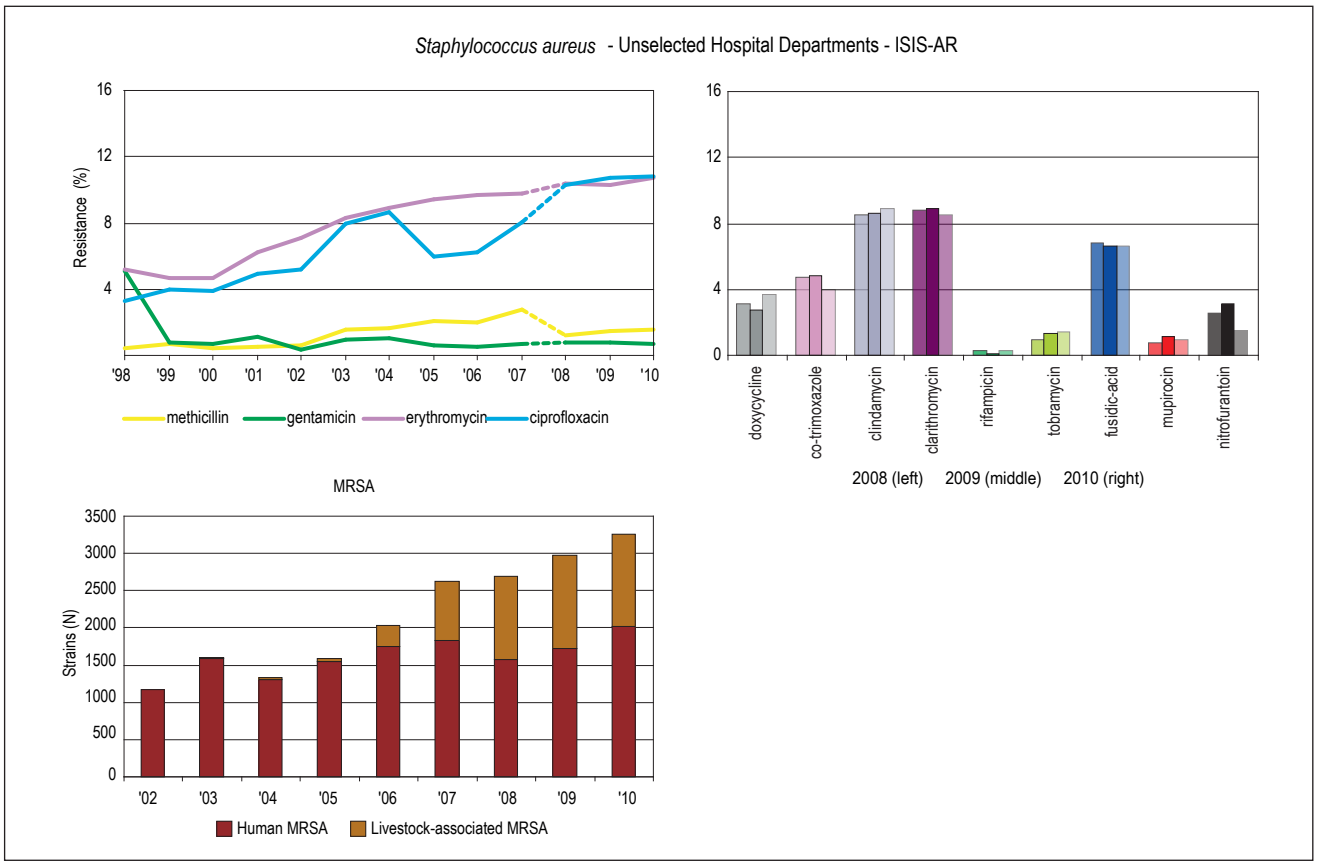

Figure 26. Trends in antibiotic resistance (1998-2010) among clinical strains of *Staphylococcus aureus* from patients of Unselected Hospital Departments (N=10.000 per year), reported to ISIS-AR and the numbers and origin of MRSA strains sent to the RIVM for typing (national MRSA surveillance).

the whole study period. Fusidic acid resistance was stable at 7% from 2008 to 2010. Mupirocin resistance was 1%.

4.2.3.7 Staphylococcus epidermidis including coagulase negative staphylococci

Resistance data of about 6.000 strains of *S. epidermidis* including coagulase negative staphylococci were reported in 2010. Resistance to methicillin, erythromycin, gentamicin, ciprofloxacin and vancomycin were collected since 1998. From 2008 also resistance data for other antimicrobial agents were reported. Methicillin resistance increased from 41% in 1998 to 53% in 2010 (figure 27). Erythromycin resistance increased slowly from 40% in 1998 to 49% in 2010; clarithromycin resistance decreased from 48% in 2008 to 45% in 2010 ($p \leq 0.05$). Clindamycin resistance was 37% in 2010 (figure 27). Doxycycline resistance was around 18% from 2008-2010, whereas that to tetracycline was 28%. Co-trimoxazole resistance reported decreased from 31% in 2008 to 28% in 2010 ($p \leq 0.05$).
Gentamicin resistance fluctuated around 30% during the whole study period; tobramycin resistance was also 30%.
Ciprofloxacin resistance fluctuated within 30-38% from 1998 to 2010 without a clear trend.
Vancomycin-resistance was less than 1% during the whole study period, but fluctuated between 0.1 and 0.8%. In the last 3 years resistance increased from 0.2% to 0.5%. Rifampicin resistance remained 7% from 2008-2010. Fusidic acid resistance was 46% in 2010, whereas resistance to mupirocin increased from 12% in 2008 to 15% in 2010 ($p \leq 0.05$).

4.2.3.8 Streptococcus pneumoniae

In 2010, susceptibility data from 2500 strains of *S. pneumoniae* were reported from Unselected Hospital Departments. *Streptococcus pneumoniae* strains resistant to penicillin (MIC > 2 mg/l) are not often isolated in the Netherlands. In 2010, 1.8% of all pneumococci from Unselected Hospital Departments were reported resistant. Over the years resistance was lower than 2%, except for 2004, 2005 and 2009, when 3% resistance was reported (figure 28).
Cefotaxime-/ceftriaxone resistance was reported until 2007 (less than 1%) and thereafter only by a few laboratories. Data are therefore not presented.
Erythromycin resistance increased from 3% in 1998 to 10% in 2007 and seems to decline gradually, to 8% in 2010. Doxycycline resistance fluctuated between 5 and 10% over time, and was 9% in 2010 (figure 28).
Ciprofloxacin resistance reported fluctuated considerably over the years (4-24%) until 2007, and was not reported since then. The difference in resistance levels of ciprofloxacin was probably due to application of different breakpoints applied. The breakpoint for susceptibility recommended by EUCAST for ciprofloxacin is very

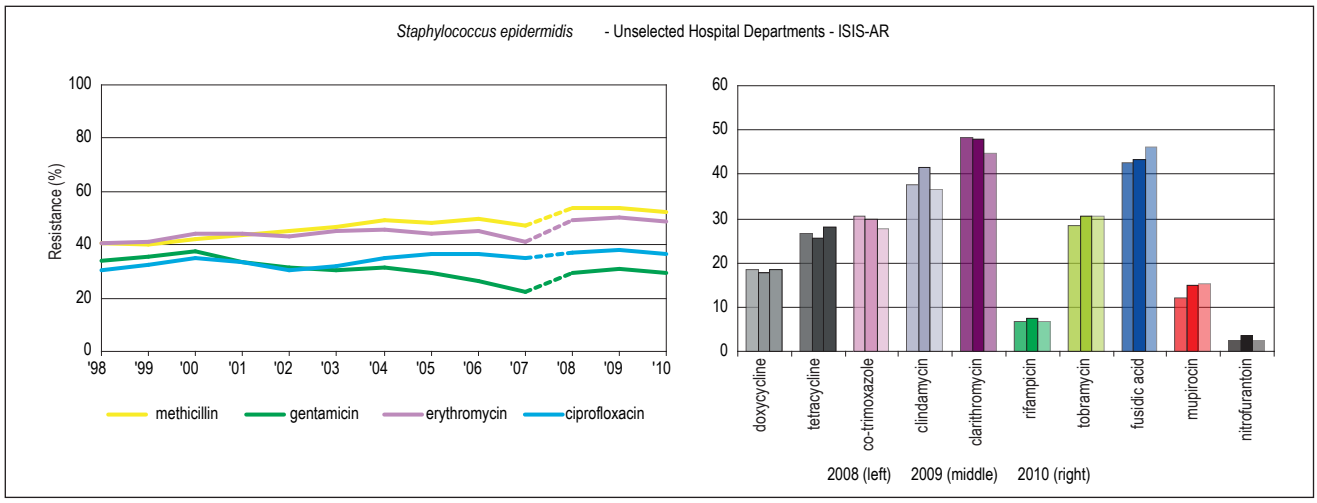

Figure 27. Trends in antibiotic resistance (1998-2010) among clinical strains of *Staphylococcus epidermidis* including coagulase negative staphylococci from patients of Unselected Hospital Departments (N=6.000 per year), reported to ISIS-AR.

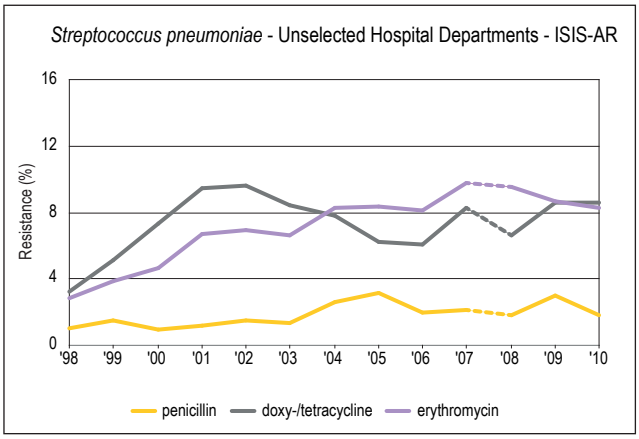

Figure 28. Trends in antibiotic resistance (1998-2010) among clinical strains of *Streptococcus pneumoniae* from patients of Unselected Hospital Departments (N=2500 per year), reported to ISIS-AR.

low (MIC < 0.125 mg/l). This implies that less than 1% should have been categorized really susceptible and that all wild type S. pneumoniae strains (MIC 0.25-1 mg/l) are categorized as intermediate. Knowing that many laboratories used automatic international systems, still based on CLSI recommendations for susceptibility (MIC < 1 mg/l), one can expect such fluctuations in testing.

4.2.3.9 Haemophilus influenzae

In 2010, resistance data of 3300 strains of *H. influenzae* were reported from Unselected Hospital Departments. Amoxicillin resistance showed an increasing trend, from 6% in 1998 to 17% in 2010 (figure 29) ($p \leq 0.05$). Co-amoxiclav was reported since 2008, and was stable at 4.5%. This implied that about 12-13% of all strains were beta-lactamase producers. Resistance to cefotaxime was less than 1% during the whole study period. Erythromycin resistance has been high since 1998, and increased to around 90% in the last four years (data not shown). Low resistance rates (1-3%) but increasing ($p \leq 0.05$) were found for doxycycline among *H. influenzae* isolates (figure 29).
Resistance data of co-trimoxazole were reported from 2008 onwards (figure 29) and suggest an increasing trend from 16% to 19% resistance in 2010 ($p \leq 0.05$). Ciprofloxacin resistance has been increasing from 0.1% in 2008 to 1.5% in 2010 ($p \leq 0.05$).

4.2.3.10 Moraxella catarrhalis

In 2010, resistance data of 1100 strains of *M. catarrhalis* were reported from Unselected Hospital Departments. Amoxicillin resistance has been between 65 and 85% during the years, so this agent can hardly be seen as a therapeutic option for *M. catarrhalis* infections. Co-amoxiclav resistance was reported from 2008 on and it was 1-2%. Cefotaxime-/ceftriaxone-/ceftazidime resistance was less than 2% during the whole study period (figure 30). Erythromycin fluctuated from 5-10% over the study period without a specific trend. Resistance to doxycycline fluctuated between 1-3 %, but dropped to less than 1% in 2010 in (figure 30). Co-trimoxazole resistance was reported from 2008 on, being 8-10%.

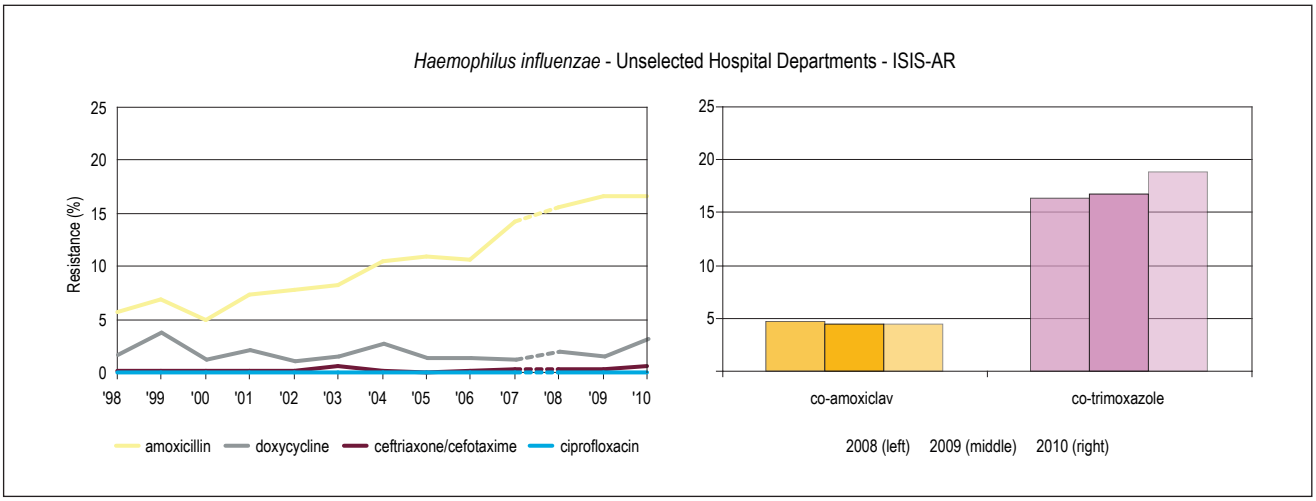

Figure 29. Trends in antibiotic resistance (1998-2010) among clinical strains of *Haemophilus influenzae* from patients of Unselected Hospital Departments (N=3300 per year), reported to ISIS-AR.

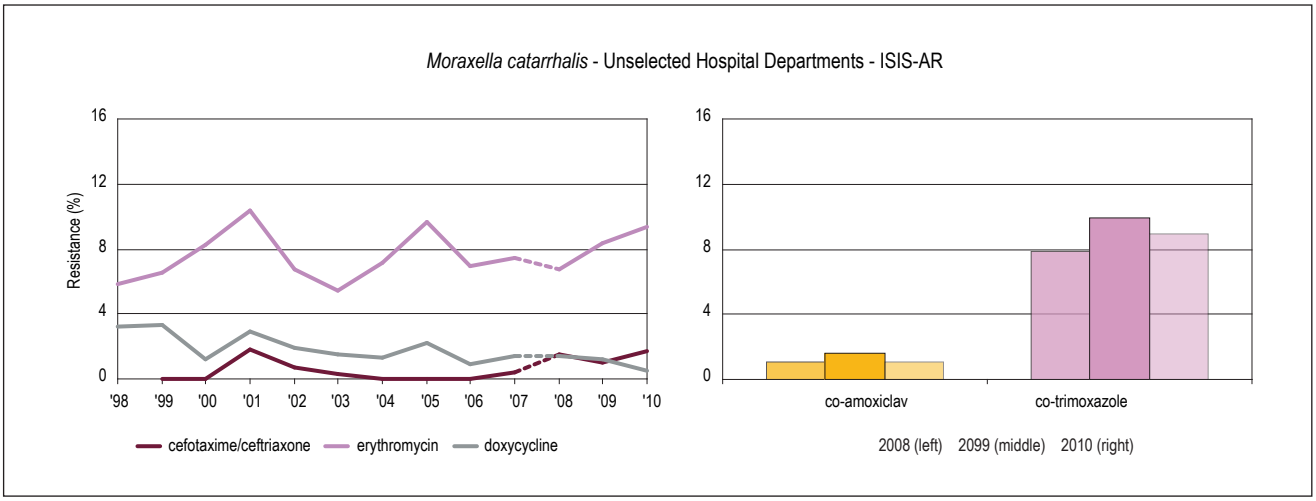

Figure 30. Trends in antibiotic resistance among clinical strains of *Moraxella catarrhalis* from patients of Unselected Hospital Departments (N=1.100 per year), reported to ISIS-AR.

Table 3. Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa in Unselected Hospital Departments in 2010.

Antibiotic	*E. coli*	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
amoxicilin	47			25	
co-amoxiclav	24	16		12	
piperacillin	44				5
pipera-tazobactam	8	11	29	3	
meropenem	0		0		5
cefuroxime	13	16		2	
cefotax/triax	5	8		2	
ceftazidime	5	8		1	6
gentamicin	6	6	7	8	8
tobramycin	5	8	7	3	3
amikacin	1	1	39	1	6
trimethoprim	29	23	10	34	
co-trimoxazole	27	19	9	30	
norfloxacin	14	7		4	
ciprofloxacin	13	8	6	7	10
nitrofurantoin	6	72	70		

increasing (p<=0.05)
stable
decreasing (p<=0.05)

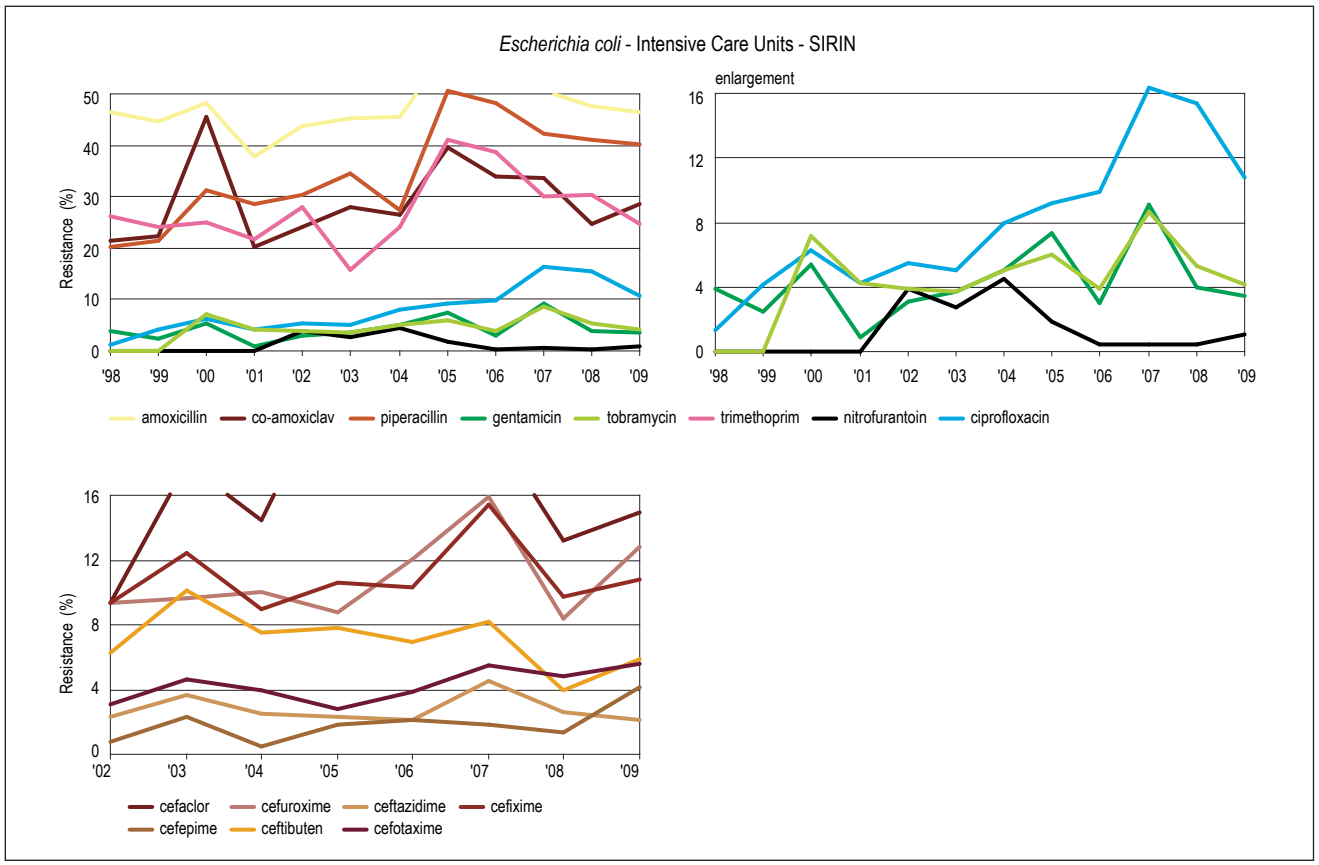

Figure 31. Trends in antibiotic resistance among clinical strains of *Escherichia coli* from Intensive Care Units (N = 2.235).

Conclusion (see also table 3)

1. Co-amoxiclav resistance was stable and highest among *E. coli* (22%) compared to the other pathogens (7-12%), which makes co-amoxiclav unusable for empiric therapy. Piperacillin resistance increased in *E. coli*.
2. Cefuroxime resistance increased in *E. coli* and *K. pneumoniae* to levels which make the drug unusable for empiric therapy. Cefotaxime- and ceftriaxone resistance was low, but increased in *E. coli* and *K. pneumoniae*.
3. Aminoglycoside resistance was low, but increased in most species
4. Trimethoprim and co-trimoxazole resistance is too high to be usable for empiric therapy.
5. A matter of concern is the increased resistance of quinolone resistance
6. Standard antibiotics for respiratory tract infections by pneumococci remain first choice (penicillin/amoxicillin, macrolide, doxycycline); the high resistance to amoxicillin, co-trimoxazole and erythromycin among *H. influenzae* and *M. catarrhalis* asks for other treatment schedules.

4.2.4 Intensive Care Units - SIRIN

SIRIN

Resistance in selected hospital departments was recorded by studying susceptibility patterns in 14 large referral hospitals participating in the longitudinal national SWAB study for Surveillance of Intramural Resistance in The Netherlands (SIRIN); the design of SIRIN differs significantly from ISIS-AR by generating quantitative susceptibility data, performed by the central laboratory of Medical Microbiology of the University Medical Centre Maastricht. The selected departments participating in SIRIN included the Intensive Care Units, being wards with high use of antibiotics and, consequently, high selective pressure favouring the emergence of resistance. Also included were the Urology Services and the Pulmonology Services, the latter two representing departments with frequent use of specific oral antibiotics. The quantitative data of all years were evaluated by use of EUCAST breakpoints according to the decision of SWAB in 2009 to adopt the EUCAST guidelines for susceptibility testing and surveillance. Results were analysed per species of common nosocomial pathogens and are presented in the accompanying figures.

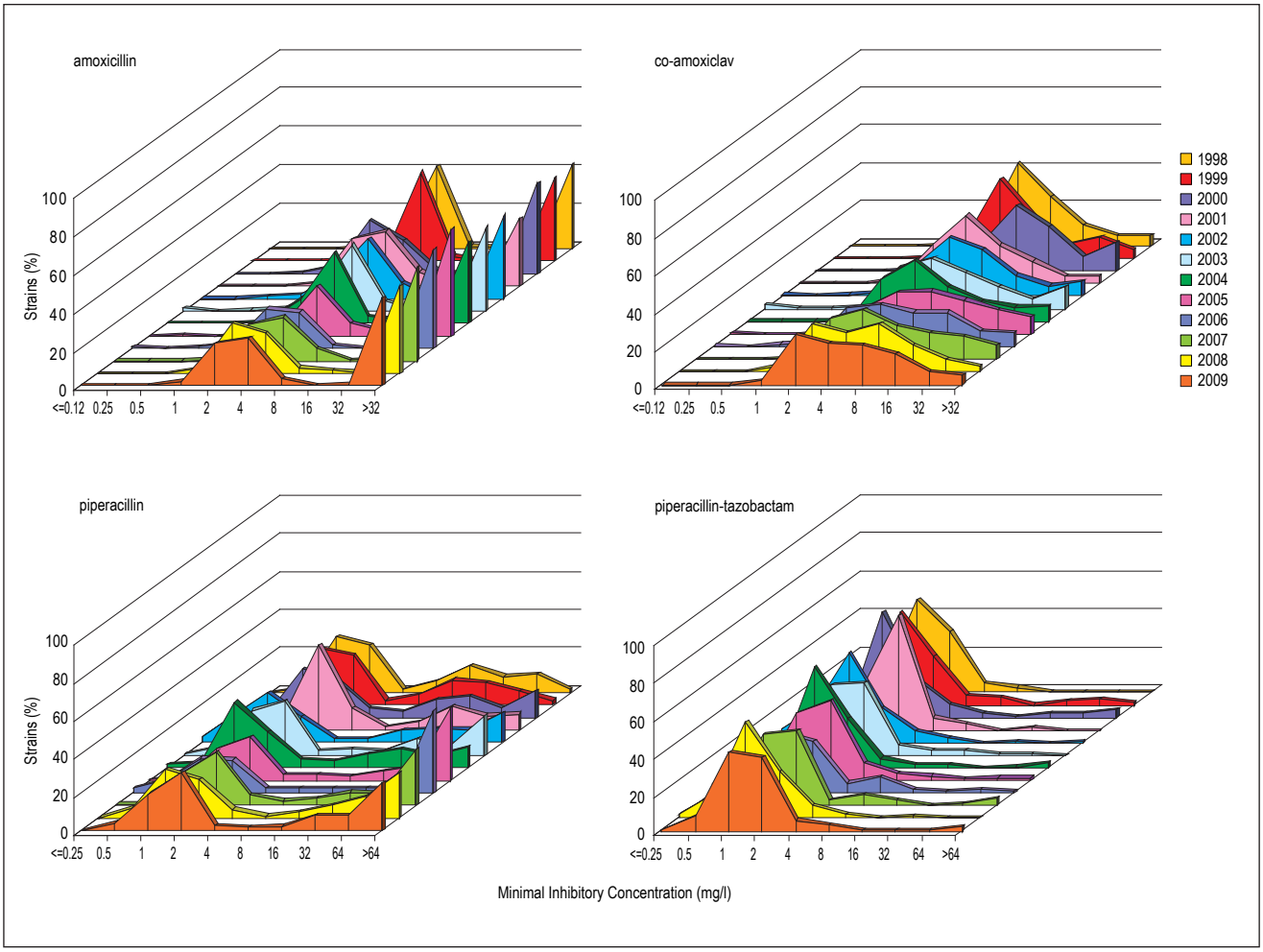

Figure 32. MIC distributions of penicillins for *Escherichia coli* from Intensive Care Units.

4.2.4.1 Escherichia coli

The numbers of strains of *E. coli* collected from Intensive Care Units were 150-200 yearly in the first five years and 225-290 yearly thereafter. Some fluctuations in resistance may be caused by the difference in numbers of isolates in a given year.

Amoxicillin resistance was roughly 45-47% during the years with considerable fluctuations between 2005 and 2007 (figure 31). The distribution of MICs (figure 32) in Intensive Care Units showed two subpopulations: a susceptible one with a broad MIC range from 0.5-8 mg/l (peak at 2-4 mg/l) and a resistant one with MICs >32 mg/l. The resistant subpopulation was slightly growing during the years, whereas the peak of the susceptible one was flattening.

Co-amoxiclav resistance in increased from 22% in 1998 to 29% in 2009 (figure 31) and showed fluctuations comparable to those of the amoxicillin trend.

The MIC distribution of co-amoxiclav was unimodal over a broad range (1-> 32 mg/l) and showed a growing number of strains with MIC = 16 mg/l (figure 32), the breakpoint for resistance as recommended by EUCAST, but classified as intermediate susceptible by CLSI. The shape of the curves changed considerably over the years: until 2000 a real peak at 4 mg/l was observed, but this disappeared completely later.

Piperacillin resistance varied between the Intensive Care Units, some had high resistance rates (30%), others low (15%) until 2004, but from 2003 onward the resistance level increased in all Intensive Care Units, resulting in an overall resistance rate of 40% in 2009.

The MIC distribution of piperacillin changed during the study period (figure 32). It was more or less bimodal over a broad range until 2001 with one susceptible subpopulation (MIC 0.5-4 mg/l) and one subpopulation containing intermediate (MIC 8-16 mg/l) and resistant strains (MIC > 16 mg/l).After 2001 bimodality became more clear with existence of one smaller subpopulation with MICs 0.5-4 mg/l and a growing subpopulation with MICs 16 - > 64 mg/l. Piperacillin showed higher activity than amoxicillin towards the same subpopulation: the peak of MICs of piperacillin in the susceptible range was at 1-2 mg/l, that of amoxicillin at 2-4 mg/l (figure 32). Resistance to piperacillin-tazobactam was 2-5% during the whole study period. The MIC distribution of piperacillin-tazobactam was unimodal (figure 32)

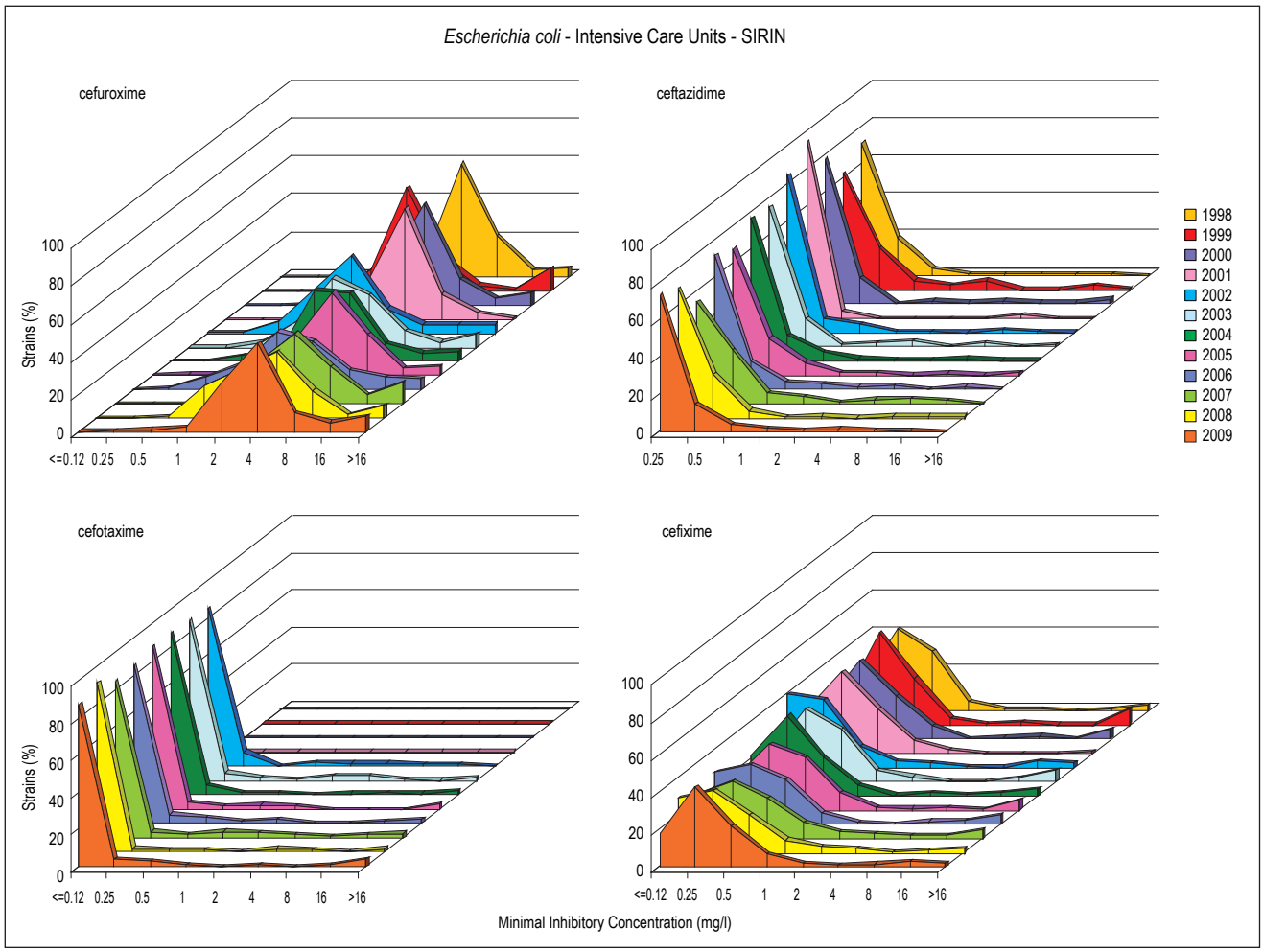

Figure 33. MIC distributions of cephalosporins for *Escherichia coli* from Intensive Care Units.

and showed an almost complete disappearance of populations resistant or intermediate to piperacillin alone. Occasionally less-susceptible strains with MICs 8-16 mg/l emerged together with some strains with MIC > 64 mg/l, but until 2009 the shape of the distribution curve as a whole did not change significantly. Sudden upcoming resistance with the current antibiotic policy for piperacillin-tazobactam use is therefore not expected in the near future.

Imipenem- and meropenem resistance was occasionally found in Intensive Care Units in 2000 and 2005 (not shown)

Cefuroxime resistance levels rose from 9% to 13% (figure 31).The MIC distribution of cefuroxime for strains of Intensive Care Units was almost unimodal over a broad range (MIC 0.5 - > 16 mg/l until 2006, except in 1999 (figure 33). Over the years the range broadened, the peak at 4 mg/l lowered (from 60% of strains in 1998 to 45% of strains in 2009) and a cluster of strains with high MICs appeared in 2007, resulting in a real bimodal distribution with a large subpopulation with MIC 1-8 mg/l, 5% of strains with MIC = 16 mg/l and with 8 % of strains with MIC >16 mg/l.

Cefotaxime resistance remained less than 5.5 % in Intensive Care Units, without a clear trend (figure 31). The resistance level was almost exclusively formed by strains with MIC > 16 mg/l, therefore the MIC distribution of cefotaxime appeared bimodal with one big subpopulation (85-90%) with MIC < 0.12 mg/l and a very small one with MIC < 16 mg/l (figure 33).

Ceftazidime resistance was low; it remained less

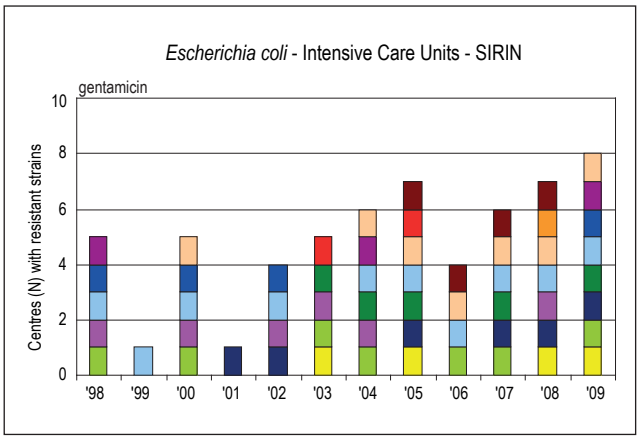

Figure 34. Number of centres with gentamicin-resistant *Escherichia coli* on Intensive Care Units. Each color represents one specific centre.

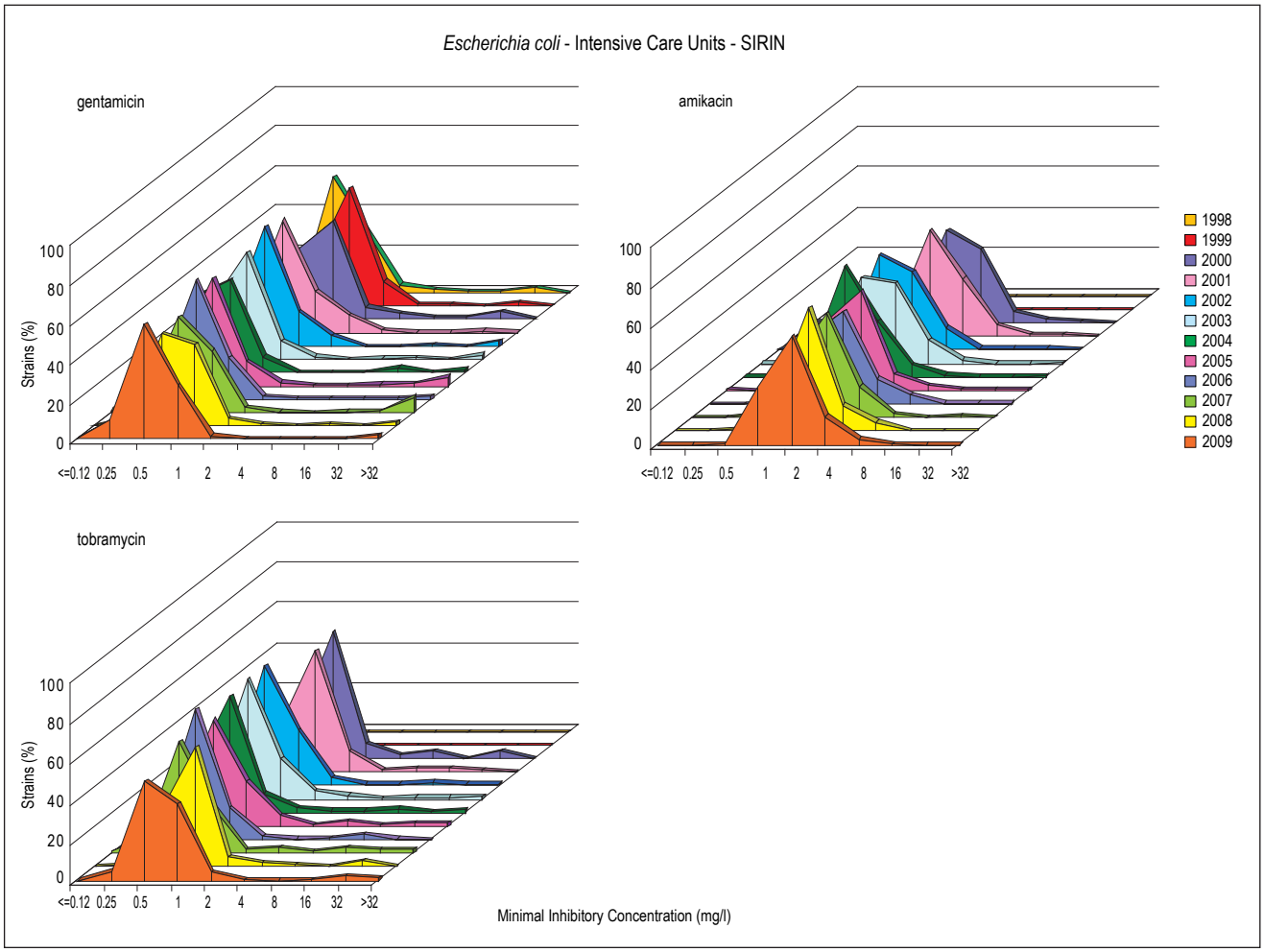

Figure 35. MIC distributions of aminoglycosides for *Escherichia coli* from Intensive Care Units.

than 4.5% without a clear trend (figure 31). The MIC distribution was not bimodal like that of cefotaxime, the range of MICs of the susceptible subpopulation was larger (0.12-1 mg/l) and most resistant strains had MIC 8-16 mg/l (figure 33). No highly resistant strains were found.

Resistance to cefepime increased from 0% in 1998 to 4% in 2009, ceftibuten resistance was 5-10% during the years without a clear trend and cefixime resistance fluctuated around 10% (figure 31). The intrinsic activity of cefepime resembled that of cefotaxime with MIC90 < 0,12 mg/l, which was 4 times more than that of ceftazidime and ceftibuten with MIC90 0.5mg/l and 8-16 times more that that of cefixime with MIC90 1-2mg/l.

Gentamicin resistance in Intensive Care Units ranged from 1-9% with 3.5% resistance in 2009 (figure 31). The fluctuations were associated with an unusual high resistance level in some centres (up to 15%) and the experience that gentamicin resistance was not found in all centres. The number of centres with gentamicin-resistant strains (MIC >4 mg/l) varied yearly: only one centre in 1999 and 2001, but seven centres in 2004 en 2005, four in 2006 and eight in 2009 (figure 34). Resistance was not associated with certain centres and it was not permanent in most centres. Therefore the increasing trend presented does not reflect a real national trend. This underlines the importance of local surveillance of resistance. The MIC distribution of gentamicin was bimodal with a large susceptible subpopulation with MIC 0.25-1 mg/l and a small subpopulation with MIC > 32 mg/l (figure 35).

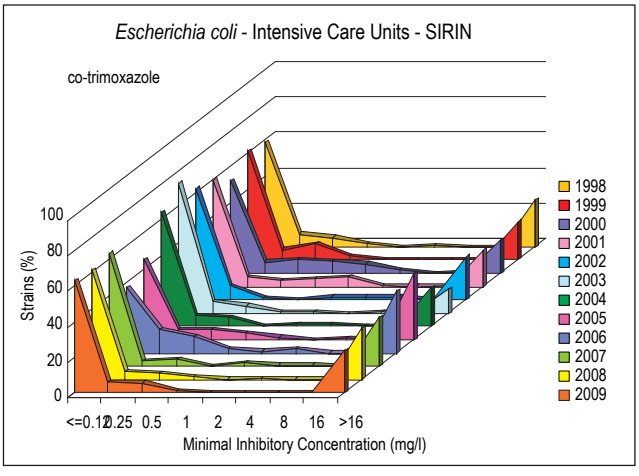

Figure 36. MIC distributions of co-trimoxazole for *Escherichia coli* from Intensive Care Units.

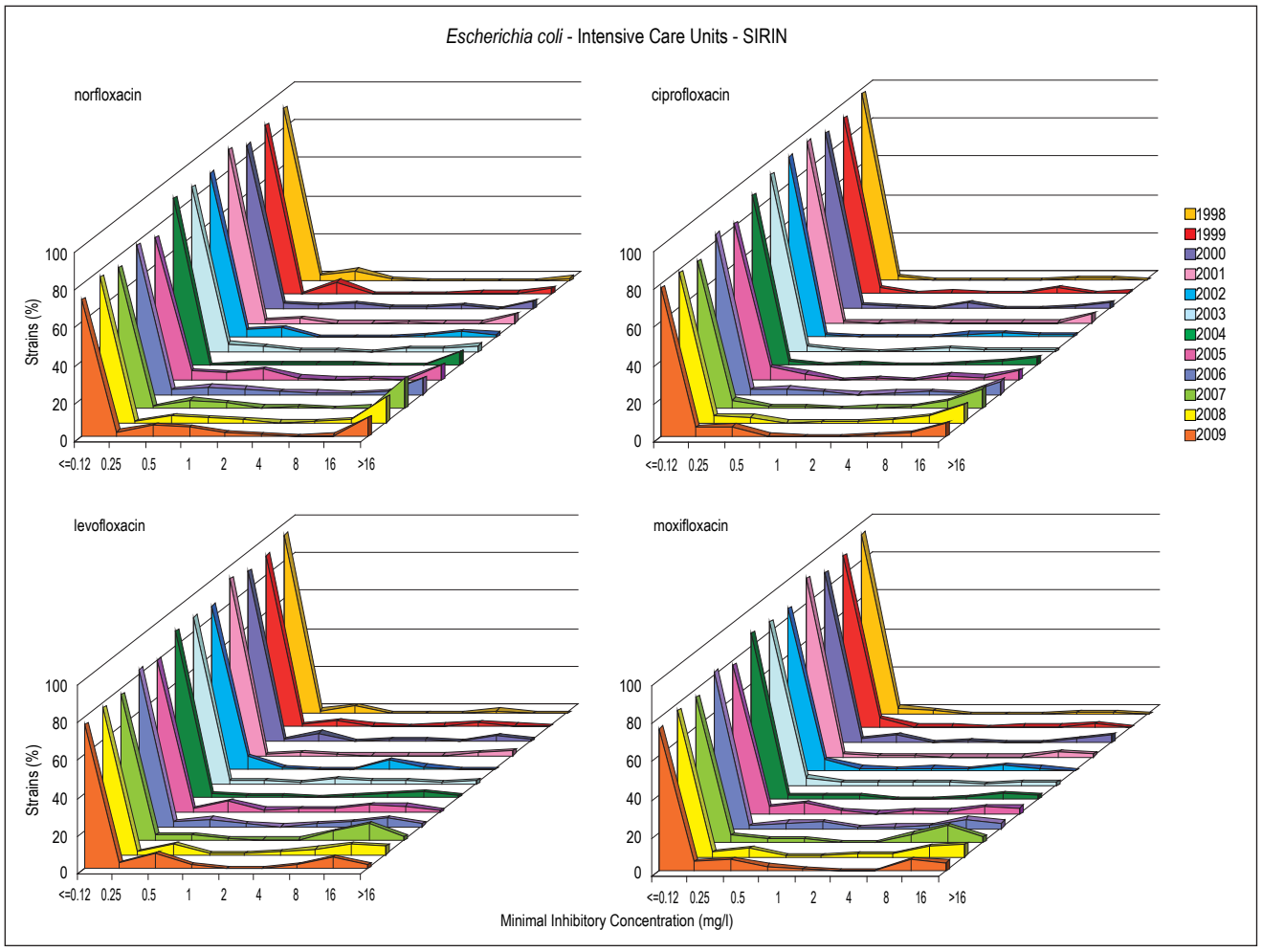

Figure 37. MIC distributions of quinolones for *Escherichia coli* from Intensive Care Units.

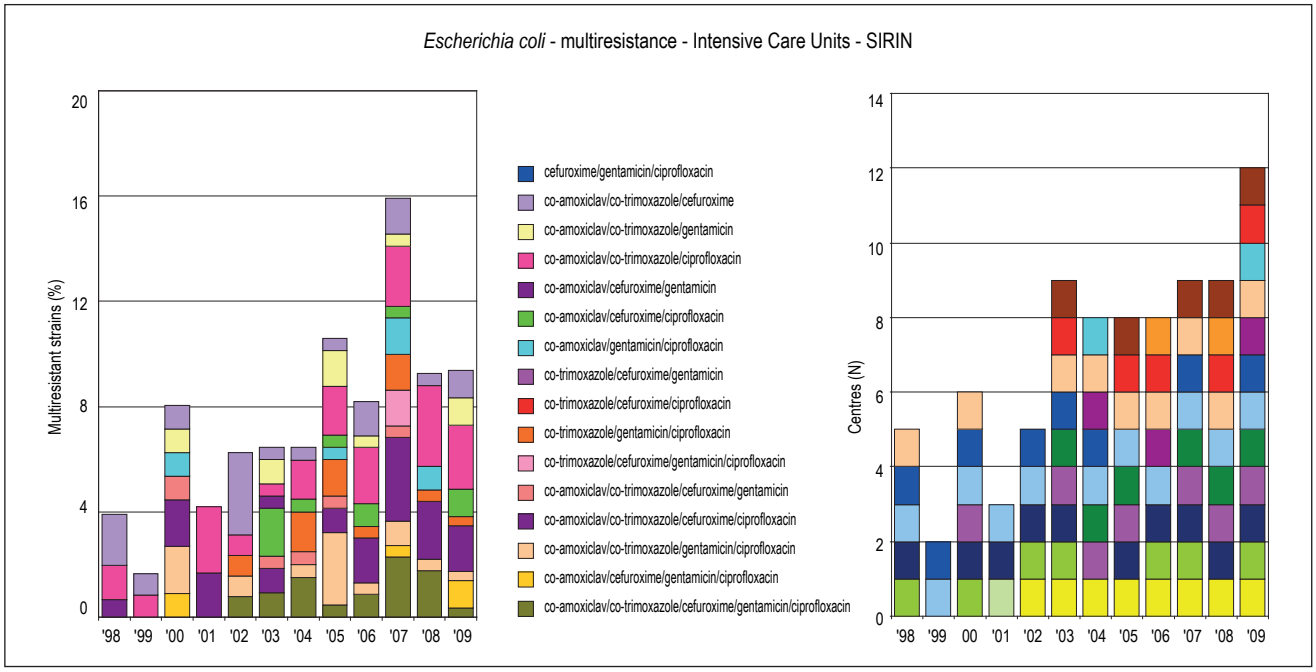

Figure 38. Trends in multiresistance among *Escherichia coli* from Intensive Care Units and the number of centres. Each color represents one specific centre.

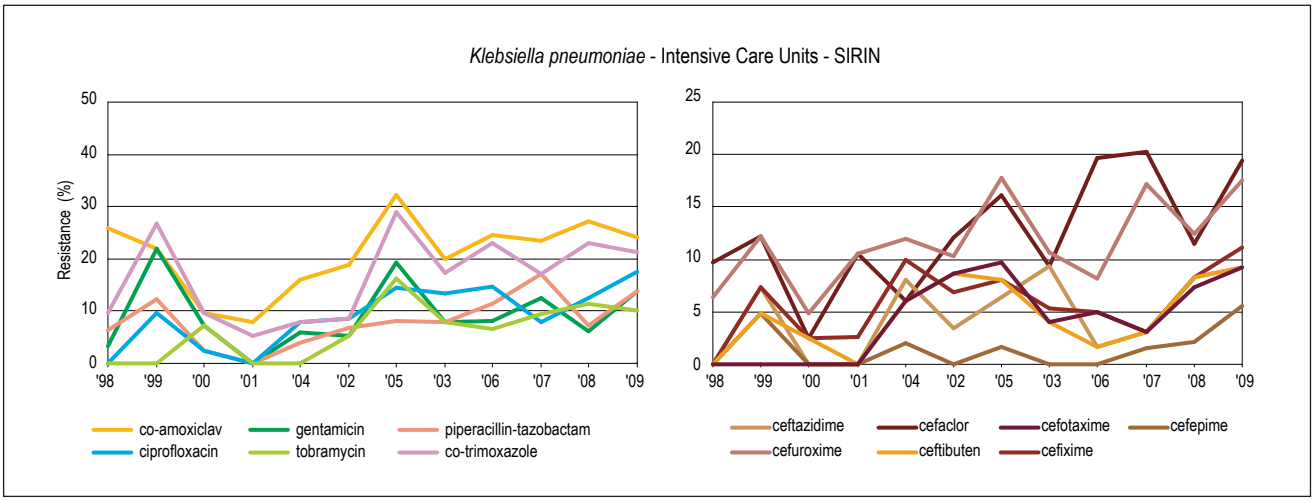

Figure 39. Trends in antibiotic resistance among clinical strains of *Klebsiella pneumoniae* from Intensive Care Units (N = 725).

Tobramycin resistance was higher (4-9%) with 4% in 2009 (figure 31). The MIC distribution of tobramycin was not bimodal like that of gentamicin. Apart from the large susceptible population with MIC 0.25-2 mg/l incidentally resistant strains emerged with MIC 8-32 mg/l (figure 35). Amikacin resistance was 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 35).
The level of trimethoprim resistance increased with some fluctuations from 26% in 1998 to 41% in 2005 and decreased thereafter to 25% in 2009 (figure 31). Co-trimoxazole resistance trend in Intensive Care Units followed that of trimethoprim at a somewhat lower level. The MIC distributions for co-trimoxazole (figure 36) showed a bimodal shape with two subpopulations: one susceptible with MIC < 0.25 mg/l and one highly resistant, with MIC > 16 mg/l.
Nitrofurantoin resistance fluctuated; it was not found until 2001, increased thereafter to 4.5% in 2004 and decreased 1% in 2009 (figure 31).
Ciprofloxacin resistance increased from 1% in 1998 to 16% in 2007 and decreased to 11% in 2009 (figure 31). The resistance percentages of norfloxacin, levofloxacin and moxifloxacin were similar to those of ciprofloxacin. The MIC distributions of the quinolones for *E. coli* from Intensive Care Units 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 37). The intrinsic activity of ciprofloxacin was superior to that of the other quinolones with 61% susceptible to <0.03 mg/l in 2009 compared to 51% for levofloxacin, 36% for moxifloxacin and 7% for norfloxacin. Only few strains had MICs in the intermediate area. The majority of the resistant strains had MICs > 16 mg/l. Quinolone resistance was common in all departments in 2009, but the level of quinolone-resistant *E. coli* varied between the centres from 3-25%.

Multiresistance of *Escherichia coli* in Intensive Care Units

Resistance to three or more classes of antibiotics (multiresistance) in Intensive Care Units within SIRIN was recorded for various combinations at increasing levels. Before 1998 no multiresistance was observed. The annual percentages of multiresistant strains were less than 7% from 1998-2004 it increased to 11% in 2005 and 16% in 2007 and decreased to 9% in 2008 and 2009 (figure 38). A total of 182 multiresistant strains from Intensive Care Units were isolated between 1998 and 2009. Resistance to the combination co-amoxiclav/co-trimoxazole with another drug was prevalent (6.7%). These other drugs were either cefuroxime or ciprofloxacin, since 2000 resistance to these four classes of antibiotics was found and from 2002 on this combination was expanded with resistance to gentamicin as well.
Similar observations were made with the co-trimoxazole combinations (others than those with co-amoxiclav). Resistance to the combination co-trimoxazole / gentamicin / ciprofloxacin with or without cefuroxime emerged since 2002 in 1-1.5% of the isolates.
The number and origin of Intensive Care Units with multiresistant strains varied over the study period, but increased in 2009; 12 of 14 Units followed since the beginning had multiresistant strains.
Resistance to four and five antibiotics was recorded from 2000 on at low percentages (2-5% of the total), but it raised significantly in 2007 to 8% of the total amount of strains collected in that year ($p< 0.02$) and decreased to less than 3.5% in 2009. These fluctuations can be explained by the incidental appearance of such strains in some Intensive Care Units.
Multiresistance to four of more classes of antibiotics was observed in a limited number of Intensive Care Units per year and often not yearly. Maybe the high resistance rate in 2007 was due to a local problem in three Units, which did not occur in the years before. So we have to conclude that multiresistance to four or more classes in Intensive

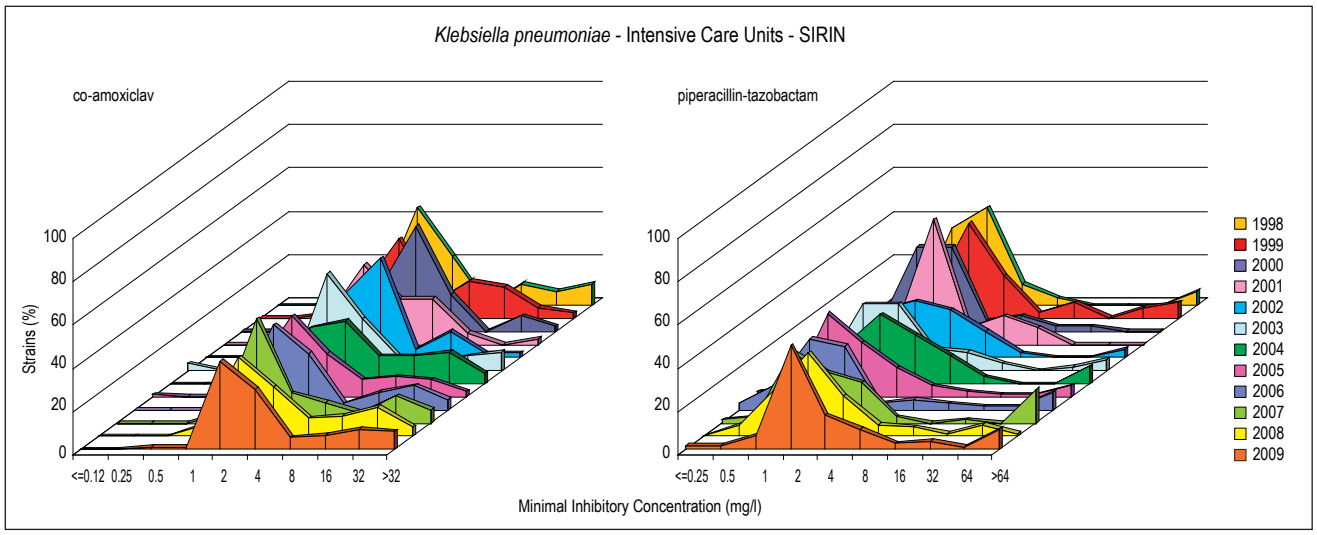

Figure 40. MIC distributions of co-amoxiclav and piperacillin-tazobactam for *Klebsiella pneumoniae* from Intensive Care Units.

Care Units is more a local than a national problem at this moment (figure 38).

4.2.4.2 Klebsiella pneumoniae

Co-amoxiclav resistance among *K. pneumoniae* from Intensive Care Units fluctuated around 20% resistance with 24% resistance in 2009 (figure 39), which is much higher than in all other study populations. The fluctuations might be caused by the existence of varying numbers of strains with MIC around the breakpoint (8 mg/l), as was observed in the MIC distribution (figure 40).

Overall piperacillin-tazobactam resistance in Intensive Care Units varied from 0-15% over the years without significant increase (not shown). Piperacillin-tazobactam resistance was not common in all centres yearly. We recorded 0-5 centres per year with resistant strains without a clear pattern. No centre had a "clear piperacillin-tazobactam problem" over time. This may explain the fluctuations during the study period. So the piperacillin-tazobactam resistance found did not reflect the resistance level for all Intensive Care Units.

Carbapenem resistance was rare in Intensive Care Units. It was found once in 2006 in one centre.

Resistance to cefuroxime increased from 7% in 1998 to 18% in 2009 (figure 39), which is higher than to the resistance level found in Intensive Care Units. The MIC distribution of cefuroxime (figure 41) was more or less bimodal with one subpopulation over a broad range (0.5-16 mg) and a small subpopulation with MIC > 16 mg/l, which increased since 2007. A total of 59% of all strains had MIC 2 mg/l in 1998, which was only 39% in 2009. In general a movement to the intermediate and resistant area was made from 2007 on, which resulted in a higher resistance level in 2009. Resistance to ceftazidime in Intensive Care Units increased from 1% in 1998 to 9% in 2009 (figure 39). Ceftazidime resistance was not always present in all Intensive Care Units. It fluctuated between 0% (1998 and 2001) and 16% (2002) and it was 9% in 2009 (figure 39). The high rate and fluctuations were exclusively due to a high resistance rate in two Intensive Care Units in 2002 and in seven others occasionally thereafter. So the resistance level is not representative for the Intensive Care Units as a whole. Resistance to cefotaxime was measured since 2002 in Intensive Care Units; it fluctuated around 8-9% and seems to increase. Ceftibuten and cefixime resistance resembled the trends observed for cefotaxime. Cefepime showed the lowest resistance levels but an increase from 0% in 1998 to 6% in 2009. From the MIC distributions of all cephalosporins (figure 41) it can be concluded that the intrinsic activity of cefotaxime, cefixime, cefepime and ceftibuten is higher against *K. pneumoniae* than that of ceftazidime: 45% of strains are inhibited by < 0.12 mg/l of ceftazidime whereas 80% or more are inhibited by the other cephalosporins at the same concentration.

Trimethoprim resistance fluctuated considerably until 2005 (13-36% resistance), but showed a more stable pattern since then with 30-32% resistance (figure 39). The fluctuations may be explained by the distribution of MICs (figure 42) with a varying subpopulation with MIC around the breakpoint (4 mg/l) and the rather low numbers of strains in the first years. The resistance to co-trimoxazole followed the trend of trimethoprim and was 21% in 2009. Co-trimoxazole is an alternative drug combination for *Klebsiella* infections in Intensive Care UnitsQuinolone resistance showed an increasing trend from less than 1% for the four quinolones tested in 1998 to 26% (norfloxacin), 19% (moxifloxacin), 18% (ciprofloxacin) and 14% (levofloxacin) in 2009 (figure 39).

The MIC distributions of all quinolones tested showed a susceptible subpopulation over a broad range (MIC < 0.03 – 0.5 mg/l) and a small subpopulation with MIC 1-8 mg/l whereas only few strains had MICs > 16 mg/l (figure 43). This differed from the MIC distributions of quinolones for

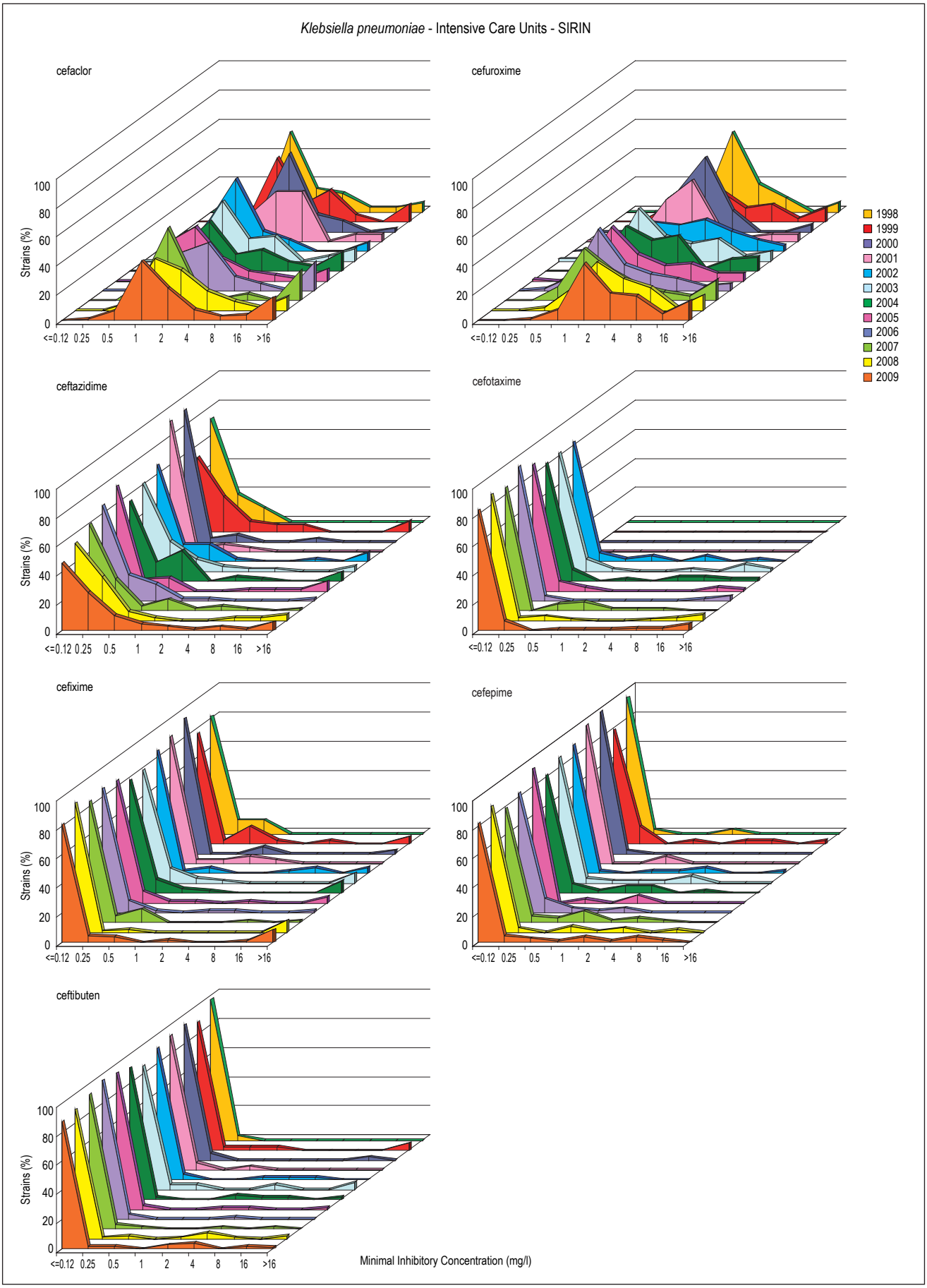

Figure 41. MIC distributions of cephalosporins for *Klebsiella pneumoniae* from Intensive Care Units.

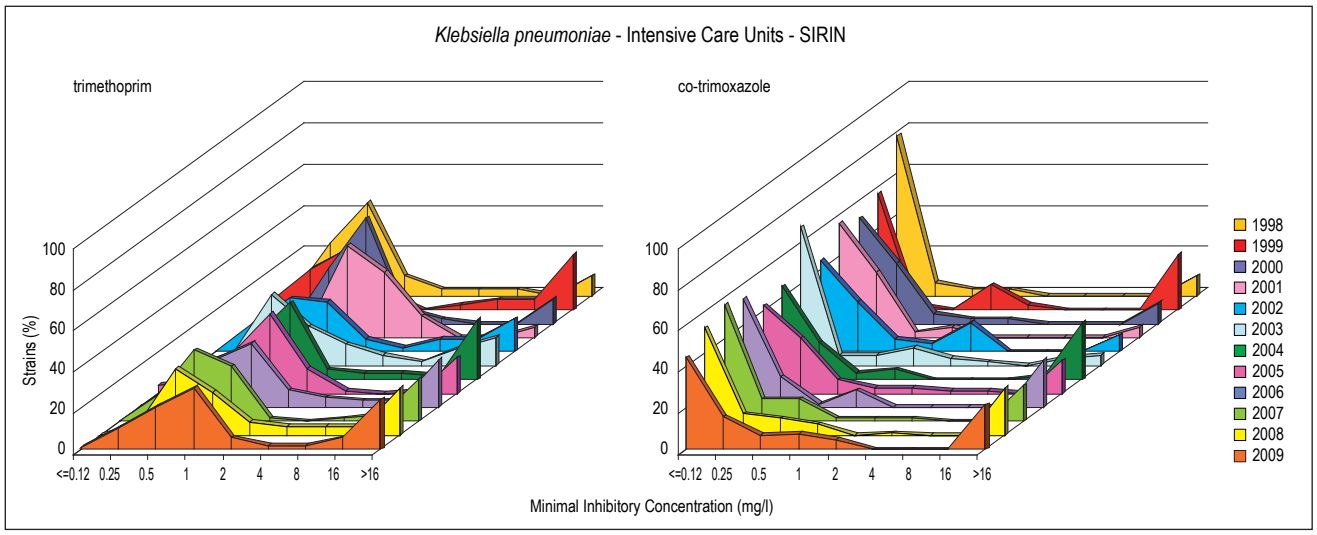

Figure 42. MIC distributions of trimethoprim and co-trimoxazole for *Klebsiella pneumoniae* from Intensive Care Units.

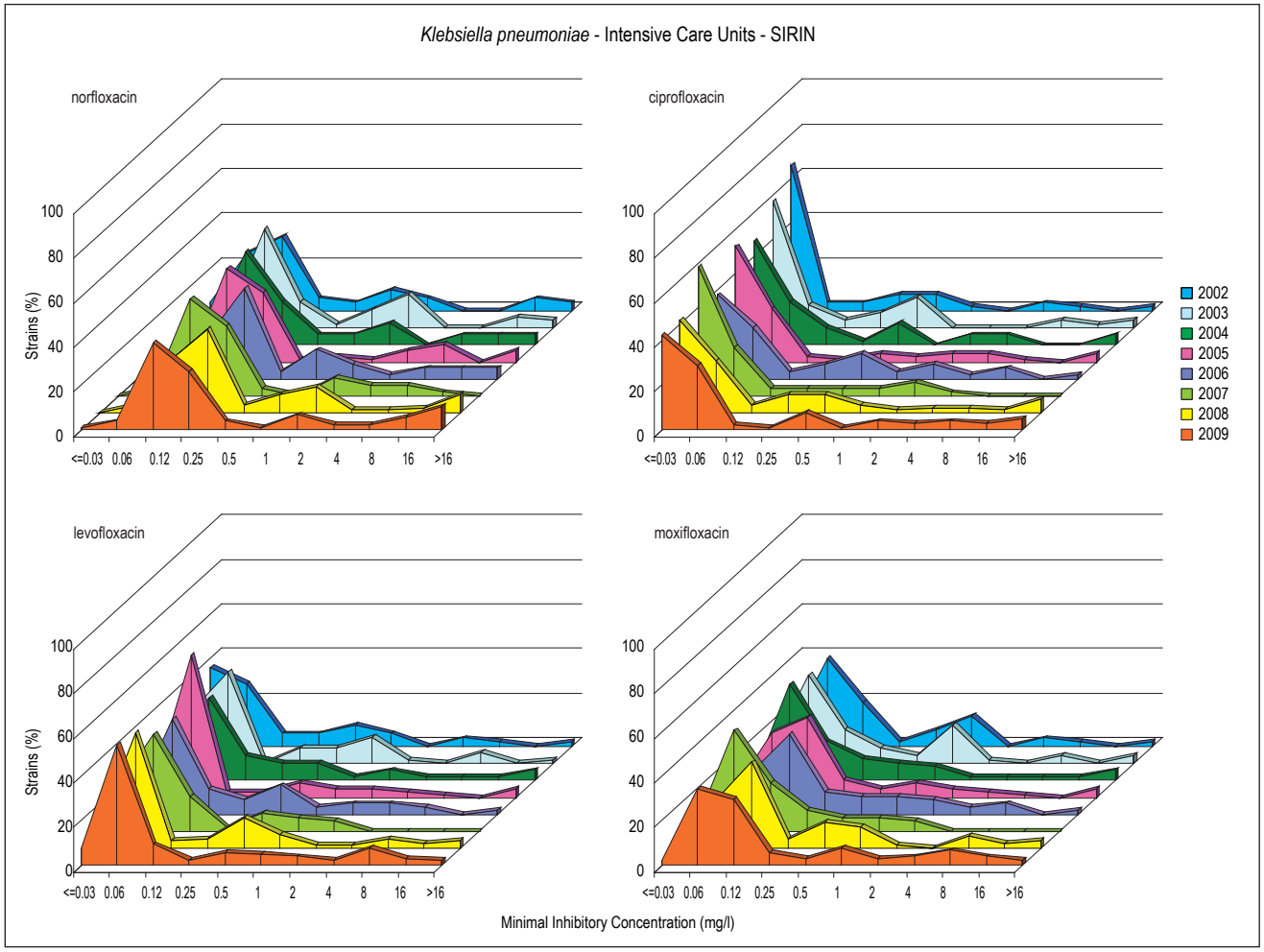

Figure 43. MIC distributions of quinolones for *Klebsiella pneumoniae* from Intensive Care Units.

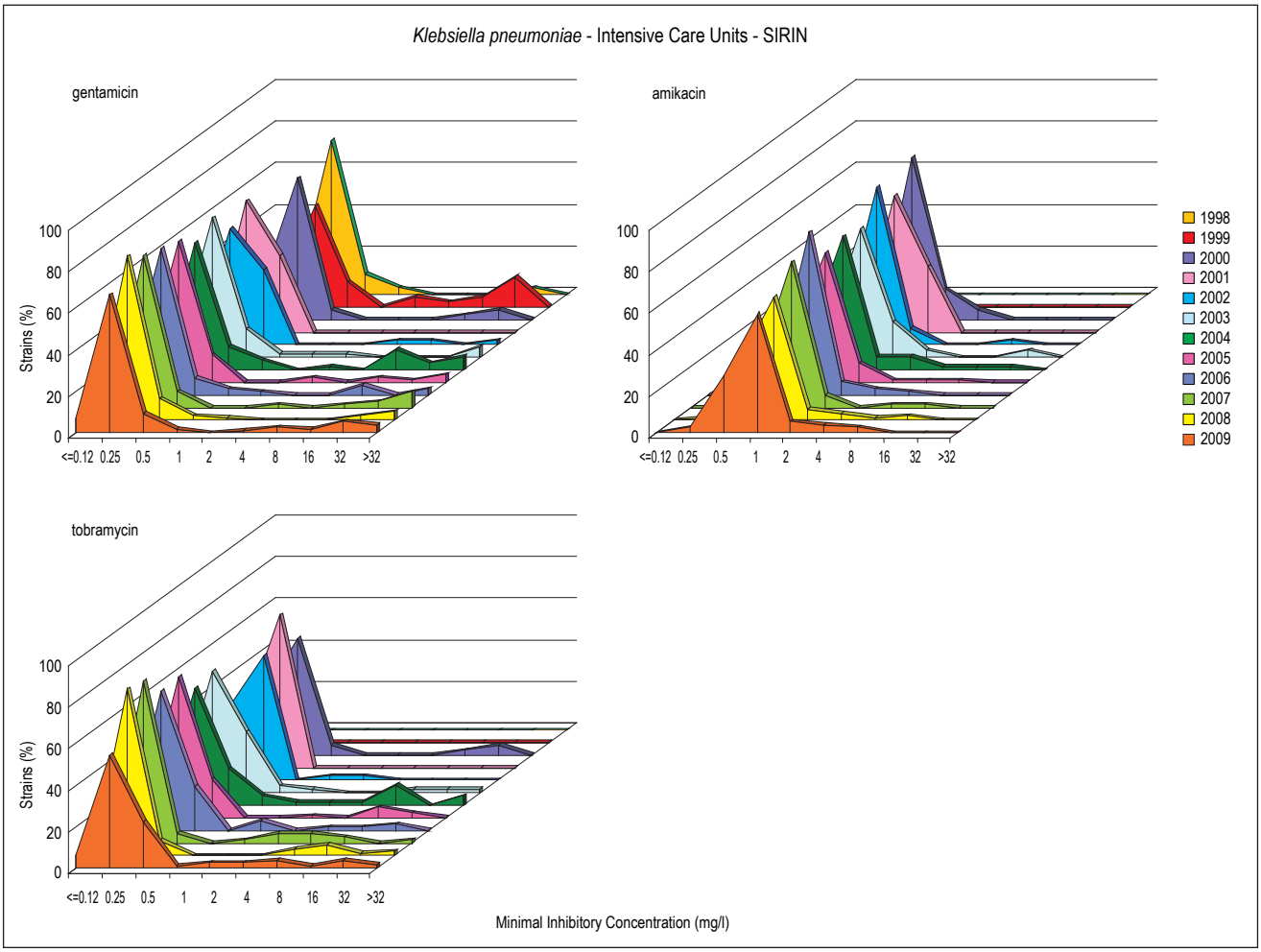

Figure 44. MIC distributions of aminoglycosides for *Klebsiella pneumoniae* from Intensive Care Units.

E. coli where a real bimodal distribution was observed. The MIC distributions of ciprofloxacin, levofloxacin and moxifloxacin were identical; the lower resistance level of levofloxacin calculated was due to the different breakpoint for levofloxacin, which is 2 mg/l compared to that of 1 mg/l for ciprofloxacin and moxifloxacin. Gentamicin-resistant strains were observed continuously in two Intensive Care Units from 1999 onward and intermittent in eight others resulting in large fluctuations in gentamicin resistance rates (0-16%) over the years of surveillance with a mean resistance rate of 6% in 2008 and 12% in 2009 (figure 39). These figures are therefore not representative for all Intensive Care Units. This underlines the need for local surveillance. Tobramycin resistance followed the pattern of gentamicin increasing from 7% in 2000 to 10% in 2009; Amikacin resistance was found sporadically in 2003 and 2004. The MIC distributions of gentamicin and tobramycin (figure 44) were bimodal with a large subpopulation with MIC 0.12-0.5 mg/l and a small subpopulation with MIC > 32 mg/l, that of amikacin was unimodal with the peak at 1 mg/l.

ESBL among *Klebsiella pneumoniae* in Intensive Care Units

All isolates from Intensive Care Units with MIC ¬> 1 mg/l for ceftazidime and/or cefotaxime were considered putative ESBL producers. A total of 61 strains were found from 1998 to 2009. ESBL production was demonstrated in 34 strains. The prevalence per year is presented in figure 45. It turned out that suspected strains were found every year at varying percentages. ESBL producers were demonstrated in 1999 and from 2002 on. The level in 2002 was 6% and this remained until 2009, where a 9% level was recorded. It appeared that these ESBL producers were a local problem of some centres and not a uniform problem in all.

Multiresistance of *Klebsiella pneumoniae* in Intensive Care Units

Multiresistance (resistance to three or more classes of antibiotics) in Intensive Care Units was recorded yearly except in 2001 at varying percentages (3-23% of all *K. pneumoniae* strains). A real trend was not visible, although the percentages of multiresistance remained 12% or more from 2004 onwards, suggesting a more stable situation compared to the years before (figure

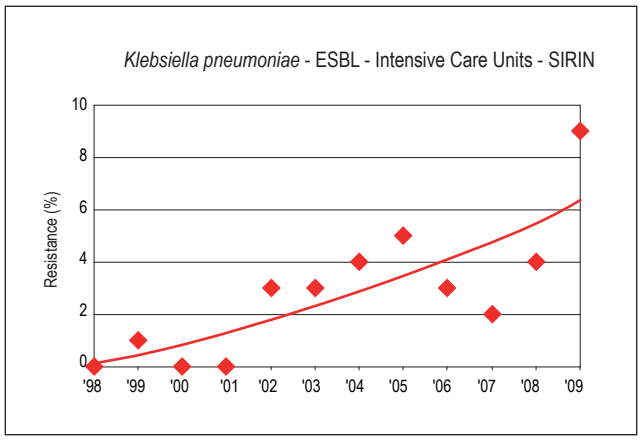

Figure 45. Prevalence of ESBL producing *Klebsiella pneumoniae* strains in Intensive Care Units.

46). The highly fluctuating numbers of multiresistant strains may be associated with high resistance levels for e.g. gentamicin in some Intensive Care Units in some but not all year, as described above. The antibiotic combinations for which resistance was recorded differed in some way from those found in *E. coli* strains. For *E. coli* the combinations co-amoxiclav/co-trimoxazole with either cefuroxime or ciprofloxacin predominated whereas the combination co-amoxiclav/co-trimoxazole/ gentamicin for *K. pneumoniae* predominated with or without cefuroxime or ciprofloxacin. Unlike in *E. coli* the proportion of strains resistant to four or five classes of antibiotics was higher (10% of all *K. pneumoniae* isolates in 2009).
Multiresistance was not common in all centres and not found every year in a given centre (figure 46). The number of centres positive increased from one in 1998 to seven in 2009. Therefore these figures are not representative for the country as a whole, but the increasing trend is a matter of concern.

4.2.4.3 Enterobacter cloacae

The numbers of strains of *E. cloacae* isolated from patients in Intensive Care Units during 1998-2002 were less than 40 yearly; therefore we evaluated the resistance trends from 2003 on. Quantitative data were obtained from 411 strains. Between 2003 and 2009 90% or more of *E. cloacae* strains from Intensive Care Units were resistant to co-amoxiclav. Resistance to piperacillin increased with fluctuations from 23% in 2003 to 32% in 2009; that to piperacillin-tazobactam fluctuated around 15% (figure 47). The fluctuations were clearly related to the emergence of resistant strains in some Intensive Care Units. These strains were recorded occasionally in all centres often only for a short period and not every year. Therefore the overall resistance percentage does not reflect the general situation in Intensive Care Units and does not indicate a trend.
Imipenem- and meropenem resistance was exceptional in Intensive Care Units (not shown).
Cefuroxime resistance increased from 41% in 2003 to 58% in 2007 and remained stable thereafter. Ceftazidime resistance fluctuated around 25% that of cefotaxime increased from 26% in 2003 to 38% in 2009. Resistance to cefixime and ceftibuten were also increasing to high levels (50-70%); the resistance level of cefepime fluctuated between 0% and 13%. The fluctuations were related to existence of resistant strains in some Intensive Care Units. Enterobacter cloacae is known for circulation of resistant clones in closed departments. Because of the

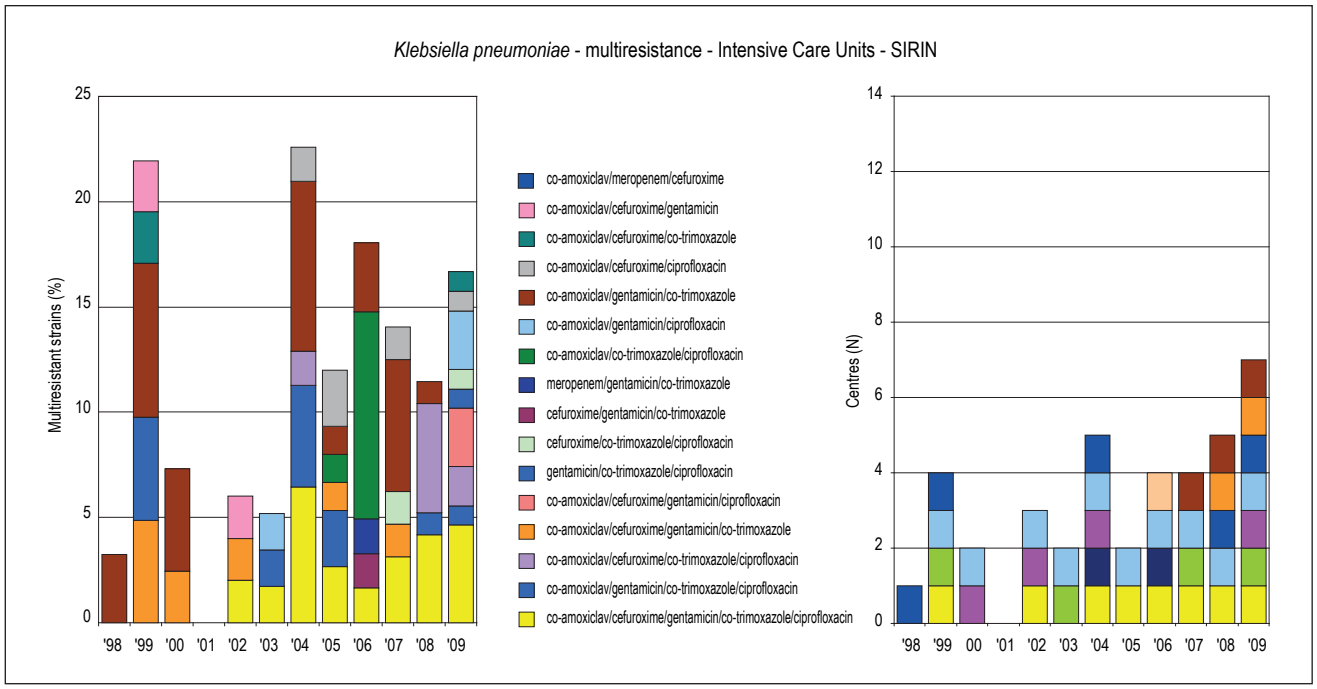

Figure 46. Trends in multiresistance among *Klebsiella pneumoniae* and number of centres (each color represents one specific centre) with multiresistant *Klebsiella pneumoniae* from Intensive Care Units.

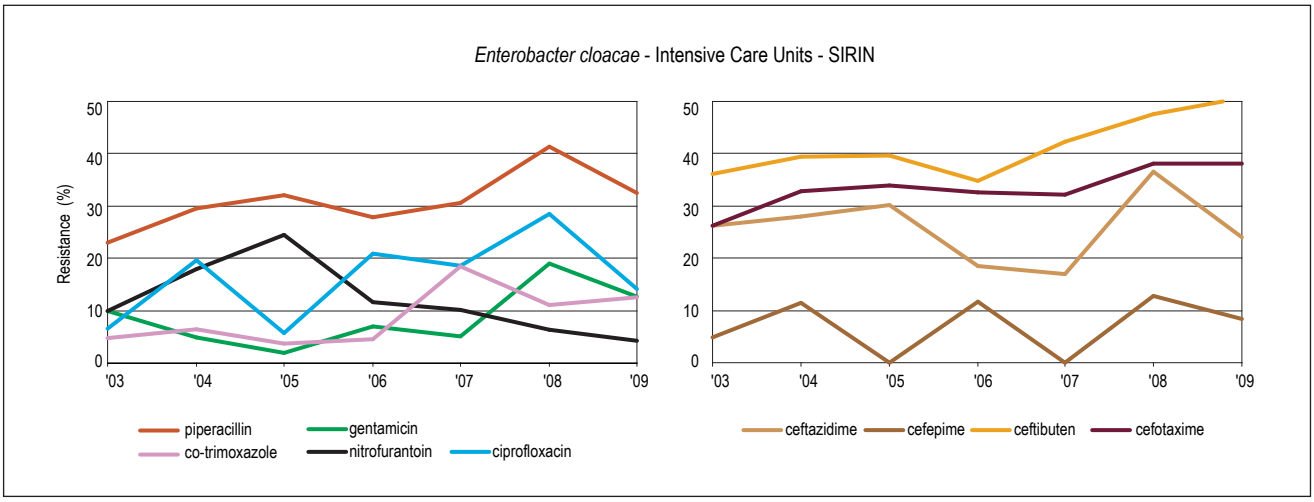

Figure 47. Trends in antibiotic resistance (2003-2009) among clinical strains of *Enterobacter cloacae* from Intensive Care Units (N=411).

high resistance rates, any cephalosporin is therefore not recommended as empiric therapy in Intensive Cares with circulating *E. cloacae* strains.

Co-trimoxazole resistance level in Intensive Care Units increased with annual fluctuations from 5% in 2003 to 13% in 2009.

Ciprofloxacin resistance was 7% in 2003 and increased highly fluctuating to 28% in 2008 with a sharp decrease to 14% in 2009. These fluctuations were due to the existence of strains with MICs = 2 mg/l, which is just above the breakpoint for resistance (1 mg/l) (figure 48). The MIC distributions of the quinolones showed a decreasing

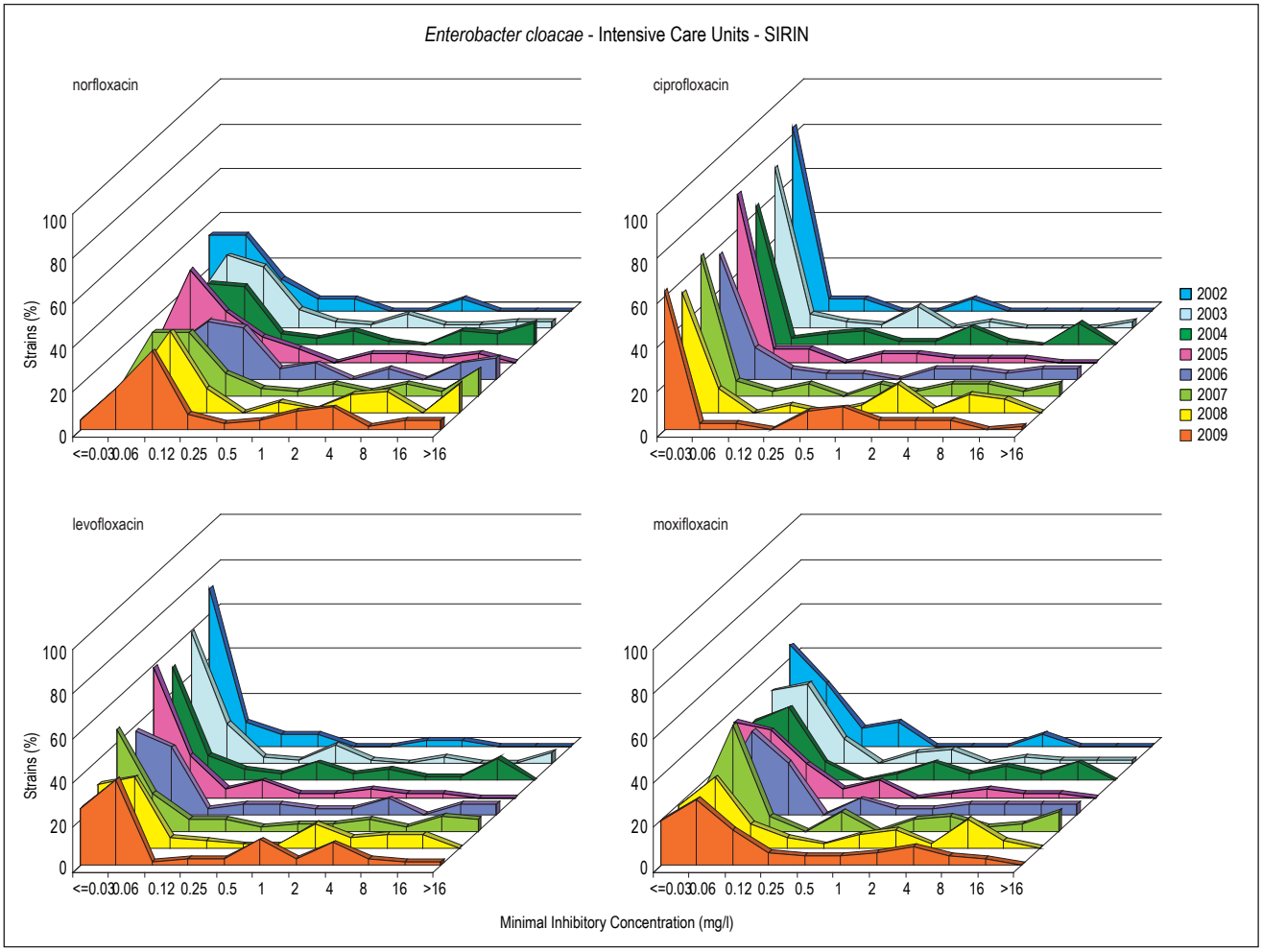

Figure 48. MIC distributions of quinolones for *Enterobacter cloacae* from Intensive Care Units.

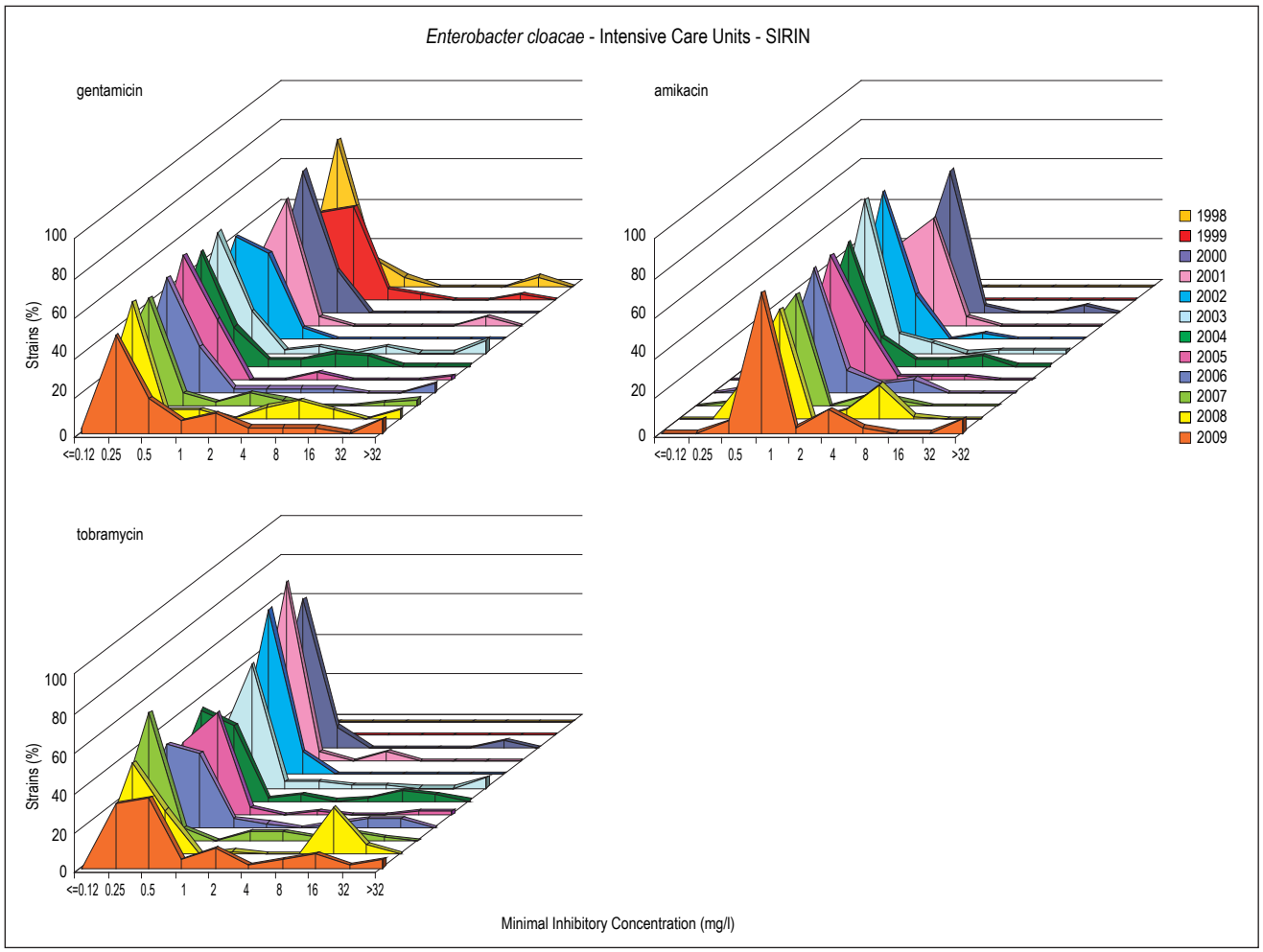

Figure 49. MIC distributions of aminoglycosides for *Enterobacter cloacae* from Intensive Care Units.

subpopulation with MIC < 0.12 -0.25 mg/l and increasing small subpopulations with MIC 1-2 mg/l and with MIC > 8 mg/l. Levofloxacin- and moxifloxacin resistance followed the patterns of ciprofloxacin at some higher level with 16% resistance for levofloxacin and 21% for moxifloxacin in 2009. Co-resistance with gentamicin and tobramycin occurred in 50% or more of ciprofloxacin-resistant strains.
Gentamicin resistance fluctuated between 5-10% from 2003 to 2007. In 2008 an unusual high resistance level of 19% was recorded, which was due to exclusive emergence of resistant strains in three Intensive Care Units; these strains were also resistant to tobramycin. Resistant strains were found in these centres from 2004 on. In 2009 13% resistance was found. The MIC distribution for gentamicin was bimodal with a susceptible subpopulation with MIC < 2 mg/l) and a small resistant one with MIC > 16mg/l (figure 49). In 2004 a small subpopulation with MIC 4- 8 mg/l appeared, predicting upcoming resistance and in 2008 a real cluster with MIC 4-16 mg/l existed between the two subpopulations, which flattened in 2009 with creation of a larger subpopulation with MIC > 32 mg/l. These resistant strains circulated exclusively in the three centres mentioned before. So longitudinal evaluation of the MIC distributions may give information on emergence of resistance long before this will become apparent. There was complete cross resistance with tobramycin, but not with amikacin. The MIC distribution of tobramycin resembled that of gentamicin, although the resistant subpopulation was larger in 2008. The MIC distribution of amikacin (figure 49) showed sporadic resistant strains (MIC > 16 mg/l).

4.2.4.4 Proteus mirabilis

The number of strains of *P. mirabilis* collected from Intensive Care Units was less than 40 per year in the first four years; evaluation over the whole period is therefore not reliable. A total of 287 strains were collected from 2003 and some trends could be observed (table 4). Amoxicillin resistance was stable at 27%; co-amoxiclav resistance was low. No resistance to imipenem, meropenem and cephalosporins was observed; gentamicin resistance increased to 9% in 2009. Co-trimoxazole resistance was 27%, which is lower than in the other study populations and quinolone resistance was 4-5% during the last three years.

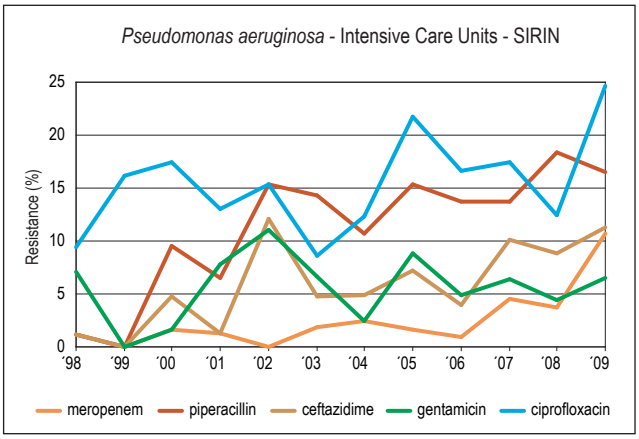

Figure 50. Trends in antibiotic resistance among clinical strains of *Pseudomonas aeruginosa* from Intensive Care Units (N = 1.257).

4.2.4.5 Pseudomonas aeruginosa

Piperacillin resistance among *P. aeruginosa* was not found until 2000; then an increasing number of Intensive Care Units delivered resistant strains, resulting in an overall increase trend to 17% in 2009 (figure 50). The MIC distributions of piperacillin and piperacillin-tazobactam are given in figure 51. They were unimodal from 1998 to 2000. In 2001 a shoulder in the area MIC 8-16 mg/l and a small subpopulation of strains with MIC > 64 mg/l emerged. The following year the resistant subpopulation had increased and the distribution became bimodal. In 2005 the distribution broadened over the area 0.25-8 mg/l with a shift of the median to higher MICs and in 2007 a shoulder appeared again in the range 8-32 mg/l which flattened in 2008 and 2009 with again a shift to the right. The same phenomenon was observed for piperacillin-tazobactam.

Meropenem resistance was less than 2% until 2006, increased to 4% in 2008 and was 11% in 2009 (figure 50). This sudden rise was due to appearance of many resistant strains from one specific centre. It appeared that resistant strains were found in five of 14 centres only so this resistance figure reflected a local problem in some Intensive Care Units and was therefore not representative for The Netherlands as a whole.

Ceftazidime resistance fluctuated but the trend was increasing from 1% in 1998 to 11% in 2009. An incidental 12% resistance was recorded in 2002 because of an unusual high resistance rate in five centres. Six of 14 centres had delivered ceftazidime-resistant strains in 2008 and 2009. So the current resistance levels are not representative for Intensive Care Units in general but reflect a local problem with a highly resistant population. This underscores the importance of local surveillance.

Gentamicin resistance in Intensive Care Units was found yearly in one to six centres responsible for the fluctuations in the overall resistance rate of 6% (figure 50). Amikacin resistance in Intensive Care was less than 4% during the whole study period whereas that of tobramycin showed more fluctuations (1-9%) reflecting however more local problems in some Intensive Care Units than a general trend. There was no complete cross-resistance between the three aminoglycosides: 59% of gentamicin-resistant strains were also tobramycin-resistant and 27% were amikacin-resistant; 81% of tobramycin-resistant strains were gentamicin-resistant and 36% were amikacin-resistant. The MIC distributions of gentamicin and tobramycin were bimodal with one subpopulation with MICs over a broad from 0.12-4 mg/l and a very small subpopulation with MIC > 16 mg/l (figure 52). The MIC distribution of amikacin was unimodal over a broad range from 0.5-> 16 mg/l. In general MICs of tobramycin were two-fold lower than those of gentamicin, reflecting its higher intrinsic activity against *P. aeruginosa* and four-fold lower than those of amikacin.

Quinolone resistance fluctuated strongly (figure 50) between 9-25% for ciprofloxacin and 11-28% for levofloxacin. The MIC distributions (figure 53) were

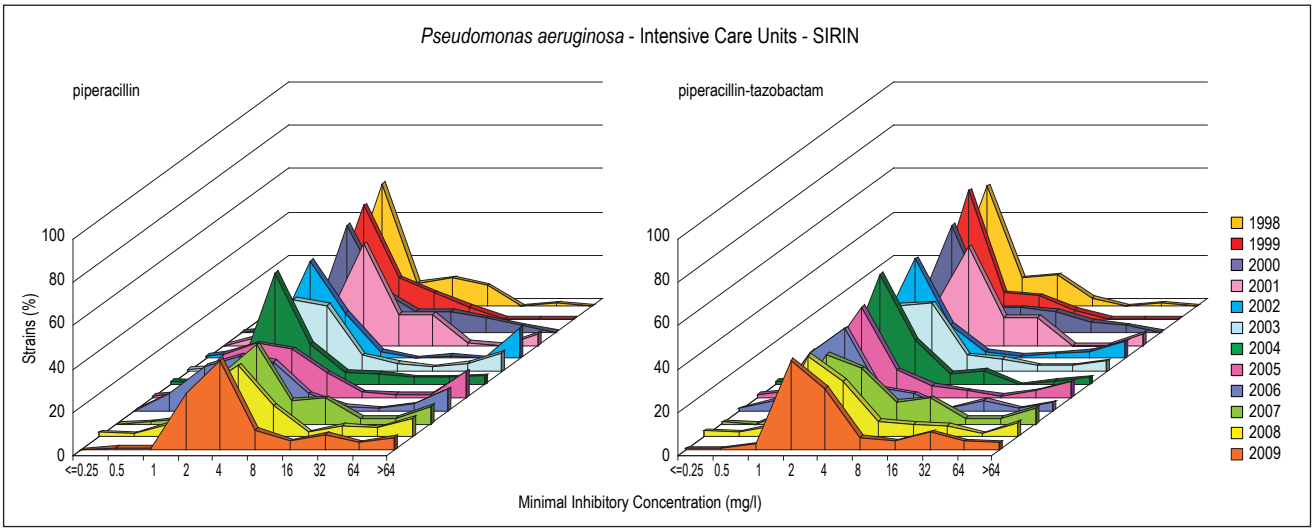

Figure 51. MIC distributions of piperacillin and piperacillin-tazobactam for *Pseudomonas aeruginosa* from Intensive Care Units.

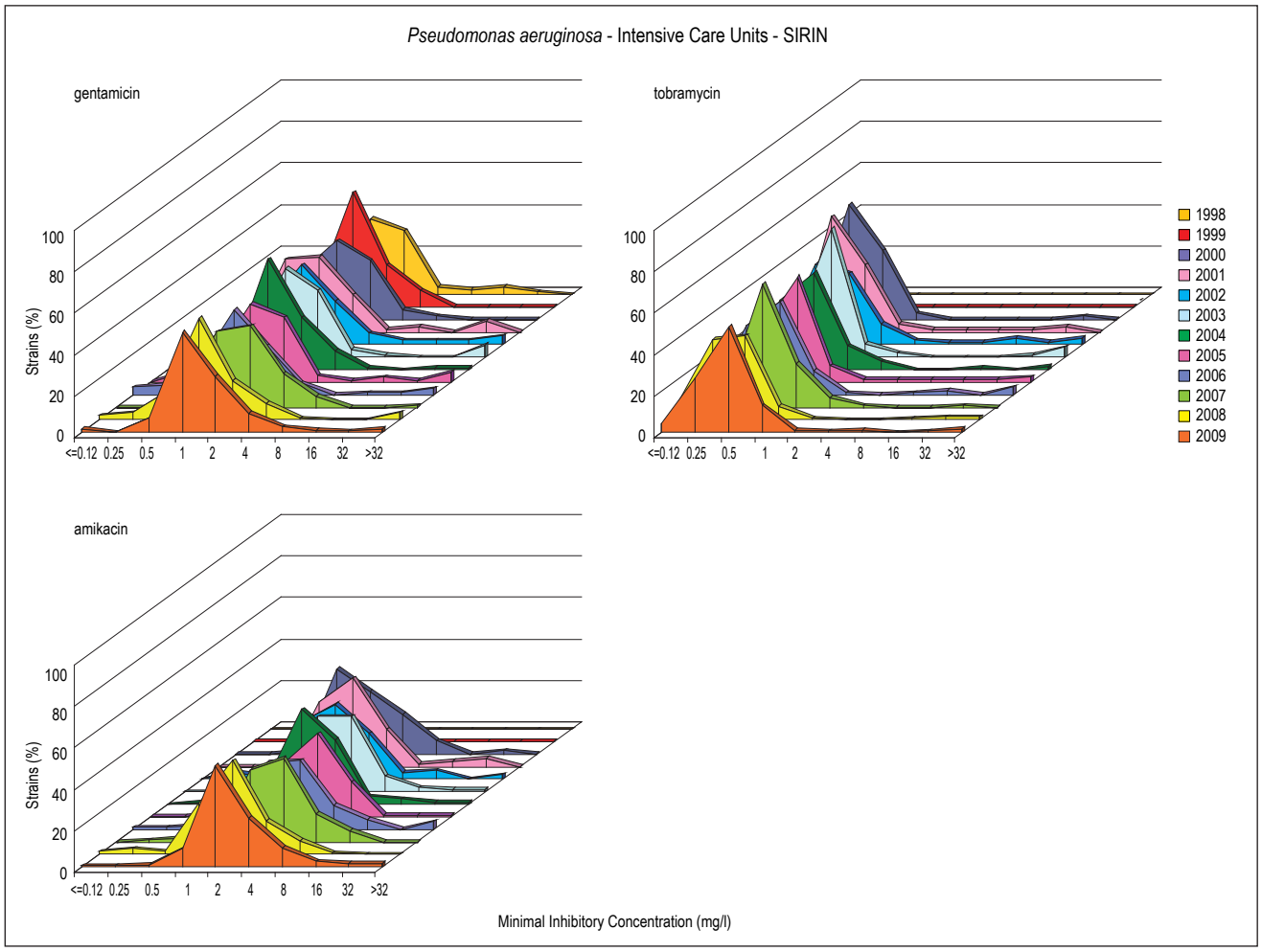

Figure 52. MIC distributions of aminoglycosides for *Pseudomonas aeruginosa* from Intensive Care.

unimodal over a broad range of MICs with a varying amount of strains around the breakpoint MIC (1mg/l for ciprofloxacin and 2mg/l for levofloxacin), which may explain the varying resistance percentages calculated. The intrinsic activity of ciprofloxacin was higher than that of levofloxacin: MIC50 for ciprofloxacin was 0.12 mg/l, for levofloxacin 0.5 mg/l.

4.2.4.6 Enterococcus faecalis

A total of 845 strains of *E. faecalis* were isolated from Intensive Care Units. Before 2002 no amoxicillin-resistant *E. faecalis* were found. From 2002 on these strains (N = 16) were found in five centres: once in centre G (2003), L (2003) and D (2007), three times in centre A (2002, 2004, 2007) and P (2004, 2007, 2009).
Fifteen strains 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, chloramphenicol and quinolones.
Imipenem resistance fluctuated between 1% and 14% without a real trend; such strains were incidentally found in 11 centres over the years, but not yearly. It is therefore a local problem rather than a national phenomenon.

Ciprofloxacin resistance was a common finding in all Intensive Care Units (23-84% resistance) without a trend. The same was found for moxifloxacin (23-76% resistance). The MIC distribution of ciprofloxacin was bimodal and showed a susceptible subpopulation with MIC 0.5-1 mg/l which is near the breakpoint and an insusceptible subpopulation with MIC > 16 mg/l (figure 54). The MIC distribution of moxifloxacin was also bimodal, but the susceptible subpopulation had MIC 0.25 mg/l and the insusceptible had MICs ranging from 4-16 mg/l.
Vancomycin resistance was sporadic, it was found in one centre in 2003 and in another in 2007.

4.2.4.7 Staphylococcus aureus

Penicillin resistance in *S. aureus* was rather stable and was 74% in 2009. Sporadically MRSA strains were isolated from the Intensive Care Units (N = 12 from 1998-2009 out of a total of 1113 isolates). Eight out of 12 MRSA strains from Intensive Care Units were ciprofloxacin resistant of which six were also clarithromycin-resistant, one was also gentamicin-resistant.
Cefuroxime resistance in Intensive Care Units was rare,

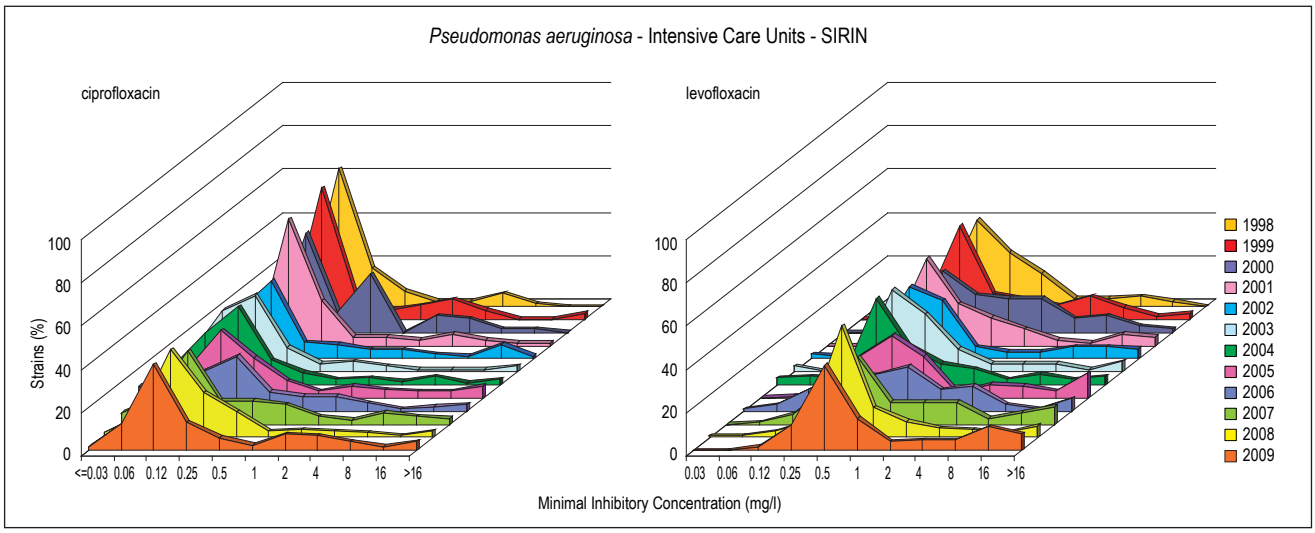

Figure 53. MIC distributions of quinolones for *Pseudomonas aeruginosa* from Intensive Care Units.

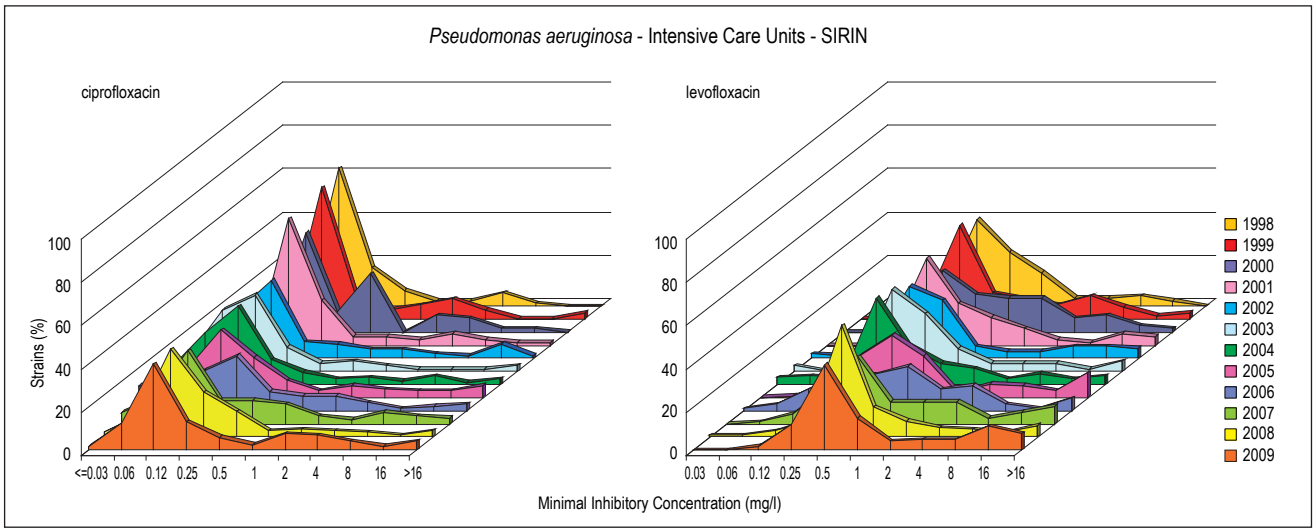

Figure 54. MIC distributions of ciprofloxacin and moxifloxacin for *Enterococcus faecalis* from Intensive Care Units (N=845).

2% or less and not recorded yearly (figure 55). Clarithromycin resistance among strains from Intensive Care Units increased from 5% in 1998 to 10% in 2009, which is comparable to the level found in Unselected Hospital departments. Clindamycin resistance was lower, it fluctuated around 3-4% over the years without a shift or clear trend. Doxycycline in Intensive Care Units in 2009 was higher (6%) than that found for Unselected Hospital Departments (3.5%). The opposite was found for co-trimoxazole with 3% resistance in Intensive Care Units.
Gentamicin resistance was 1-4% from 1998 to 2004 and sporadically found thereafter (not shown).
Ciprofloxacin resistance increased from 4% in 1998 to 16% in 2005 and decreased thereafter to 7% in 2009. Moxifloxacin resistance followed this trend, although at a lower level (4% in 2008). Resistant strains had MIC 16 mg/l for ciprofloxacin and MIC 2-4 mg/l for moxifloxacin.

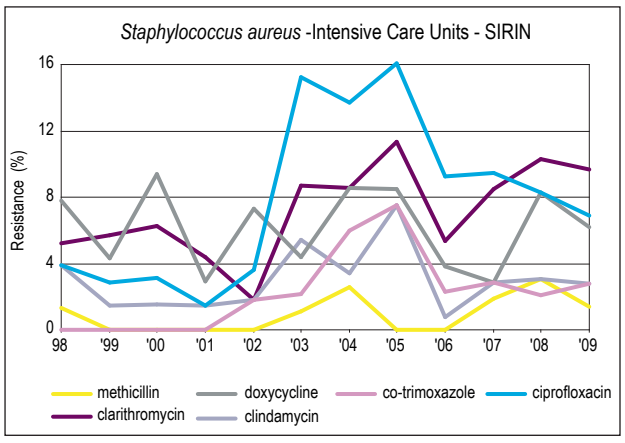

Figure 55. Trends in antibiotic resistance among clinical strains of *Staphylococcus aureus* from Intensive Care Units (N =1.127).

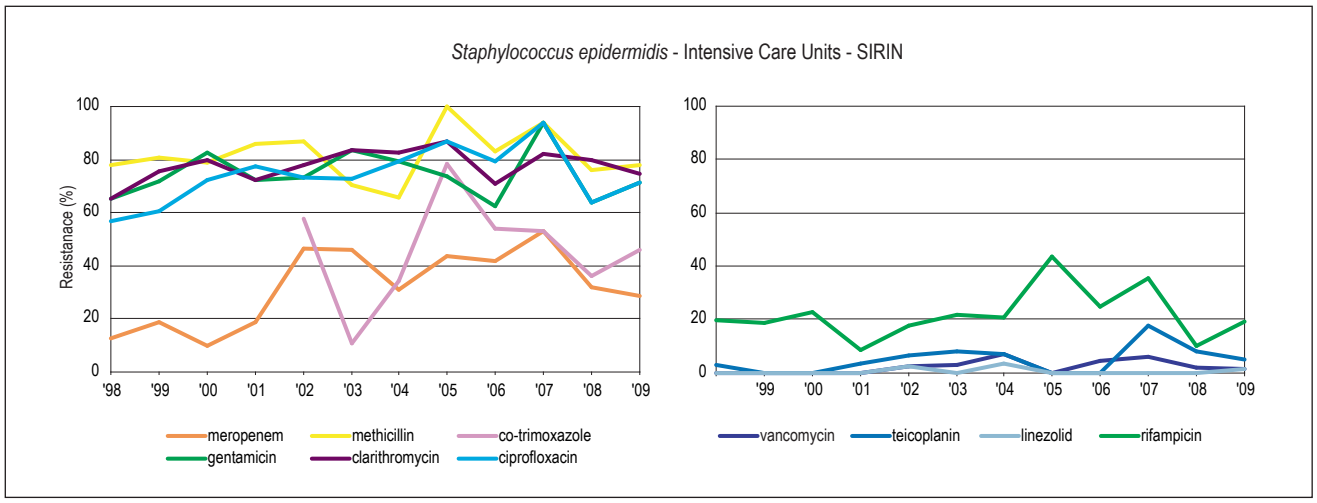

Figure 56. Trends in antibiotic resistance among clinical strains of *Staphylococcus epidermidis* from Intensive Care Units (N = 511).

Resistance rates to carbapenem, rifampicin, linezolid and quinupristin/dalfopristin were less than 1% (not shown). Vancomycin resistance was once recorded in 2006, teicoplanin resistance was once found in 2003 being less than 0.1%.

4.2.4.8 Staphylococcus epidermidis

About 80% of all strains of *S. epidermidis* (N=511) from Intensive Care Units were methicillin-resistant. Methicillin-resistant strains were often co-resistant to erythromycin, clarithromycin, gentamicin, ciprofloxacin and meropenem. The emergence of resistance to meropenem (figure 56) in Intensive Care Units was impressive being less than 20% until 2001 and increasing to 32% in 2008 and 29% in 2009. The MIC distribution (figure 57) was more or less bimodal until 2005 with a small subpopulation of strains with MIC < 0.25 mg/l and another subpopulation over a large range (MIC 1- >16 mg/l) with the median at 2 mg/l. A clear shift to the right was observed from 2002 onward with appearance of a cluster of strains with MIC > 8 mg/l.

Clarithromycin resistance in Intensive Care Units was much higher than that reported for Unselected Hospital Departments and fluctuated around 80%. The MIC distribution was bimodal with a large cluster with MICs >16 mg/l and a very small cluster with MICs of 0.5 mg/l or less (figure 57). Clindamycin resistance was around 55% (not shown). Doxycycline resistance fluctuated between 20% and 30% without a real trend.

Gentamicin resistance fluctuated around 70% with a peak of 94% in 2008.

Ciprofloxacin resistance was around 70%, which was much higher than the level in Unselected Hospital departments.

Co-trimoxazole resistance rates were 35-45%; rifampicin resistance fluctuated around 20%. Vancomycin-resistance was occasionally found in Intensive Care Units in 1-2 centres per year from 2002 on. Three vancomycin-resistant strains were also teicoplanin-resistant (MIC 8, 64, 256 mg/l, respectively). Teicoplanin resistance was

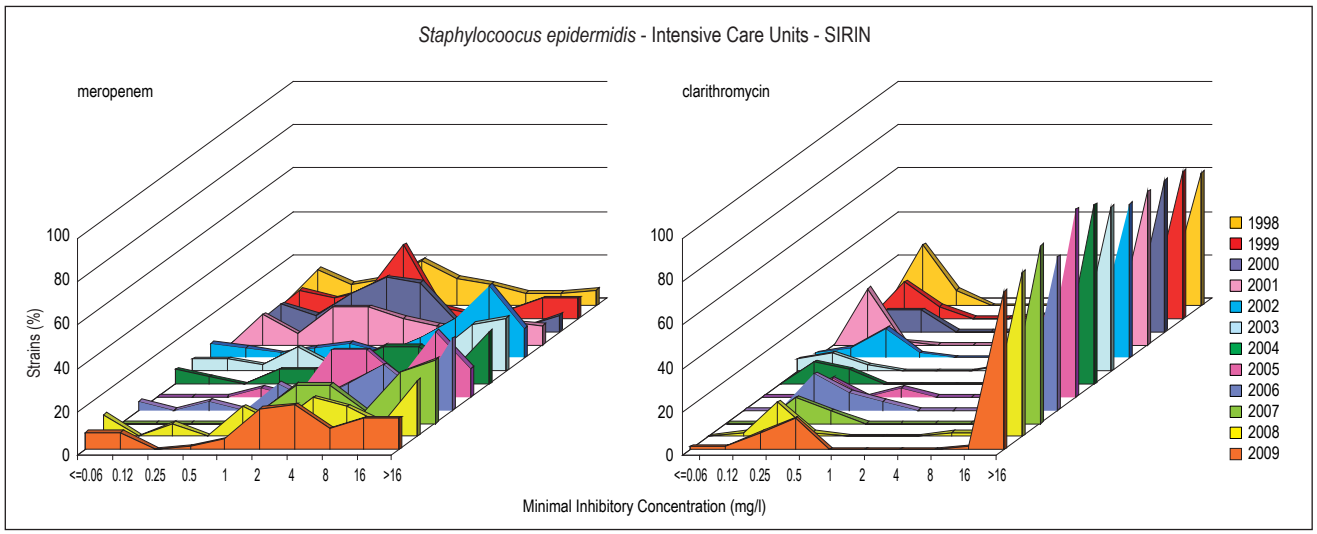

Figure 57. MIC distributions of meropenem and clarithromycin for *Staphylococcus epidermidis* from Intensive Care Units.

Table 4 Resistance levels among Enterobacteriaceae and Pseudomonas aeruginosa in Intensive Care Units in 2009.

Antibiotic	*E. coli*	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*
amoxicillin	47			27	
co-amoxiclav	29	24	87	7	
piperacillin	40		32	9	17
pip-tazobactam	3	14	15	0	16
meropenem	0	0	0	0	11
cefuroxime	13	18	58	0	
cefotaxime/ceftriaxone	6	9	38	0	
ceftazidime	2	9	24	0	11
ceftibuten	6	9	51	0	
cefixime	11	11	70	0	
cefepime	4	6	9	0	
gentamicin	4	14	13	9	7
tobramycin	4	10	17	2	4
amikacin	0	0	7	2	4
trimethoprim	25	32	16	40	
co-trimoxazole	24	21	13	27	
norfloxacin	14	26	28	7	
ciprofloxacin	11	18	14	4	25
levofloxacin	11	14	16	0	28
moxifloxacin	12	18	21	7	
nitrofurantoin	1	78			

increasing
stable
decreasing

observed intermittent in nine centres with 5% overall resistance. No trend could be observed. Linezolid resistance was sporadic.
High resistance levels to many drugs among *S. epidermidis* from Intensive Care Units are common and reflect the high selective pressure in these wards. Often these strains belong to specific populations circulating in Intensive Care Units and colonizing many patients. Resistance levels within these populations may differ from Unit to Unit and therefore the overall resistance levels reported may not be representative for a given Intensive Care 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.

Conclusion (see also table 4)

1. Increasing and high resistance to co-amoxiclav and piperacillin among *Enterobacteriaceae* except in *P. mirabilis*; upcoming resistance of piperacillin-tazobactam.
2. Only 3rd and 4th generation cephalosporins still usable for empiric therapy, except for Enterobacter infections
3. Aminoglycoside resistance low among *E. coli*, increasing among *K. pneumoniae* and *E. cloacae*, not found in all centres.
4. Co-trimoxazole- and quinolone resistance high, increasing in *K. pneumoniae*, decreasing in *E. coli*.
5. Carbapenem resistance not found among Enterobacteriaceae; carbapenems resistance among *P. aeruginosa* not found in all centres.
6. Multiresistance is increasing; ESBL producing *K. pneumoniae* are increasing.
7. Differences in local resistance patterns found underline the value of local surveillance.
8. Empiric therapy for Gram-negative infections in Intensive Care Units should be combination therapy.
9. Resistance among *S. aureus* is low for all antibiotics tested. MRSA play a minor role in Intensive Care Units in general.
10. Resistance among *S. epidermidis* is high for most antibiotics tested and requires insight in local resistance patterns before adequate therapy can be advised.

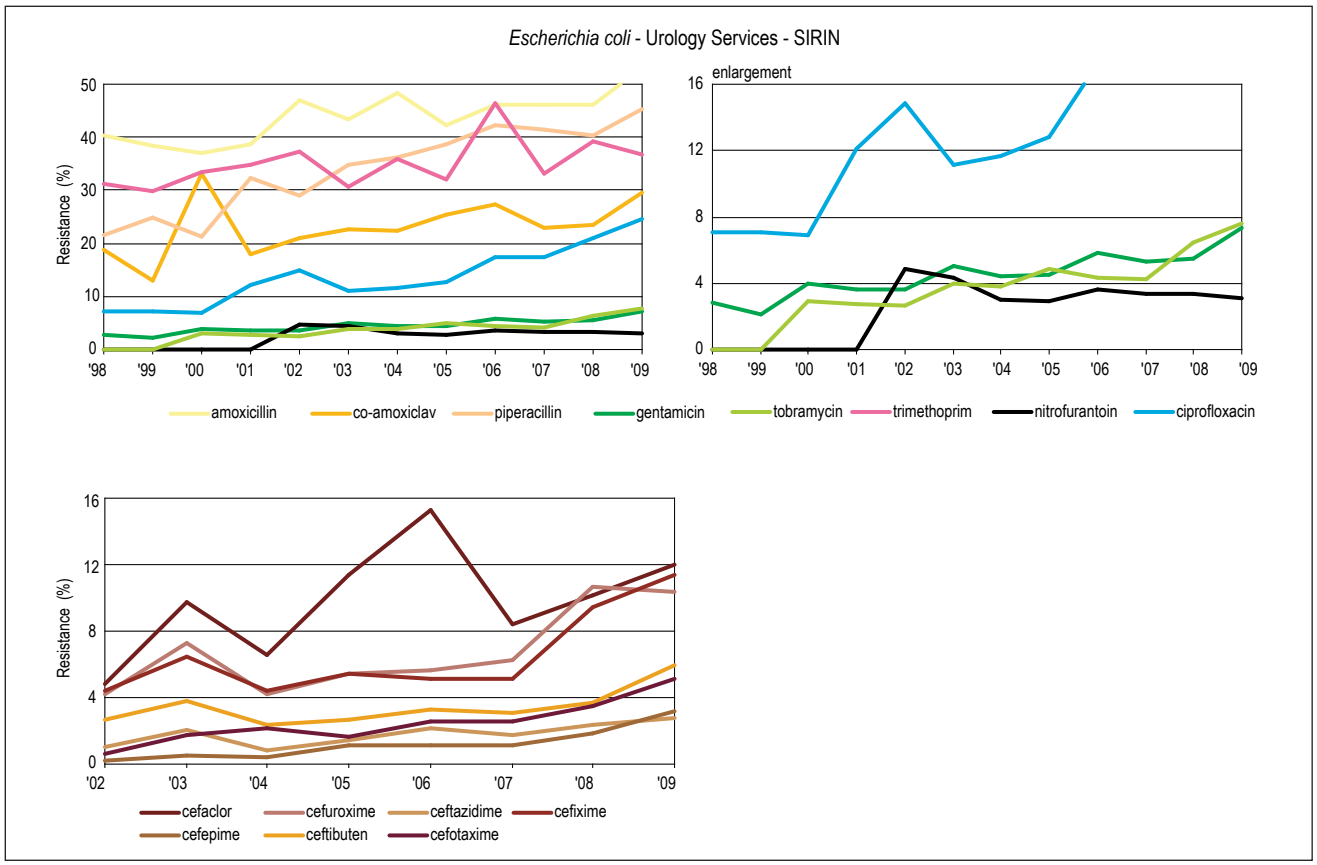

Figure 58. Trends in antibiotic resistance among clinical strains of *Escherichia coli* from Urology Services (N = 8.686).

4.2.5 Urology Services - SIRIN

4.2.5.1 Escherichia coli

The numbers of strains of *E. coli* collected from Urology Services were 500-700 yearly. The resistance to amoxicillin fluctuated around 40% until 2001 and increased to 53% in 2009 (figure 58), now being higher than the resistance level found in Intensive Care Units during the whole study period. The MIC distribution was similar to that found for strains from Intensive Care Units. Co-amoxiclav resistance was steadily increasing from 19% in 1998 to 30% in 2009, being as high as the level in Intensive Care Units in 2009, but with the difference that the latter level was decreasing since 2005. Piperacillin- and piperacillin-tazobactam resistance was similar to those found in Intensive Care Units. This was also found for imipenem and meropenem. Cefuroxime resistance increased to 10% in 2009, being consistently somewhat lower than that found in Intensive Care Units. Resistance to cefotaxime increased to 5%, to cefixime to 11%, cefepime to 3%, ceftibuten to 6% and to ceftazidime to 3%. These levels are equal to those found in Intensive Care Units, whereas they used to be consistently lower during the years before. Gentamicin resistance increased from 3% in 1998 to 7% in 2009, which is higher than in Intensive Care Units (3.5%). It appeared that almost all centres had gentamicin-resistance strains since 2003 (figure 59). This was also found for tobramycin: 8% resistance in Urology Services compared with 4% in Intensive Care Units. Trimethoprim resistance increased from 31% to 17% with some fluctuations. The fluctuations must be caused by the existence of a subpopulation with MIC around the breakpoint (4 mg/l), which was observed by the MIC distributions in 2002 and 2006 (figure 60). Co-trimoxazole resistance showed the same trend at a lower level. We found consistently higher resistance levels of trimethoprim and ciprofloxacin in Urology Services compared to those in Intensive Care Units.

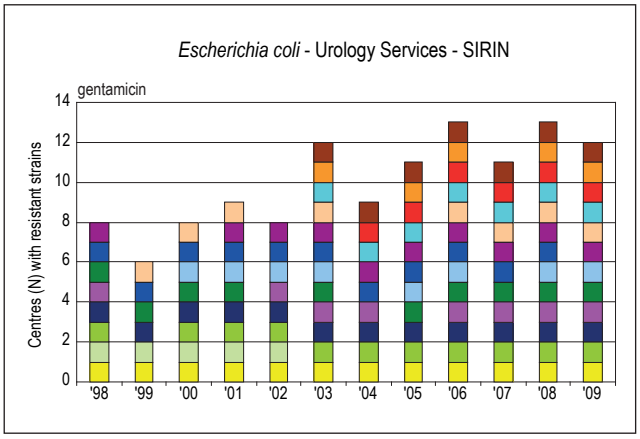

Figure 59. Number of centres with gentamicin-resistant *Escherichia coli* on Urology Services. Each color represents one specific centre.

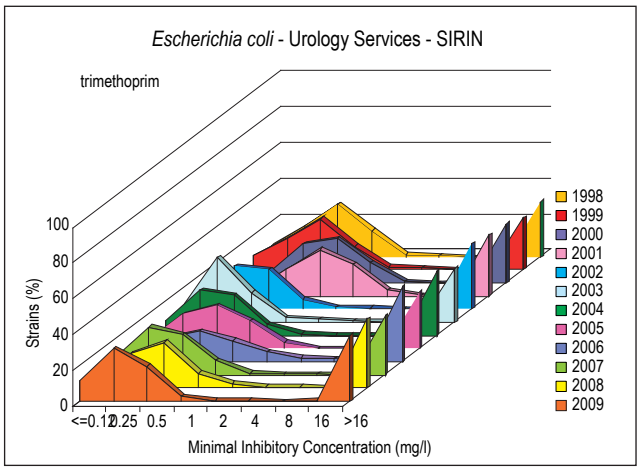

Figure 60. MIC distributions of trimethoprim for *Escherichia coli* from Urology Services.

Ciprofloxacin resistance level increased more rapidly from 7% in 1998 to 25% in 2009. The resistance percentages of norfloxacin, levofloxacin and moxifloxacin were similar to those of ciprofloxacin.
The resistance level of all quinolones (ciprofloxacin, norfloxacin, levofloxacin and moxifloxacin) was higher and increased more rapidly in Urology Services than in Intensive Care Units, from 7% in 1998 to 25% in 2009. The MICs showed the same distributions as found in strains from Intensive Care Units. Nitrofurantoin resistance increased to from 0% in 1998 to 5% in 2002 and decreased thereafter to 3% in 2009.

Multiresistance in *Escherichia coli*
Surprisingly a higher rate of multiresistance was found in Urology Services compared to Intensive Care Units (figure 61). It increased from 6% of all strains in 1998 to 16.2% in 2009. A total of 613 multiresistant strains were isolated during the years (9.5% of all). Resistance to the combination co-amoxiclav/co-trimoxazole/ciprofloxacin with or without gentamicin and cefuroxime was most prominent increasing from 2.9% of all strains tested in 1998 to 10.9% in 2009. From 1998 on resistance to four classes of antibiotics was recorded, which also increased from 1.9% in 1998 to 5.1% of all strains in 2009, whereas resistance to five classes increased from 0.4% to 2.3%. This affected almost all centres since 2006 (figure 61) and is therefore not a local problem, but rather a national one.

4.2.5.2 Klebsiella pneumoniae
Co-amoxiclav resistance among *K. pneumonaie* from Urology Services (N=858) was lower compared to that in Intensive Care Units but showed also an increasing trend from 4% in 1998 to 15 % in 2008 (figure 62).
Piperacillin-tazobactam resistance in Urology Services fluctuated at a low level (0-5%) with only a few centres yearly delivering resistant strains. So the piperacillin-tazobactam resistance found did not reflect the resistance level for Urology Services as a whole.
Carbapenem resistance was not found.
Resistance to cefaclor and cefuroxime fluctuated during the years without a clear trend (figure 62). They were significant lower than the levels found in Unselected Hospital Departments and Intensive Care Units. The MIC distributions were similar to those found for strains from Intensive Care Units. Ceftazidime and cefotaxime resistance were 2% or less, which is also lower than the levels found in the other hospital departments.
Trimethoprim resistance increased slowly with fluctuations from 29% in 1998 to 34% in 2009, which

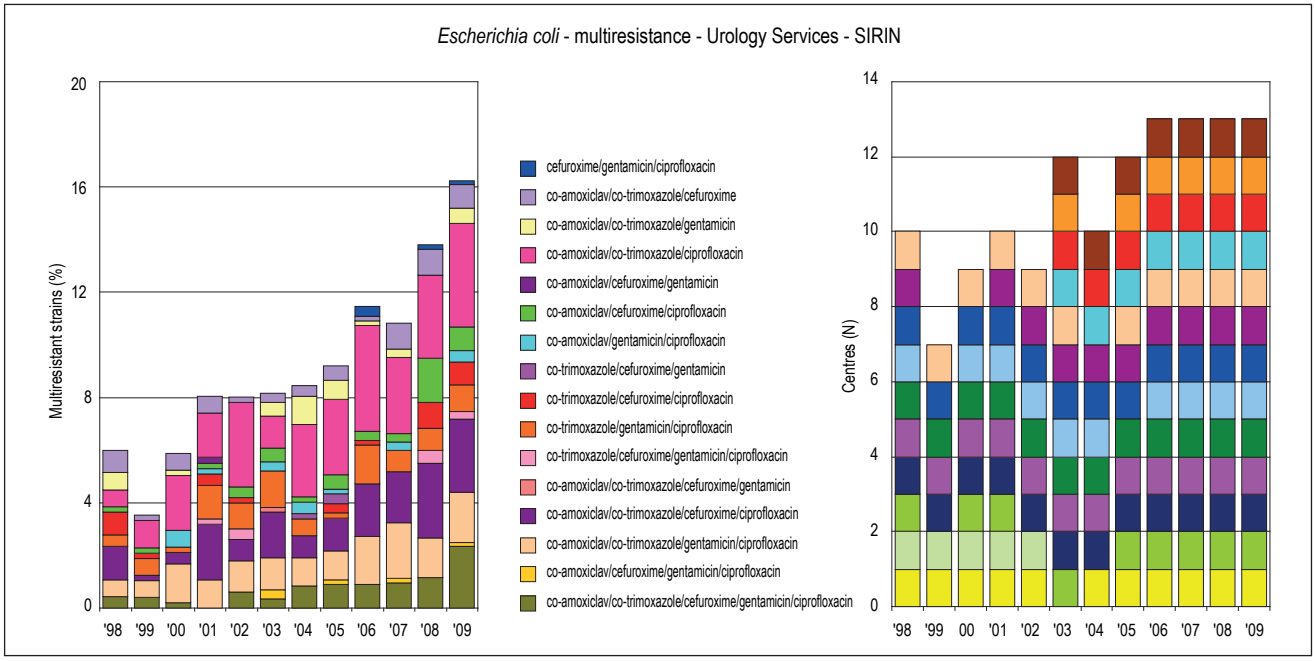

Figure 61. Trends in multiresistance among *Escherichia coli* from Urology Services and the number of centres. Each color represents one specific centre.

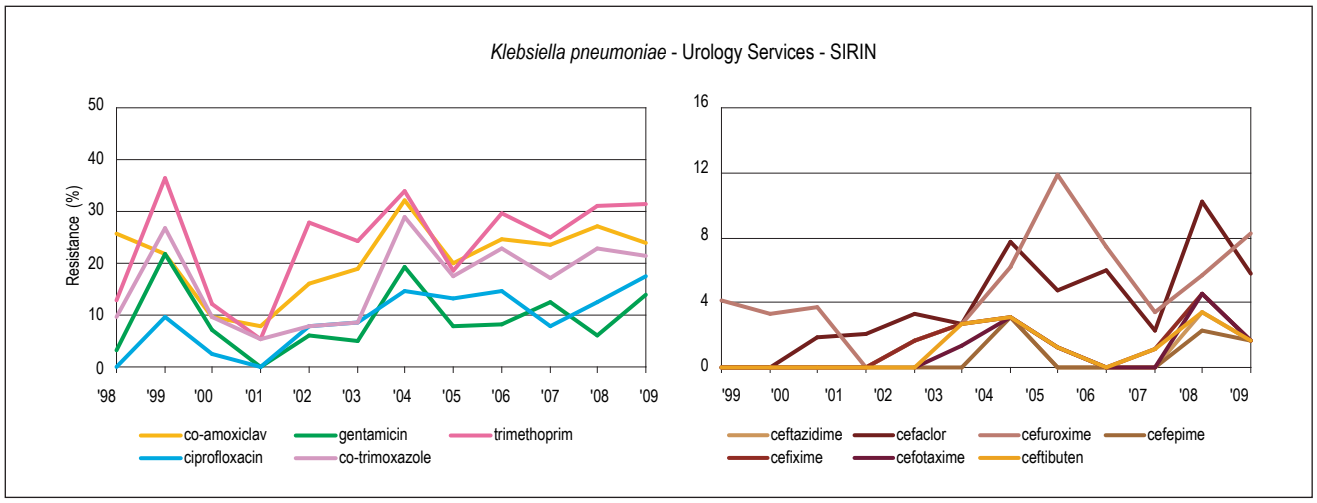

Figure 62. Trends in antibiotic resistance among clinical strains of *Klebsiella pneumoniae* from Urology Services (N = 858).

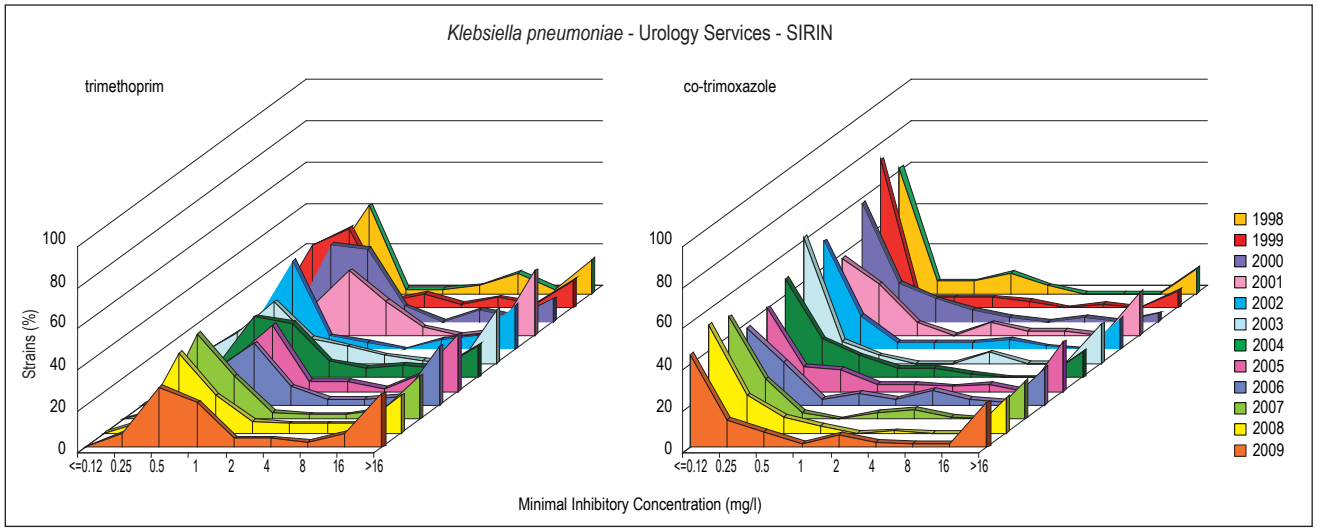

Figure 63. MIC distributions of trimethoprim and co-trimoxazole for *Klebsiella pneumoniae* from Urology Services.

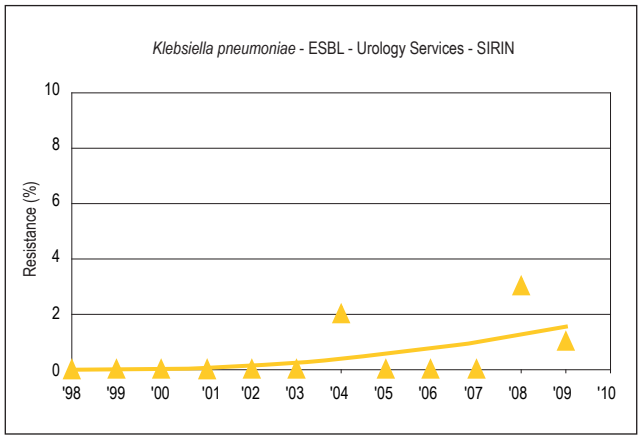

Figure 64. Prevalence of ESBL producing *Klebsiella pneumoniae* strains in Urology Services.

level was the highest compared to the levels found in the other populations. Trimethoprim was the drug of first choice in GP patients until 2005. The higher resistance rates observed in urinary strains from Urology Services may reflect frequent use of this drug alone or in the combination co-trimoxazole in the previous years. The fluctuations may be explained by the distribution of MICs (figure 63). That of trimethoprim looks bimodal with one subpopulation with MICs 0.25-2 mg/l and one subpopulation with MICs > 16 mg/l, but there is another subpopulation in between with MICs 4-8 mg/l, being within the area of the breakpoint for resistance. Variations in laboratory procedures or number of strains per year may make that strains in the intermediate area become categorized resistant or susceptible and influence thus the resistance level in a given year.

The resistance to co-trimoxazole followed the trend of trimethoprim with an increase from 12% in 1998 to 25% in 2009. Co-trimoxazole is an alternative drug combination for complicated urinary tract infections *Klebsiella*

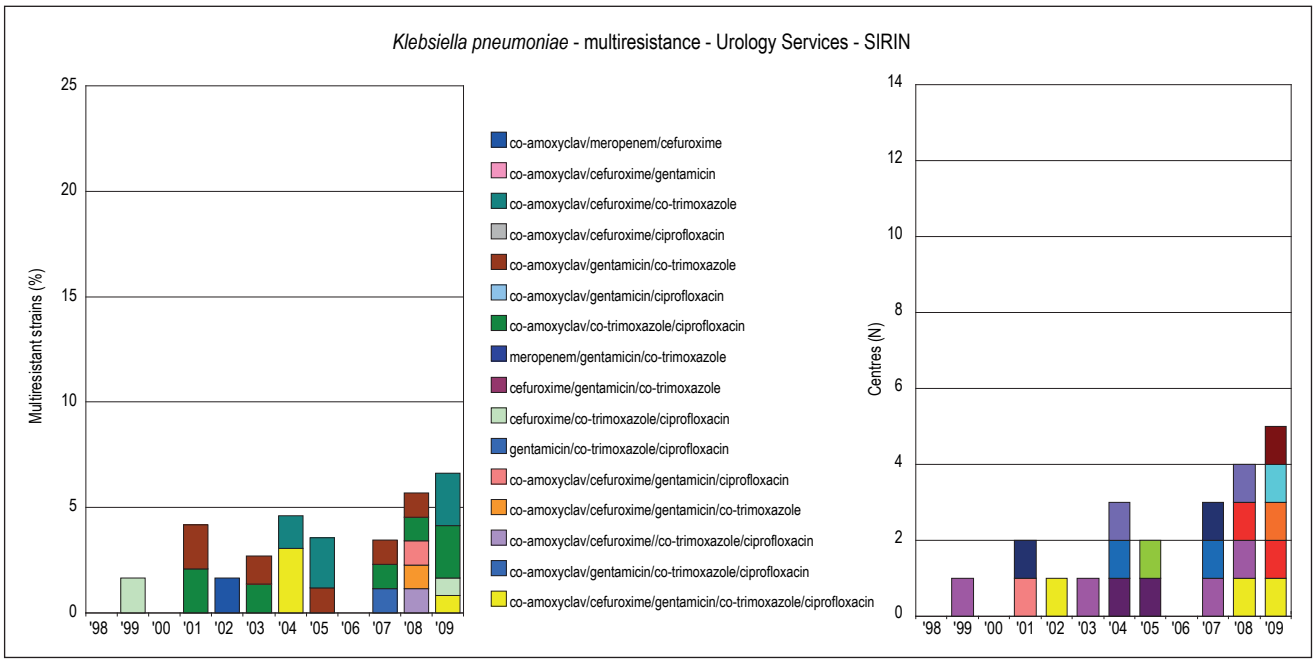

Figure 65. Trends in multiresistance among *Klebsiella pneumoniae* and number of centres with multiresistant *Klebsiella* from Urology Services.

infections in Urology Services and Paediatric Departments. Use of co-trimoxazole in these settings should be reconsidered in view of the high resistance levels found. The MIC distribution of strains from Urology Services (figure 63) showed a clear bimodal shape without the intermediate subpopulation as we noticed for trimethoprim. Gentamicin and tobramycin resistance in Urology Services was less than 3% and not common in all Urology Services. Amikacin resistance was not found. Nitrofurantoin resembled those of the levels in Unselected Hospitals (not shown).
Norfloxacin resistance increased from 13% in 1998 to 23% in 2009, which is much higher than the levels found in selected GP patients, Outpatients department and Unselected Hospital Departments. Previous quinolone use in these patients may explain these high levels. Ciprofloxacin resistance was 9% which is lower than the level in Intensive Care Units but higher than those in other patients groups. The MIC distributions of all quinolones were comparable to those found for strains from Intensive Care Units (figure 43)

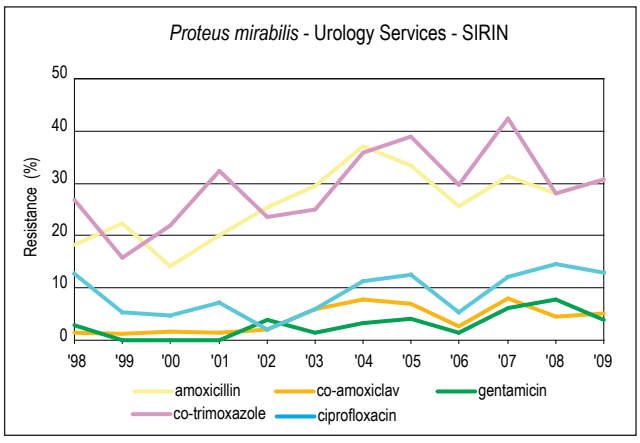

Figure 66. Trends in antibiotic resistance among clinical strains of *Proteus mirabilis* from Urology Services (N=901).

ESBL among *Klebsiella pneumoniae*
A total of 16 strains with MIC > 1 mg/l for cefotaxime and /or ceftazidime were tested for ESBL production; six of them were ESBL producers: two strains in 2004, three in 2008 and one in 2009 (figure 64).

Multiresistance of *Klebsiella pneumoniae*
Multiresistance in Urology Services occurred (6.5% in 2009), but to a much less extend than that in Intensive Care Units and it reached never the level of that found in *E. coli* strains in Urology Services (figure 65). It occurred incidentally in most centres.

4.2.5.3 Proteus mirabilis
A total of 901 strains of *P. mirabilis* were collected from Urology Services. Amoxicillin resistance increased from 18% in 1998 to 37% in 2004 and decreased subsequently to 31% in 2010 (figure 66). The distribution of MICs of the strains from Urology Services was bimodal and showed two subpopulations: a susceptible one over a small range in most years (MIC 0.5-1.0 mg/l) and a resistant one with MICs >8 mg/l (figure 67). Co-amoxiclav resistance increased from 1% in 1998 to 5% in 2010. The MIC distribution of co-amoxiclav (figure 67) showed a change from 2000 on including a broadening of the susceptible subpopulation (MIC 0.25-8 mg/l) and flattening of the peak at 1 mg/l with appearance of small and growing subpopulations with MIC 8 >16 mg/. This continued in the following years resulting in a 5-6% resistance in 2003 and further. So the increase of resistance observed in 2003 could already be predicted

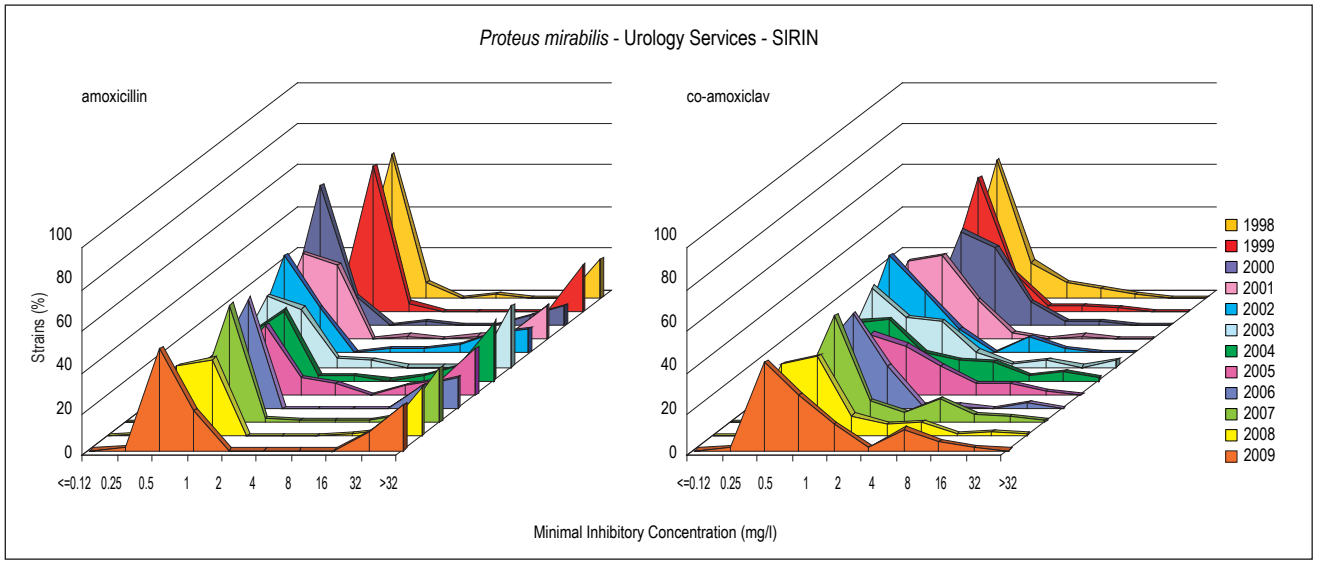

Figure 67. MIC distributions of amoxicillin and co-amoxiclav for *Proteus mirabilis* from Urology Services.

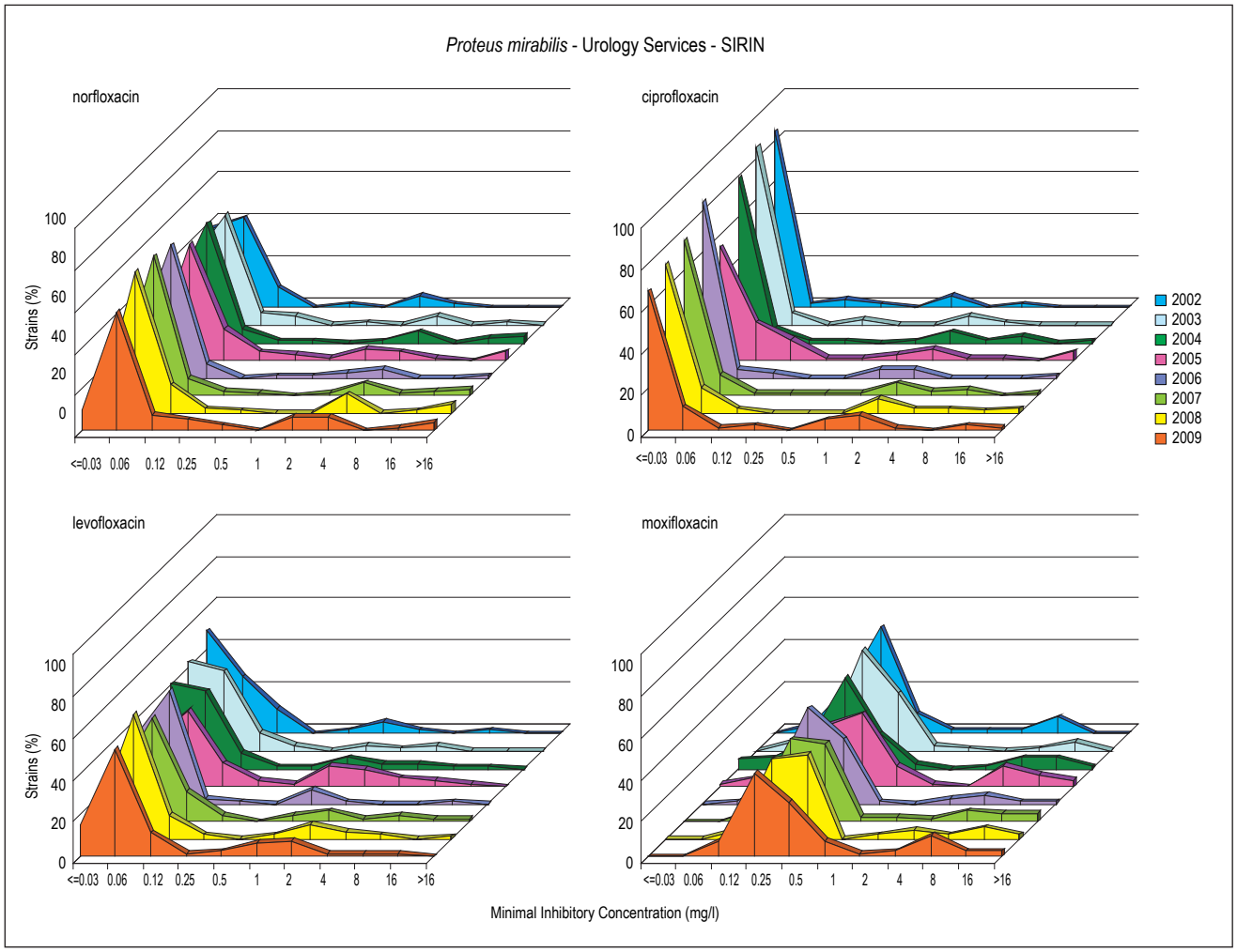

Figure 68. MIC distributions of quinolones for *Proteus mirabilis* from Urology Services.

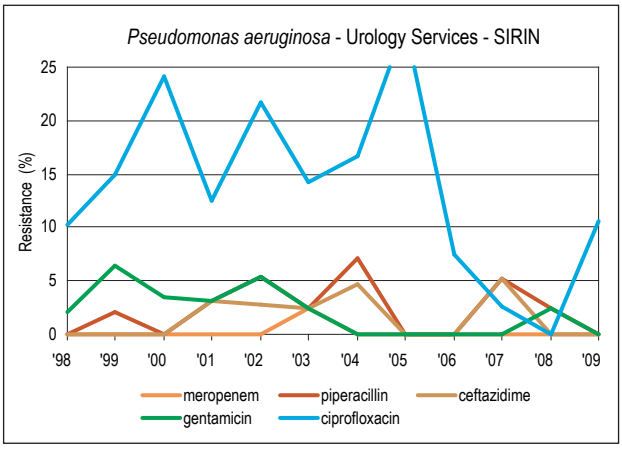

Figure 69. Trends in antibiotic resistance among clinical strains of *Pseudomonas aeruginosa* from Urology Services (N = 473).

three years earlier by analyzing the MIC distributions. This underlines the importance of quantitative susceptibility testing.
Piperacillin resistance increased from 3% in 1998 to 10% in 2009; 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, upto levels of 70% in 2005, for which we have no explanation. Since 2006 around 50% of all strains were resistant to trimethoprim. Co-trimoxazole resistance increase from 27% in 1998 to 31% in 2009 (figure 66), which is comparable with the levels found in Unselected Hospital departments.

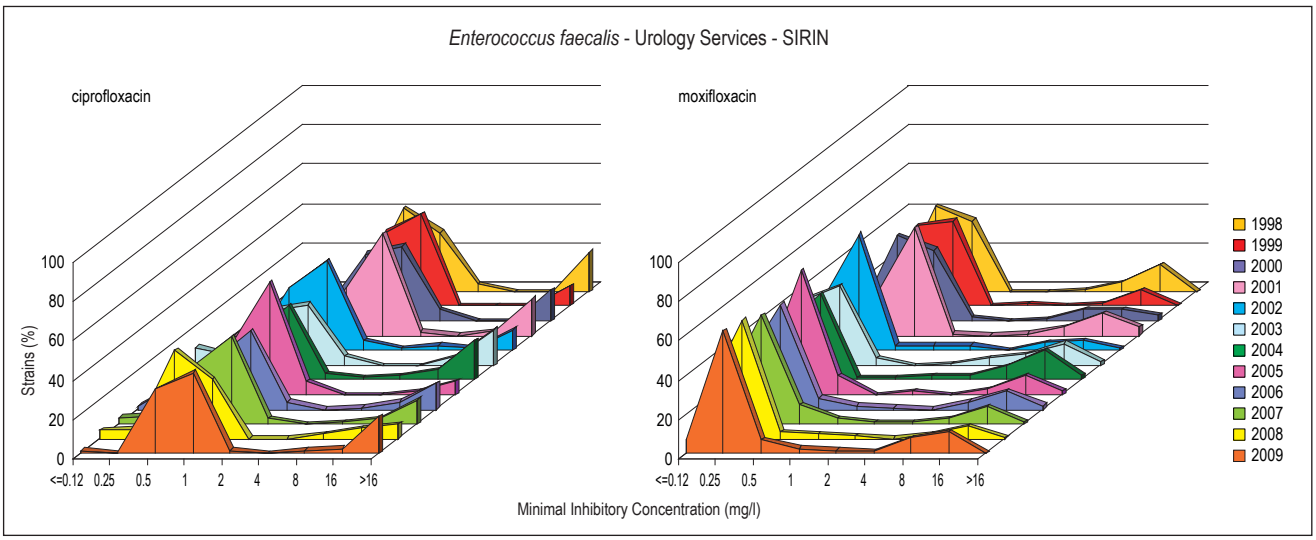

Figure 70. MIC distributions of ciprofloxacin and moxifloxacin for Enterococcus faecalis from Urology Services (N=1313).

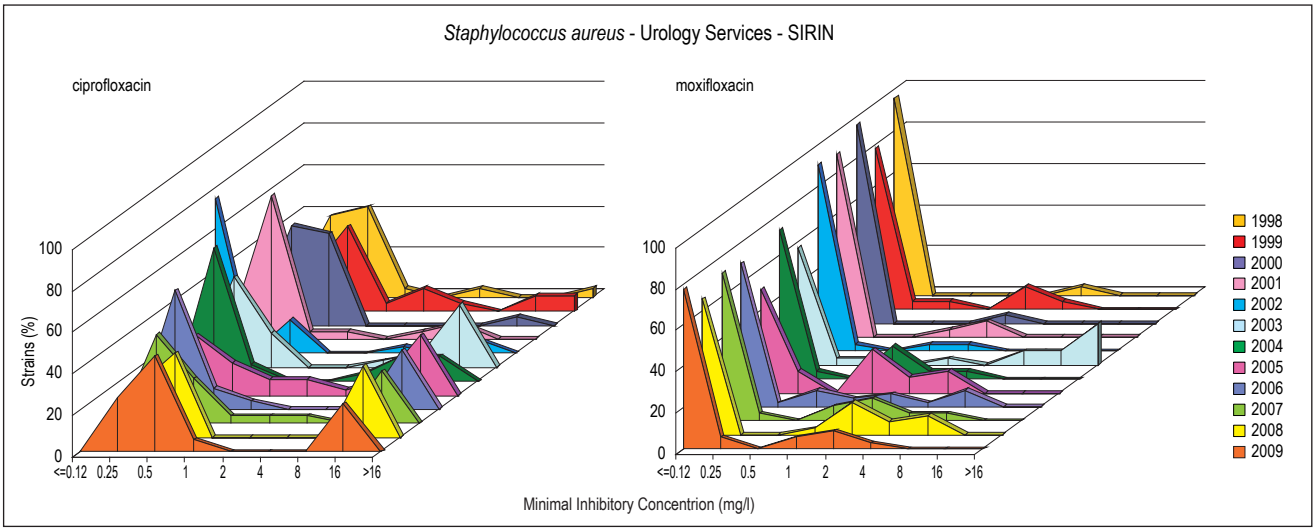

Figure 71. MIC distributions of ciprofloxacin and moxifloxacin for *Staphylococcus aureus* from Urology Services (N=369).

Table 5 Resistance levels (%) among Enterobacteriaceae and Pseudomonas aeruginosa in Urology Services in 2009.

Antibiotic	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P.aeruginosa*
amoxicillin	53		31	
co-amoxiclav	30	15	5	
piperacillin	45		10	0
pip-tazobactam	3	3	0	0
carbapenem	0	0	0	
cefuroxime	10	8	0	
cefotaxime/ceftriaxone	5	2	1	
ceftazidime	3	2	0	0
ceftibuten	6	2	1	
cefixime	11	2	1	
cefepime	3	2	0	
gentamicin	7	1	4	0
tobramycin	8	2	1	0
amikacin	0	0	1	0
trimethoprim	37	34	47	
co-trimoxazole	35	25	31	
norfloxacin	25	23	18	
ciprofloxacin	25	9	13	11
levofloxacin	24	8	4	13
moxifloxacin	25	12	19	
nitrofurantoin	3	76		

increasing
stable
decreasing

Gentamicin resistance increased from 3% in 1998 to 8% in 2008 and decreased to 4% in 2009 (figure 66). These fluctuations were due to emergence of resistant strains in 2-6 centres yearly. This underlines the value of local surveillance.
Quinolone resistance fluctuated considerably and this was exclusively due to the existence of a varying number of strains with MICs = 2mg/l, which is near the breakpoints of norfloxacin (1 mg/l), ciprofloxacin (1 mg/l) and levofloxacin (2 mg/l) (figure 68). The MIC distributions of ciprofloxacin do not differ significantly, but the difference in breakpoints resulted in an 18% resistance to norfloxacin, 13% for ciprofloxacin and 4% for levofloxacin. However the intrinsic activity of ciprofloxacin is the highest with 66% of strains inhibited by 0.03mg/l or less compared to 15% by levofloxacin, 10% by norfloxacin and 0% by moxifloxacin.
The MIC distribution of moxifloxacin was bimodal with one subpopulation with MIC 0.12-1 mg/l and one subpopulation with MIC 8 mg/l or more (figure 68).

4.2.5.4 Pseudomonas aeruginosa
The numbers of strains isolated were around 40 yearly, which are low for evaluation, yet some trends could be observed. Piperacillin resistance in Urology Services was accidental, fluctuating between 0% and 7% (figure 69). Meropenem resistance was found only once in Urology Services in 2003. Ceftazidime resistance was consistently low (0-5%) without a trend.
Aminoglycoside resistance was found sporadically in some Urology Services. Ciprofloxacin resistance fluctuated strongly between 10% in 1998 and 28% in 2005, showed a remarkable decrease thereafter to 7% in 2006 3% in 2007 and 0% in 2008 and a reappearance of 10% in 2009 (figure 69). The levels of resistance to levofloxacin paralleled those of ciprofloxacin but were mainly 2-3% higher with 13% resistance in 2009. Like for strains from Intensive Care Units, we found existence of many strains around the breakpoint of resistance which may explain the strong fluctuation in resistance percentages calculated. There was no significant change in the shape of the MIC distributions during the study period.

4.2.5.5 Enterococcus faecalis
The number of strains isolated from patients from Urology Services was 1313, 17 of them were amoxicillin resistant. These strains were isolated in eight centres between 2002 and 2007, only once per centre. Seven (out of 17) were co-resistant to vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin, but susceptible to ciprofloxacin and moxifloxacin.
Vancomycin resistance (12 strains) was found once in three centres in 2003 and 2004. All but one were also teicoplanin resistant, which is evidence for clonal spread of a VanA gene positive strain. MICs for both drugs were >128 mg/l.
Ciprofloxacin resistance decreased from 54% in 1998 to 25% in 2009, that to moxifloxacin fluctuated around 20%. The MIC distributions of both quinolones were bimodal; ciprofloxacin showed a large susceptible subpopulation with MIC 0.5-1 mg/l which is near the breakpoint and a small insusceptible subpopulation with MIC > 16 mg/l (figure 70). The MIC distribution of moxifloxacin had a large susceptible subpopulation with

MIC 0.25 mg/l and a small insusceptible one with MICs ranging from 4-16 mg/l.

4.2.5.6 Staphylococcus aureus

The numbers of strains collected yearly from Urology Services were less than 40, which is too low for general evaluation. Yet some findings were striking. Resistance levels to most antibiotics were lower than those found for strains from Intensive Care Units, except for co-trimoxazole and the quinolones. Co-trimoxazole resistance fluctuated between 6% and 19% compared to 3% in Intensive Care Units and ciprofloxacin resistance increased from 8% in 1998 to 40% in 2003 and showed a decrease to 23% in 2009, whereas that of moxifloxacin rose from 4% to 11% with considerable fluctuations. The MIC distributions of both quinolones (figure 71) were bimodal with a susceptible subpopulation with MIC < 0.25 mg/l and a resistant population with MIC 2-16 mg/l for moxifloxacin and at 16 mg/l for ciprofloxacin. Apparently these resistance levels are result of frequent use of co-trimoxazole and quinolones on Urology Services.

Conclusion (see also table 5)

1. High and increasing resistance to penicillins, trimethoprim, co-trimoxazole and quinolones among *Enterobacteriaceae* is matter of high concern.
2. Empiric therapy cannot be advised, except for suspected infection by *P. mirabilis*.
3. Nitrofurantoin still drug of 1st choice in uncomplicated *E. coli* infections

4.2.6 Pulmonology Services - SIRIN

4.2.6.1 Streptococcus pneumoniae

Penicillin resistance among *S. pneumoniae* (N=1695) was less than 1% during the whole study period and was not found in 2009 (figure 72).

The resistance to cefaclor (MIC > 0.5 mg/l) increased quickly from 4% in 1998 to 56% in 2009, that to cefuroxime (MIC > 1 mg/l) was less than 4% during the whole study period. The MIC distribution (figure 73) showed a shift to the right side (higher MICs) in 2007 and 2008 moving many strains into the resistant area (MIC > 0.5 mg/l). The MIC distribution of cefuroxime showed no change over the years. Ceftazidime resistance was less than 1% in 2009 (not shown). Increasing resistance to clarithromycin was observed from 1998 (6%) to 2006 (10%), which remained stable thereafter. Clindamycin resistance increased from 2% in 1998 to 5% in 2009 (not shown). MIC distributions of clarithromycin and clindamycin were clearly bimodal with 90% of strains with MIC < 0.25 mg/l and a resistant population mostly with high MIC (> 8 mg/l) (figure 74). Doxycycline resistance level was already 12% in 1998 and remained on that level during the whole study period although

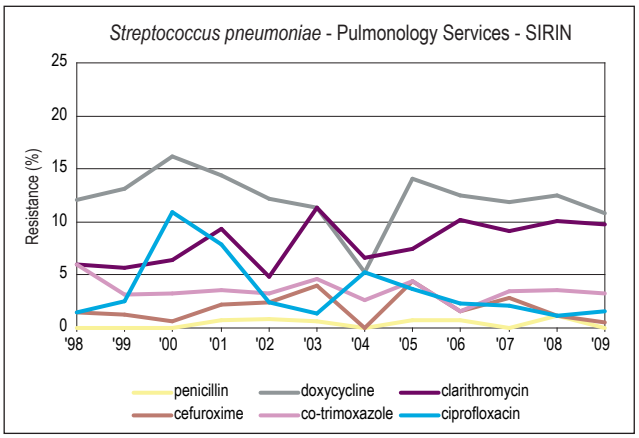

Figure 72. Trends in antibiotic resistance among clinical strains of *Streptococcus pneumoniae* from patients of Pulmonology Services (N= 1.695).

some fluctuations were observed (figure 72). The MIC distributions (figure 74) showed a change from 2001 onward. Until that time a large subpopulation with MIC < 0.25 mg/l and a small subpopulation over a broad range (MIC 1-16 mg/l) were observed. These small subpopulations are responsible for the fluctuations, as they are around the breakpoint for resistance and may fall into the susceptible category one year and into the resistant category the other year when their MIC is one dilution step higher. From 2002 onward the distribution became clearly bimodal with one susceptible subpopulation (MIC < 0.5 mg/l) and one resistant with MIC > 16 mg/l.

Co-trimoxazole resistance was 3-5% during the whole study period with 3% resistance in 2009.

Ciprofloxacin resistance showed some fluctuations, but remained less than 5% from 2002 on with 2% in 2009.

Moxifloxacin resistance was 1-3% during the whole study period. From these figures on cannot distinguish the difference in activity of both drugs. The MIC distributions showed however that the intrinsic activity of moxifloxacin was eight times higher than that of ciprofloxacin. Comparing MIC90: this was 0.12 mg/l for moxifloxacin versus 1 mg/l for ciprofloxacin.

4.2.6.2 Haemophilus influenzae

Amoxicillin resistance among *H. influenzae* (N=2730) was consistently higher than in Unselected Hospital Departments and increased with fluctuations from 8% in 1998 to 23% in 2009, whereas co-amoxiclav resistance increased from 3% in 1998 to 18% in 2009 (figure 75). The latter resistance is not based on beta-lactamase production. Data from 2004 were excluded from evaluation because of the low number of strains collected that year. The MIC distributions (figure 76) of amoxicillin showed a shift in 2005 from an almost unimodal shape over a broad range (MIC 0.1-1 mg/l) to a bimodal shape and a move to the right side. This resulted in a susceptible subpopulation with an MIC range 0.5-2 mg/l and a second subpopulation with MIC >

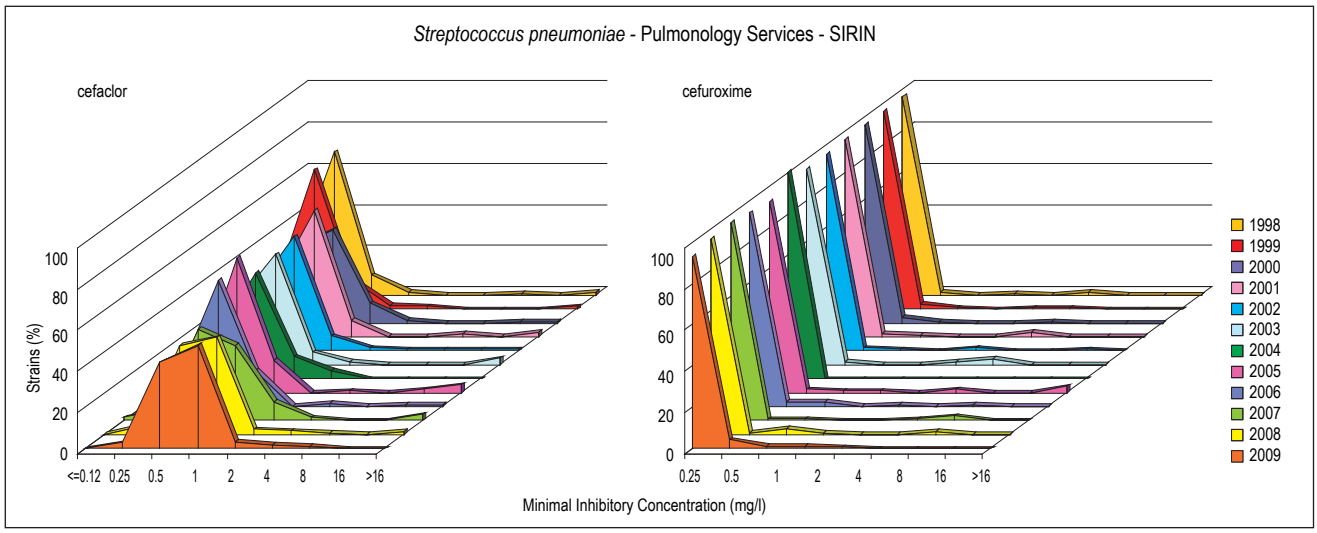

Figure 73. MIC distributions of cefaclor and cefuroxime for *Streptococcus pneumoniae* from Pulmonology Services.

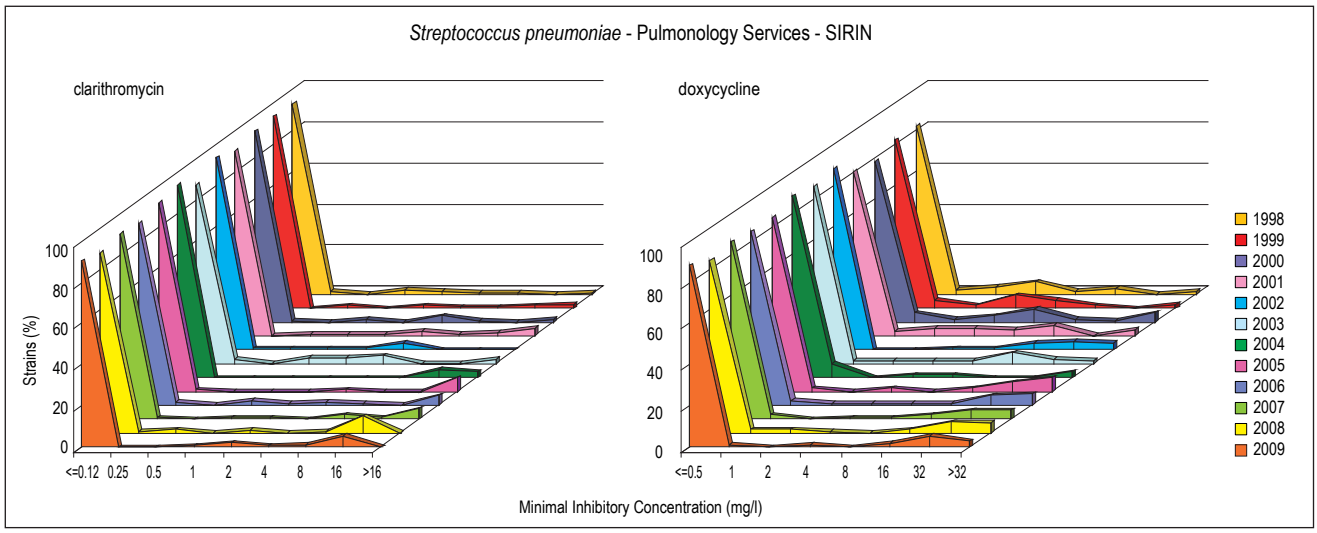

Figure 74. MIC distributions of clarithromycin and doxycycline for *Streptococcus pneumoniae* from Pulmonology Services.

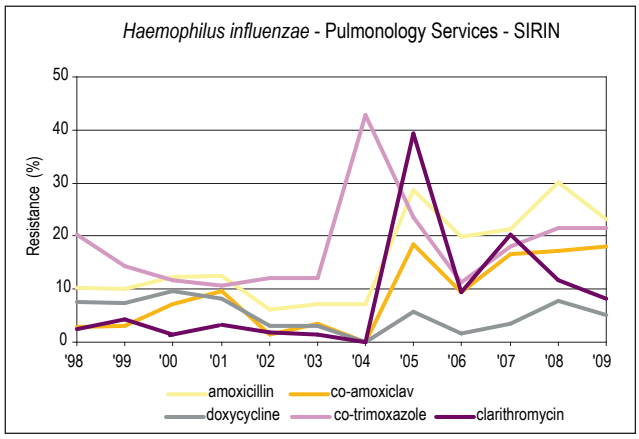

Figure 75. Trends in antibiotic resistance among clinical strains of *Haemophilus influenzae* from patients of Pulmonology Services (N=2.730).

16 mg/l. The same move to the right was observed with co-amoxiclav. The shift to the right resulted in higher resistance level because the breakpoint for resistance is MIC > 2mg/l. The increasing amoxicillin- and co-amoxiclav resistance is a matter of concern. It makes both antibiotics unusable for empiric therapy.
Clarithromycin resistance in Pulmonology Services increased with fluctuations from 3% in 1998 to 12% in 2008 and decreased to 8% in 2009. Doxycycline resistance was 8-10% in the first years of study and decreased to 5% in 2009.
A matter of concern is the high resistance to co-trimoxazole, which is one of the drugs used in COPD exacerbations. The resistance level fluctuated between 11-24% with 21% resistance in 2009. These resistance levels in both Unselected Hospitals and Pulmonology Services are too high for use of co-trimoxazole as empiric therapy.

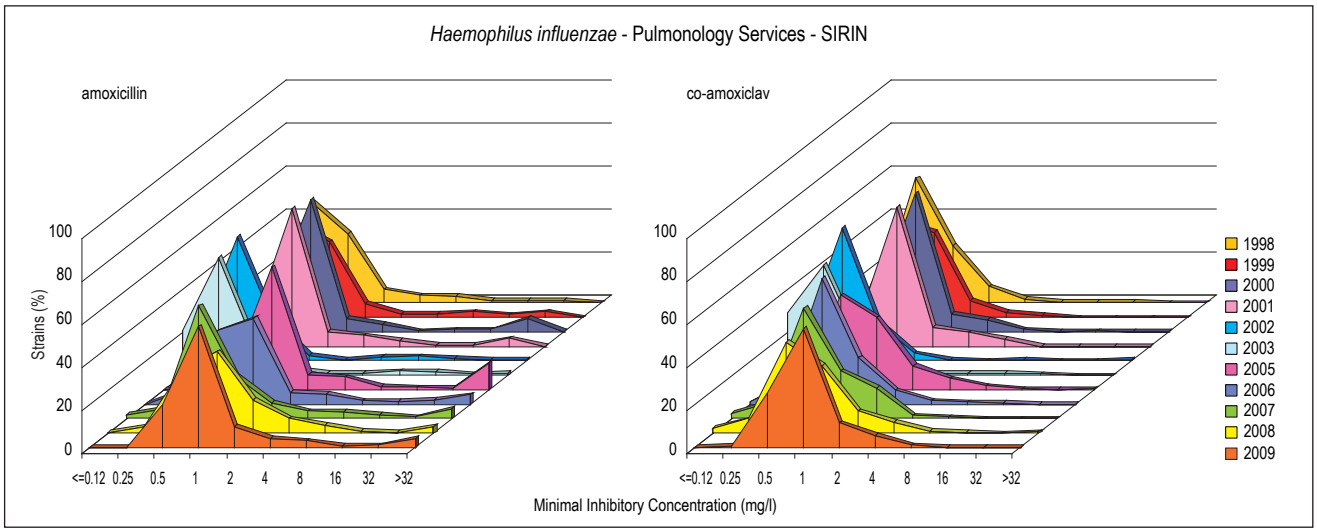

Figure 76. MIC distributions of amoxicillin and co-amoxiclav for *Haemophilus influenzae* from Pulmonology Services.

4.2.6.3 Moraxella catarrhalis

Amoxicillin resistance among *M. catarrhalis* (N=1097) fluctuated around 45% over the whole study period (figure 77). This resistance was completely due to beta-lactamase since resistance to co-amoxiclav did not occur. Cephalosporin resistance was low in all hospital departments. Cefaclor resistance in Pulmonology Services decreased from 8% in 1998 to 2% in 2009. Cefuroxime resistance was 0-5% over the years without a clear trend (figure 77), but it appeared that a growing number of strains had MIC 2mg/l, which is just below the breakpoint of resistance (> 2mg/l) (figure 78). This may predict upcoming resistance. Cefotaxime- and ceftazidime resistance was sporadic. Clarithromycin resistance was 1-3% and did not show any trend of development of resistance; 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),

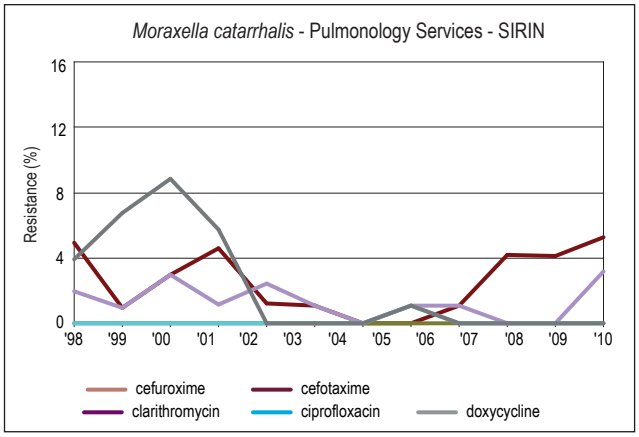

Figure 77. Trends in antibiotic resistance among clinical strains of *Moraxella catarrhalis* from patients of Pulmonology Services (N=.1097).

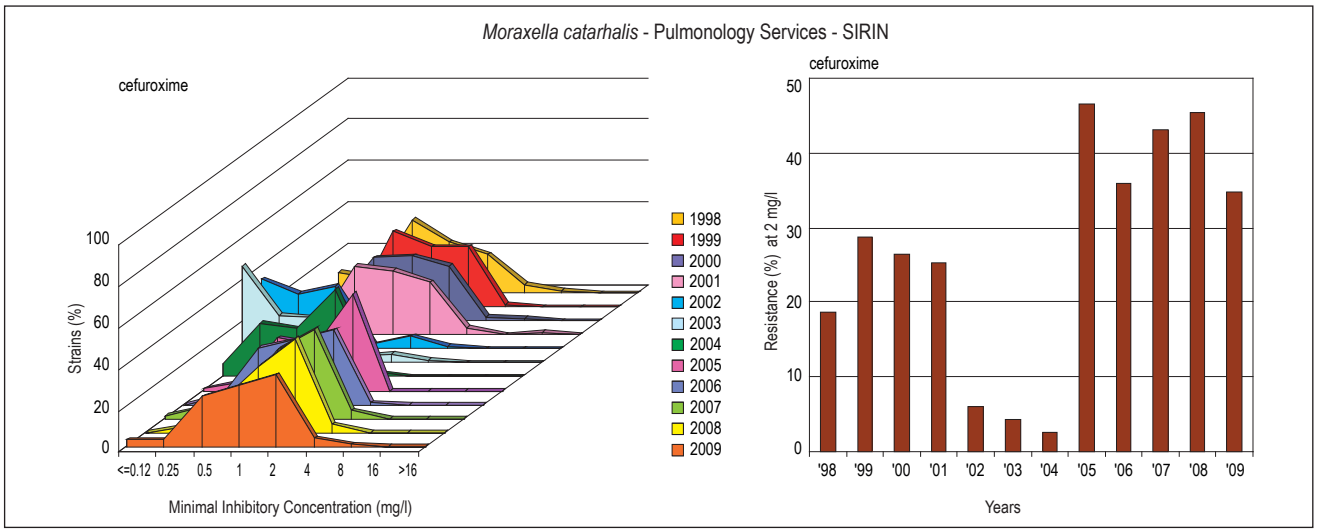

Figure 78. MIC distributions of cefuroxime for *Moraxella catarrhalis* from Pulmonology Services.

Table 6 Resistance levels (%) in respiratory pathogens in Pulmonology Services in 2009.

Antibiotic	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
penicillin	0		
amoxicillin		23	36
co-amoxiclav		18	0
cefaclor	56		2
cefuroxime	1		5
clarithromycin	10	8	3
clindamycin	5		0
doxycycline	11	5	0
co-trimoxazole	3	21	3
ciprofloxacin	2		0
moxifloxacin	2		0

increasing
stable
decreasing

which is lower than the level found in Unselected Hospital Departments, reported from 2008 on (10%). Ciprofloxacin resistance was found once in 2005, moxifloxacin resistance was not found.

Conclusion (see also table 6)

1. Penicillin/amoxicillin for treatment of respiratory tract infections by pneumococci remain first choice
2. Empiric therapy with penicillin/amoxicillin/co-amoxiclav is not advised when other pathogens may play a role. Then clarithromycin or doxycycline may be usable alternatives.

4.3 Conclusions: comparison of resistance in patients populations

The resistance levels of the species tested in the various patient populations were compared. Since strains in the SIRIN and SERIN projects were collected in 2009 we took the resistance level recorded in 2009 and looked in addition at the trend observed over the whole study period in each population. When comparing the resistance levels please keep in mind that the data from the community, selected GP patients, outpatient clinics and urology services are solely based on urine samples and the data from the pulmonology services are merely respiratory specimens. Data from the unselected hospital departments and ICUs include various clinical materials.

4.3.1.1 Escherichia coli

Amoxicillin resistance was very high in all patient populations studied (34-53%) (table 7); it is still increasing in the community, Unselected Hospital Departments and Urology Services. The level of amoxicillin resistance in 2009 was highest in Urology Services, but the difference with these other patient populations was not large (44-48% resistance) (table 7). This indicates that all these patients have been exposed to or were treated with antimicrobial drugs before. In all populations the level of amoxicilin resistance is too high to use amoxicillin as empiric therapy for any infection by *E. coli* and can only be used after susceptibility testing. The high resistance levels for co-amoxiclav lead to the same conclusion (table 7). The co-amoxiclav resistance pattern followed the same trend as amoxicillin, but at a lower level. However, even the lowest level found in the community (12%) makes the drug unsuitable for empiric therapy. A matter of concern for all hospital departments is also the increasing resistance to piperacillin (table 7). Resistance to piperacillin-tazobactam is still relatively low (table 7). Concerning cephalosporins: resistance to cefuroxime is 10-13% in hospitals and increasing (table 7). Resistance to the oral newer cephalosporins, cefixime (11%) and ceftibuten (6%), is increasing in Urology Services, which may reflect use of these cephalosporins instead of the older ones. Resistance to 3rd and 4th generation is low, but showed also a slow increase over the study period. Carbapenem resistance is still very exceptional, but the number of ESBL producing strains is increasing in all populations.

Aminoglycoside (gentamicin and tobramycin) resistance is 5% or less in hospitals except in Urology Services, where a 7-8% resistance was found (table 7).

It appeared that aminoglycoside resistance in Intensive Care units was mostly a local problem and these figures do not reflect a general situation in The Netherlands.

Trimethoprim- and co-trimoxazole resistance was the highest in strains from Urology Services, Outpatient Departments and selected GP patients (table 7). These strains were isolated from urine from patients with a (history of) a urinary tract infection. It is clear that these high resistance rates are result of previous treatment. Resistance of both drugs was decreasing in Intensive Care Units and the community. Both drugs are not routinely used in Intensive Care Units and the decrease in the community may be the result of the change in the Dutch Standard for treatment of urinary tract infections in 2005 when trimethoprim was replaced by nitrofurantoin as a first choice. The continuing high resistance level of trimethoprim and co-trimoxazole make these drugs unsuitable for empiric therapy.

Table 7. Resistance levels (%) in Escherichia coli in various patients populations in 2009.

Antibiotic	Community	Selected GP Patients	Outpatients Departments	Unselected Hospital Departments	Intensive Care Units	Urology Services
amoxicillin	34	44	48	47	47	53
co-amoxiclav	12	19	22	21	29	30
piperacillin				33	40	45
pip-tazobactam					3	3
carbapenem				0	0	0
cefuroxime				12	13	10
cefotaxime/ceftriaxone				5	6	5
ceftazidime				4	2	3
ceftibuten					6	6
cefixime					11	11
cefepime					4	3
gentamicin	3			5	4	7
tobramycin				5	4	8
amikacin				1	0	0
trimethoprim	19	30	34	29	25	37
co-trimoxazole	17	28	31	27	24	35
norfloxacin	4	10	17	12	14	25
ciprofloxacin	4	10	17	12	11	25
levofloxacin					11	24
moxifloxacin					12	25
nitrofurantoin	1	6	8	5	1	3
fosfomycin	0	0,3	0,2			

increasing
stable
decreasing

Quinolone resistance was (again) the highest in Urology Services and Outpatient Departments. Quinolone resistance is significantly higher in selected GP patients than in GP patients from the community (table 7), which may indicate that selected GP patients have been treated before for their UTI. The high resistance levels in Outpatient Departments and hospital departments make quinolones unusable for empiric treatment.
A matter of concern is the increasing multiresistance in Intensive Care Units and Urology Services, which leads to limited treatment options.

4.3.1.2 Klebsiella pneumoniae

Co-amoxiclav resistance was either stable or slightly increasing among the different patient populations, and was highest in Intensive Care Units (24%), whereas the levels in the other patient groups were in the same range (12-15%). Increasing resistance was also found for piperacillin-tazobactam. Carbapenem resistance remained exceptional.
Resistance to most cephalosporins increased slowly, but the resistance levels are still below 10%, except for cefuroxime and cefixime. Carbapenem resistance remains exceptional.
Special attention is needed for the high level of aminoglycoside resistance, which is 10-14% in Intensive Care Units and increasing in Unselected Hospital Departments.
Co-trimoxazole (15-25%) and trimethoprim resistance (18-34%) is high in all patient populations and still increasing. Therefore co-trimoxazole is unsuitable for empiric therapy for infections by *Klebsiella* spp. Increasing resistance was also recorded for all quinolones in all study groups, with the highest levels in Intensive Care Units (26% norfloxacin resistance) and Urology Services (23% norfloxacin resistance). A matter of concern is the increasing resistance to fosfomycin in selected GP patients (9% in 2010) and Outpatient Departments (12% in 2010); this may reflect frequent use of this drug as an alternative therapy for UTI after failure of a 1st choice drug like nitrofurantoin and trimethoprim or co-trimoxazole, for which indeed a high resistance level was found.
Multiresistance and appearance of ESBL producing strains are increasing in Intensive Care Units, although not in all, but need careful attention.

4.3.1.3 Proteus mirabilis

Amoxicillin resistance was stable and high in all study groups (25-31%); in the last decade amoxicillin was replaced by co-amoxiclav for complicated UTI and this may have led to the 9-12% resistance we find now in selected GP patients, Outpatient Departments and patients in Unselected Hospital Departments.
Alternative antibiotics for complicated UTI are quinolones and fosfomycin and here we observed an increasing resistance pattern as well, which was fastest for fosfomycin. The latter cannot be advised anymore for treatment of UTI by *P. mirabilis*. Alternatives for strains resistant to these "first choice" drugs are cephalosporins.

4.3.1.4 Enterobacter cloacae

The difference in resistance levels between Unselected Hospital Departments (figure 23) and Intensive Care

Units (figure 47) is striking and suggestive for emergence and existence of resistant and circulating clones in Intensive Care Units, which is known for this organism. Therefore local surveillance is needed to get insight in the resistance patterns dealing with and to make a correct choice for therapy. For incidental infections by *E. cloacae* in Unselected Hospital Departments co-trimoxazole or a quinolone may be still justified. Cephalosporins should be avoided in *E. cloacae* infections clinically because of their resistance-inducing and -selecting character.

4.3.1.5 Pseudomonas aeruginosa
Resistance levels to all antibiotics tested were the highest in Intensive Care Units; this was most frequently due to circulation of resistant clones in a limited number of centers, which underlines again the value of local surveillance. In general, aminoglycoside resistance remained low (<= 7%) in all study groups. Except for Intensive Care Units (25% resistance) ciprofloxacin resistance was 10-12% in all study groups including Urology Services. *Pseudomonas aeruginosa* from urine is always from a secondary or a complicated infection and this is typical for the groups of selected GP patients, Outpatient Departments and Urology Services from which urine has been sent in for identification and resistance of the pathogen. Unfortunately no alternative but parenteral antibiotics in Pseudomonas infection can be advised in case of quinolone resistance, although intramuscular tobramycin is sometimes used.

4.3.1.6 Staphylococcus aureus
The findings in Unselected Hospital Departments and Intensive Care Units show a similar low percentage of MRSA (1.6% in Unselected Hospital Departments in 2010). MRSA is well controlled and therefore not a big problem in Dutch hospitals. Macrolide resistance has increased to 10-11%. This is a matter of concern since a macrolide is the alternative for patients with known penicillin allergy. Glycopeptide resistance is exceptional.

4.3.1.7 Staphylococcus epidermidis
Infections by this organism cannot be treated without determination of the resistance pattern of the isolate itself, since they are derived from the patient itself or from circulating clones in the hospital wards. Circulating clones are often highly resistant to many antibiotics. Comparison of resistance patterns from various departments is therefore not meaningful. A common finding in the Dutch hospitals is a low resistance level to vancomycin and teicoplanin.

4.3.1.8 Streptococcus pneumoniae
No significant differences in resistance levels for penicillin, macrolides and doxycycline could be observed between strains isolated from Pulmonology Services or Unselected Hospital Departments. Macrolide- and doxycycline resistance was around 10% in these wards, which is a matter of attention in terms of use as empiric therapy. Co-trimoxazole may be a useful alternative.

4.3.1.9 Haemophilus influenzae
Resistance levels for amoxicillin, co-amoxiclav and co-trimoxazole among *H. influenzae* from patients from Pulmonology Services (figure 75) were consistently higher than those found in Unselected Hospital Departments (figure 29). In contrast, 90% resistance to erythromycin was reported from Unselected Hospital Departments compared to 8% determined for clarithromycin from Pulmonology Services. This discrepancy must be the result of application of different breakpoints. When taking all strains not susceptible for erythromycin (MIC > 0.5 mg/l) for resistant (ISIS-AR) and compare this with the outcome of the quantitative method quantitative (SIRIN) where the real breakpoint for resistance is used (MIC > 16 mg/l for erythromycin and > 32 mg/l for clarithromycin), one can expect big differences in interpretation.

4.3.1.10 Moraxella catarrhalis
Resistance levels of amoxicillin and co-trimoxazole appeared higher in Unselected Hospital Departments (figure 30) than in Pulmonology Services (figure 78).

4.4 Resistance among anaerobic pathogens

The susceptibility of clinical strains of anaerobic bacteria from patients hospitalized in the University Medical Center Groningen was determined by E-test. Strains of *Bacteroides fragilis* sp. (N=69), *Prevotella* sp. (N=32), *Fusobacterium* sp.(N=10), *Bilophila* sp.(N=8), *Clostridium* sp. (excl. *C. difficile*, N=15), gram-positive anaerobic cocci (GPAC, N=42) and *Propionibacterium* sp. (N=39) were tested. Antibiotics used were amoxicillin, co-amoxiclav (only for Gram-negative anaerobes), clindamycin and metronidazole. Breakpoints for resistance were derived from EUCAST.

4.4.1 Gram-negative anaerobes
Amoxicillin resistance was recorded in 88% of *B. fragilis* sp., all *Bilophila* sp., 22% of *Prevotella* sp. and 10% of *Fusobacterium* sp. (table 8). The MIC distribution of amoxicillin for *B. fragilis* sp. was bimodal with one subpopulation with MICs 2-64 mg/l (peak at 16 mg/l) and another subpopulation with MIC > 256 mg/l (figure 79). The MIC distributions of *Prevotella* sp., and *Fusobacterium* sp. for amoxicillin were unimodal with MICs over a broad range (MIC < 0.02 - 128 mg/l); that of *Bilophila* sp. was also unimodal with MIC range 16-64 mg/l. Co-amoxiclav resistance was not found among B. *fragilis* sp., *Prevotella* sp., *Fusobacterium* sp. and *Bilophila* sp., which indicates that resistance among these species is completely based on beta-lactamase production.

Table 8. Resistance among Gram-negative anaerobic bacteria.

Species (N)	Antibiotic resistance N (%)			
	Amoxicillin	Co-amoxiclav	Clindamycin	Metronidazole
Bacteroides fragilis sp (68-69)	61 (88%)	0	10 (14%)	0
Prevotella sp (29-32)*	7 (22%)	0	1 (3%)	0
Fusobacterium sp (10)	1 (10%)	0	0	0
Bilophila sp (7-8)*	7	0	1 (13%)	0

* not all strains were tested for all antibiotics

The MIC distributions for co-amoxiclav were unimodal for the species tested with MIC range 0.03-4 mg/l for *B. fragilis* sp. and 0.03-2 mg/l for *Prevotella* sp. (figure 79). Clindamycin resistance was found in 10 of 69 strains of *B. fragilis* sp. and one of eight strains of *Bilophila* sp. tested. The MIC distribution for clindamycin showed a bimodal shape for *B. fragilis* sp. with one subpopulation with MIC 0.015-16 mg/l (peak at 1 mg/l) and one subpopulation with MIC > 256 mg/l (figure 80). The MIC distribution of *Prevotella* may be assessed as bimodal also: 30 strains had MIC 0.015-0.5 mg/l and one strain with MIC > 256 mg/l was found. The numbers of strains tested are too low to make a definite statement. All strains of *Fusobacterium* sp. and seven of eight *Bilophila* sp. strains were susceptible to clindamycin. Metronidazole resistance was not found; the MIC distribution of metronidazole for *B. fragilis* sp showed a unimodal shape over a broad range (MIC 0.06-4 mg/l), with a small number of strains near the breakpoint for resistance (4 mg/l).

4.4.2 Gram-positive anaerobes

Amoxicillin- and co-amoxiclav resistance was not observed in the strains of *Clostridium* sp, GPAC and *Propionibacterium* sp. tested. MIC distributions showed that all strains tested were susceptible to 0.5 mg/l or less of amoxicillin (figure 81).

Two out of 14 strains of *Clostridium* sp (14%), 10% of GPAC and 3% of *Propionibacterium* sp. were resistant to clindamycin. The MIC distribution of *Clostridium* sp was spread over a broad range (0.03-32 mg/l), those of GPAC and *Propionibacterium* sp were bimodal with one susceptible subpopulation (MIC < 4 mg/l and one resistant strain with MIC > 256 mg/l (figure 82). The resistant strains of GPAC were identified as *Finegoldia magna*. All strains of *Clostridium* sp and GPAC were susceptible to metronidazole.

Conclusion

1. All anaerobic bacteria tested were susceptible to co-amoxiclav and metronidazole.
2. Clindamycin resistance was found in 9% of Gram-positive and Gram-negative anaerobic bacteria. Amoxicillin can only be used as a drug for infections by Gram-positive anaerobes.

4.5 Surveillance studies on bacterial pathogens isolated in The Netherlands

Apart from the surveillance data presented in NethMap on the basis of the surveillance system developed by SWAB, several individual studies by other authors have reported on the occurrence of antimicrobial resistances

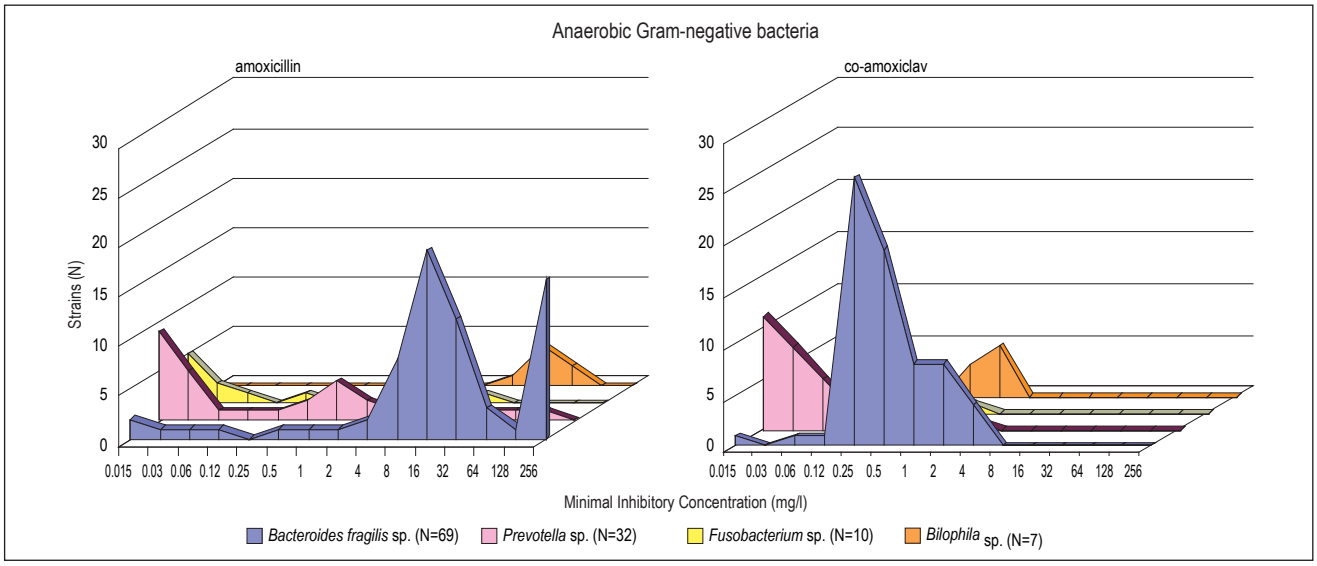

Figure 79. MIC distribution of amoxicillin and co-amoxiclav for clinical strains of anaerobic Gram-negative bacteria (N= 111).

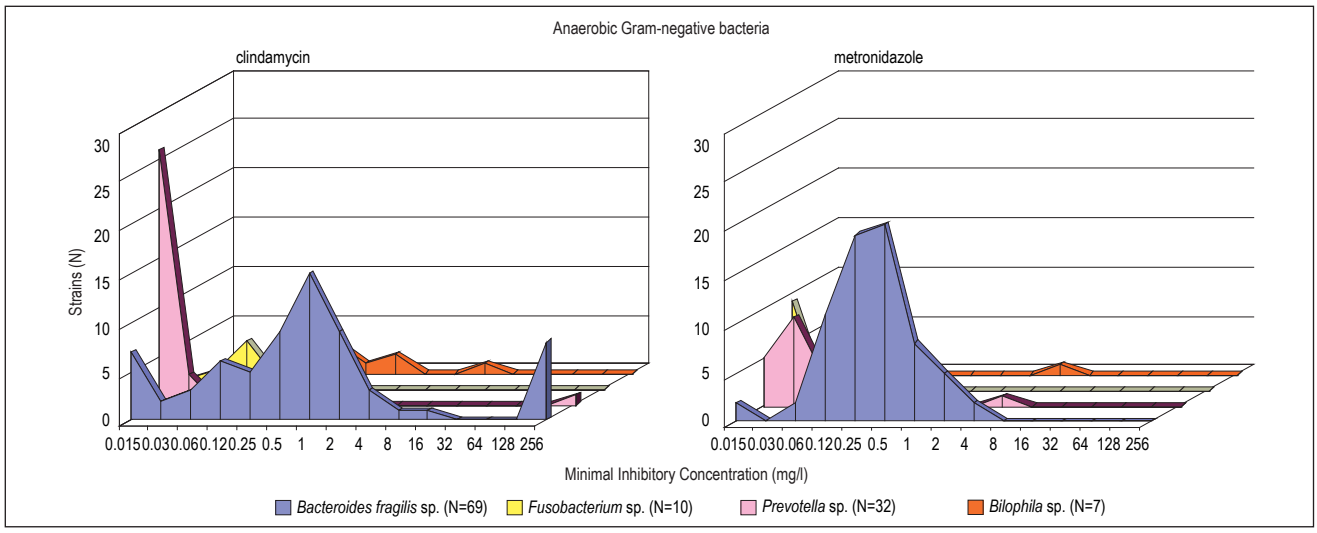

Figure 80. MIC distribution of clindamycin and metronidazole for clinical strains of anaerobic Gram-negative bacteria (N= 111).

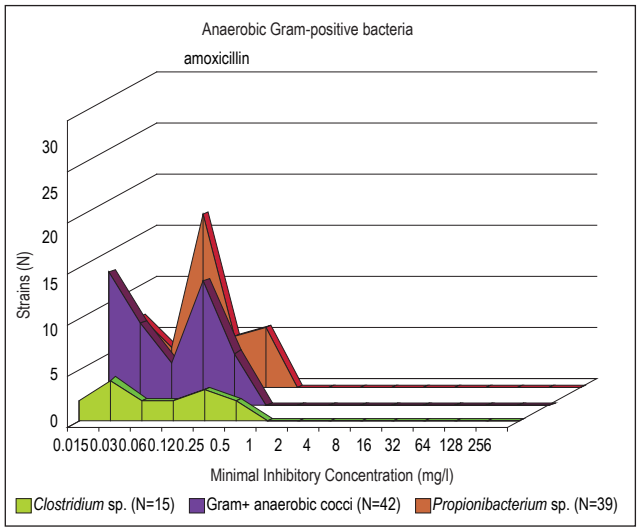

Figure 81. MIC distribution of amoxicillin for clinical strains of anaerobic Gram-positive bacteria (N= 106).

among various bacterial species in The Netherlands. These studies were selected for inclusion in NethMap if they met the following criteria: (1) all studies reported on resistance rates based on the measurements of MICs, i.e. quantitative susceptibility tests were performed on all strains; (2) all strains were collected from patients in multiple centres throughout The Netherlands and (3) the studies were reported in peer-reviewed journals, listed in the Medline database. Individually, and taken together these studies provide further insight into the prevalence and emergence of antimicrobial resistance among medically important micro-organisms in The Netherlands. In addition to the list of studies readers are helped by a cross table (table 9) that reveals the combinations of "bugs & drugs" for which data were reported in each of the listed studies.

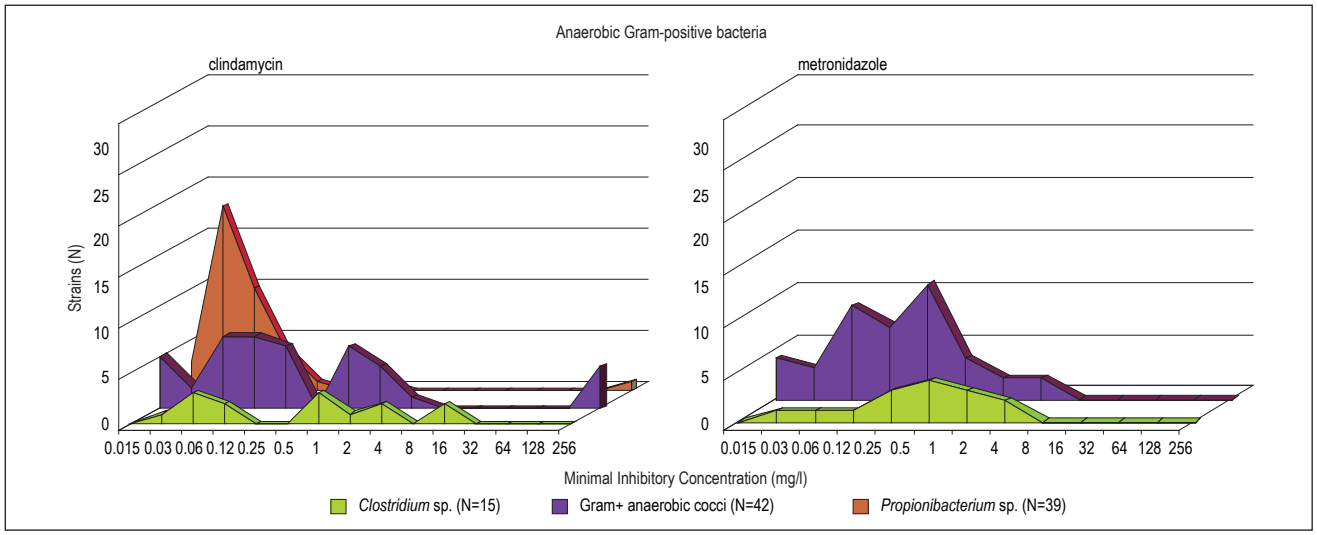

Figure 82. MIC distribution of clindamycin and metronidazole for clinical strains of anaerobic Gram-positive bacteria (N= 106).

Table 9. Cross table of combinations of species of bacteria and antibiotics for which MIC data are presented in the individual studies listed above.

	Staphylo cocci	*Strepto cocci*	*Pneumo cocci*	*Entero-cocci*	*Entero-bacte-riaceae*	Non-ferm Gram-bacteria	*Haem-philus influenzae*	*Helico-bacter pylori*	*Meningo cocci*	*Gono cocci*
Penicillin	7,8,11,48	8,11	4,6,7	7					4,6	
Oxacillin	7									
Methicillin	3,40,41, 43,44,45, 48									
Flucloxacillin	8,11,44,45									
Ampicilin				3	1,25,33	1	4			
Amoxicillin		8,11	7	7,8,11,16, 20,29	20,21,22, 32,35,36, 46,47			15		
Co-amoxiclav			10		1,7,22, 32,33,35, 36,46,47	1,7	7			
Piperacillin	3			3	1,3,17, 35,36	1,3,36				
Piperacillin/tazobactam	3,7		7	3,7	1,3,17, 35,36	1,3,36	7			
Ticarcillin/clavulanate	3			3	1,3,7	1,3,7	7			
Mezlocillin					1	1				
Cefaclor					37					
Cefazolin					1,20,21,25	1				
Cefoxitin					17					
Cefuroxime	11	11			1,7,36,49	1,7	7			
Ceftriaxone			4,6		1	1	4		4,6	
Cefotaxime		11			1,7,17, 31,36	1,7,32	2			
Ceftazidime					1,3,7,17, 22,36,49	1,3,7, 22,36	2			
Cefpirome				16	17					
Cefepime					17					
Cefixime					37					
Ceftibuten					37					
Aztreonam					1	1				
Imipenem	3,7,12	12	7,12	3,7,2,16	1,3,7,22	1,3,7,22,36	2			
Meropenem	7,12	12	7,12	7,12,16	7,17,49	7,36	7			
Vancomycin	7,8,11,12	8,11,12	7,12	7,8,11,12, 16,20,29						
Teicoplanin	8,11,12	8,11,12	12	8,11,12,16						
Linezolid	19	19	19							
Gentamicin	3,7,44,45		7	7,11,16, 20,29	1,3,4,7, 17,22,20, 21,25,36, 49	1,3,7,22,36	7			
Tobramycin					1,17	1,36				
Netilmicin					17					
Amikacin	3				1,3,17	1,3,36				
Norfloxacin					22,32,35, 33,46,47	22				

Table 9. Cross table of combinations of species of bacteria and antibiotics for which MIC data are presented in the individual studies listed above (continued).

	Staphylo cocci	*Strepto cocci*	*Pneumo cocci*	*Entero-cocci*	*Entero-bacte-riaceae*	Non-ferm Gram-bacteria	*Haem-philus influenzae*	*Helico-bacter pylori*	*Meningo cocci*	*Gono cocci*
Ciprofloxacin	2,3,7,8,12	2,8,12	2,7,10,12,	2,3,8,7, 12,16,20, 29	1,2,3,7, 22,20,21, 25,35,36, 46,47	1,2,3,7, 22,36	2,7,10			42
Ofloxacin	2,8	2,8	2	2,8,16	2,17	2	2			
Levofloxacin					35					
Trovafloxacin	8	8		8,16				15		
Sparfloxacin	8,12	8,12	10,12	8,12,16			10			
Pefloxacin	8	8		8						
Moxifloxacin				16	35					
Clindamycin	7,11,12	11	7	7,11						
Erythromycin	7,11,12	11,12,30	7,12	2,7,11,12, 20,29						
Clarithromycin	11,48	11,12,34	10,12	11,12			10	5,15		
Tetracyclin			20,29	20,29	20,21,25			15		42
Minocyclin				11						
Chloramphenicol			4,6	16	20,25		4		4,6	
Quinupristin/dalfopristin	11,12	11,12	12	2,11,12						
Rifampicin	11,12	12	12	12					4,6	
Metronidazole								5,13,15		
Trimethoprim					20,21,22, 25,32,33, 35,46,47					
Co-trimoxazole					2,32,35, 46,47,49					
Nitrofurantoin					20,22,32, 33,35,47					
Fosfomycin					46					
Fusidic acid	48									

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5 ESBL and Carbapenemase in the Netherlands

M.F.Q.Kluytmans-Van de Bergh

In the Netherlands antimicrobial resistance rates have traditionally been low as compared to most other countries (1). However, recent data suggest that the Dutch situation is deteriorating, in particular with respect to the Gram-negatives (2, 3).
Antimicrobial resistance in Gram-negatives is rapidly increasing worldwide and treatment options are becoming scarce (4). This increase pertains not only to the number of individuals that are infected or colonised with antimicrobial resistant micro-organisms, but also to the diversity of underlying resistance mechanisms (5, 6). Resistance may be mediated by several mechanisms, but the most serious threat is currently posed by plasmid-encoded resistance to beta-lactam antibiotics, such as extended-spectrum beta-lactamases (ESBL), carbapenemases and AmpC beta-lactamase (7).
Recent Dutch publications on the emergence of ESBL-producing *Enterobacteriaceae* in both healthcare and community settings are alarming. A surveillance in four Dutch hospitals showed that 4% of the patients were colonised with ESBL-producing *Enterobacteriaceae* on admission to the hospital (8). Moreover, two Dutch studies have found livestock, especially poultry, to be heavily colonised with ESBL-producing *Enterobacteriaceae*, and demonstrated that similar strains could be cultured from retail meat (9, 10). A recent study of Dutch soil samples collected from 1940 to 2008 indicated that the number of resistance genes, including genes encoding for ESBL, is steadily increasing (figure) (11). The presence of resistance genes in agricultural soil constitutes an important risk for contamination of other food sources, such as fruit and vegetables (12). With the presence of resistance genes in the food chain, exposure to antimicrobial resistance is no longer limited to the healthcare setting but has extended to the community.
Recently, the first Dutch patients that were infected with carbapenemase-producing *Enterobacteriaceae* have been reported, in all cases related to foreign travel or hospitalisation (13, 14). The emergence of carbapenemase-producing *Enterobacteriaceae* almost immediately followed the worldwide increase in ESBL-producing *Enterobacteriaceae*, and has been directly related to the increased use of carbapenems for the treatment of infections with ESBL-producing *Enterobacteriaceae* (7). Carbapenemase-producing *Enterobacteriaceae* constitute a major threat to public health, as these bacteria are resistant to almost all beta-lactam antibiotics, including cephalosporins and carbapenems.
The rapid emergence of ESBL- and carbapenemase-producing *Enterobacteriaceae* asks for drastic measures in antibiotic policy, not only in human medicine, but also in intensive livestock. Furthermore, unambiguous guidelines on the laboratory detection, treatment and prevention of infections with ESBL- and carbapenemase-producing *Enterobacteriaceae* are urgently needed.
The implementation of rapid and accurate laboratory detection methods is crucial in the prevention of nosocomial transmission of resistant bacteria within healthcare settings, and in the timely administration of appropriate antimicrobial therapy.

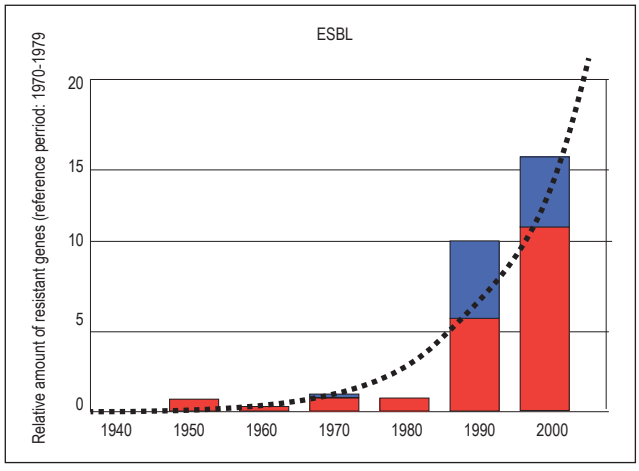

Figure. Relative increase in beta-lactamase-genes (blaTEM (red) and blaSHV (blue) in Dutch soil samples collected from 1940 – 2008 on five different locations. Values are normalised to the amount of 16S rRNA-genes. De normalised values are grouped per 10-year period and are related to the average amount in the period 1970-1979. Adapted from Knapp et al. (11).

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6 Antifungal resistance

For many years species identification was the most important diagnostic tool for treatment decisions in invasive candidiasis. In the era of fluconazole, the speciation of Candida was important as *Candida glabrata* was known to be less susceptible to fluconazole and *C. krusei* resistant. At that time in vitro susceptibility testing was not routinely performed and interpretative breakpoints were not available. In the past years much effort has been made to optimize methods for in vitro susceptibility testing and in determining breakpoints, using a systematic approach that takes into consideration pharmacodynamic and pharmacokinetic parameters. For Candida and Aspergillus, the most frequent opportunistic fungi, both the CLSI and EUCAST have established reference methods (1,2). Harmonization of the methods has resulted in relatively comparable techniques and similar interpretative breakpoints (3,4).

For invasive candidiasis it has been shown that timing of the antifungal therapy as well as treating with an appropriate drug at an adequate dose is directly related to treatment outcome (5,6). It is therefore important to know the epidemiology of infections due to Candida and to establish if acquired resistance is an issue.

We investigated the epidemiology of blood stream infections due to yeasts in the University Medical Center St Radboud on the basis of laboratory records of yeasts cultured from blood cultures. Between 2002 and 2010, 427 yeasts were cultured from the blood. Identification showed that 95.8% of positive blood cultures with a yeast was caused by Candida species. The majority was caused by *C. albicans* with *C. glabrata* as second most frequent species (table 1).

In vitro susceptibility testing was performed on most isolates. In table 2 the distribution of MICs are shown for *C. albicans* of fluconazole (151 isolates), voriconazole (146 isolates) and caspofungin (114 isolates). The concentrations that are currently considered resistant are indicated in red. The percentage of resistant isolates remains very low: only one of 151 isolates was resistant to fluconazole (0.7%), which is consistent with the consistently low prevalence reported from other countries (7). No resistant isolates were found for voriconazole, while four isolates were tested resistant of caspofungin (3.5%). However, the percentage of caspofungin resistance may be unreliable and anidulafungin was found to best distinguish between non-wild type and wild type isolates (8). The results of anidulafungin MIC-testing can be extrapolated to the other two clinically licensed echinocandins, caspofungin and micafungin. Overall, the percentage of acquired resistance in invasive *C. albicans* isolates remains low.

The MIC distribution of fluconazole, voriconazole and caspofungin for *C. glabrata* compared with *C. albicans* is shown in the figure. The MIC distribution of fluconazole is shifted to the right with a modal MIC of approximately four two-fold dilutions higher for *C. glabrata* compared with *C. albicans*. Voriconazole appears more active, but some isolates were cross resistant to this drug and fluconazole. It is generally not recommended to treat invasive *C. glabrata* infections with azoles, especially fluconazole, because of the high probability of treatment failure. Echinocandins are favored in *C. glabrata* infection, and the MIC distribution is similar to that of *C. albicans* (9).

Aspergillus species

Unlike with Candida, resistance is an emerging problem in *Aspergillus fumigatus*. This is by far the most frequent cause of Aspergillus diseases in the Netherlands, representing over 90% of Aspergillus infections. In the Netherlands resistance to medical triazoles has emerged since 1998 and is now wide-spread (10). Azole resistance was found in all Dutch University Medical Centers with a prevalence ranging between 0.8% and 9.5% (11). Azole resistance was found to be caused by changes in the Cyp51A-gene, which is the target for antifungal azoles. A combination of changes, a substitution at codon 98 (L98H) in combination with a 34 base-pair tandem repeat in the gene promoter region (TR34/L98H) was found to be a highly prevalent resistance mechanism (10). Evidence is accumulating that this resistance mechanism has developed in our environment through the use of azole fungicides (12). *A. fumigatus* isolates harboring the TR34/L98H resistance mechanism were also recovered from the environment (13). Furthermore, the majority of azole resistant isolates were recovered from patients who had not received azole therapy within the previous three months (11).

It was suggested that continued azole pressure in the environment may cause new resistance mechanisms to emerge. Very recently, a new resistance mechanism was identified through a surveillance network that involves clinical microbiology laboratories of seven University Medical centers (14). The first isolate was recovered in Utrecht in December 2009. The isolate showed an atypical resistance profile, with no activity of voriconazole (MIC >16 mg/l), and attenuated activity of itraconazole and posaconazole. This phenotype was associated with two mutations in the Cyp51A-gene and a 46 base pair tandem repeat in the gene promoter region. Within one year isolates harboring this resistance mechanism were found in five University Medical Centers, indicating rapid migration across the Netherlands. Isolates harboring the same resistance

Table 1. Identification of 427 consecutive blood cultures positive with yeasts. The blood cultures were obtained between 2002 and 2010 from patients admitted to the Radboud University Nijmegen medical Center.

Species	Number of isolates (%)
C. albicans	257 (60,1%)
C. glabrata	51 (11,9%)
C. parapsilosis	44 (10,3%)
C. tropicalis	18 (4,2%)
C. guilliermondii	16 (3,7%)
C. krusei	9 (2,1%)
C. dubliniensis	7 (1,6%)
Other *Candida species*	7 (1,6%)
Cr. neoformans	11 (2,6%)
Other yeasts	7 (1,6%)

Table 2. The distributions of MICs of fluconazole (FCZ), voriconazole (VCZ) and caspofungin (CAS) for Candida albicans blood stream isolates

MIC	Number of isolates		
mg/l	FCZ	VCZ	CAS
<0,016	0	128	1
0,03	3	12	0
0,06	0	4	26
0,125	38	1	44
0,25	69	1	28
0,5	31	0	11
1	8	0	2
2	1	0	2
4	0	0	0
8	1	0	0
16	0	0	0
Total	151	146	114

resistant
intermediate
susceptible

mechanism have been recovered from the environment, indicating that this resistance mechanism has emerged through the environmental route, similar to TR34/L98H. Lack of activity of voriconazole, is a major problem as this drug is currently first choice treatment option in invasive aspergillosis.
The emergence and migration of a second resistance mechanism in *A. fumigatus* through environmental use of azole compounds is alarming and requires reassessment of the use of triazole compounds in the environment. Persistent azole pressure will continue to cause new resistance mechanisms to emerge. The acquisition of azole resistance does not to be associated with a significant fitness costs as resistant isolates cause Aspergillus diseases in humans. Furthermore, TR34/L98H has been able to survive in the environment for over 13 years in competition with wild type isolates, which would not have been possible if TR34/L98H are less virulent. Similar to phytopathogenic fungi, resistance will migrate through airborne spores, resulting in a resistance problem of global proportions.
Research is urgently warranted that enables evidence-based interventions to be made that prevent the emergence and migration of azole resistance in *A. fumigatus*.

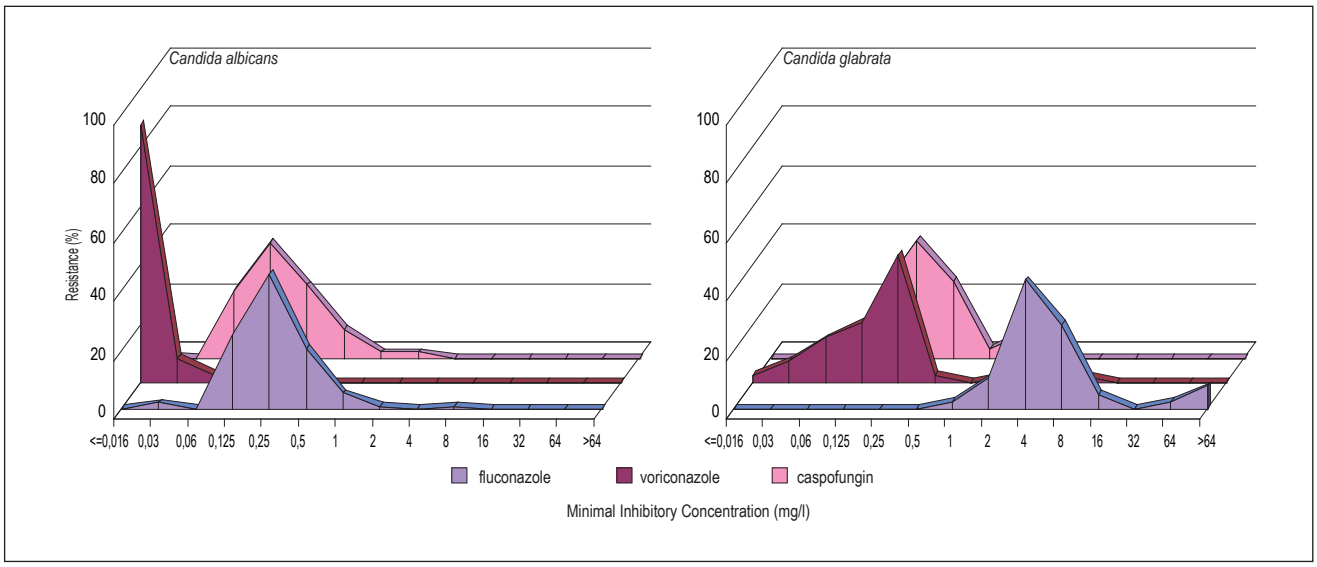

Figure. Comparative MIC distributions of *C. albicans* and *C. glabrata* cultured from blood cultures.

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7 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 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) 2009 pandemic 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 (figure). Before the A(H1N1) 2009 pandemic, highest prescription of 6,641 courses oseltamivir was noted in October 2005 (figure), possibly due to personal stockpiling in response to the emergence of highly pathogenic avian influenza A(H5N1) in Turkey. During the first wave in the summer of 2009 of the A(H1N1) 2009 pandemic, oseltamivir was widely prescribed for therapy and prophylaxis on indication fitting the case definition, mainly to limit the spread of the pandemic virus (figure). During the epidemic phase in late 2010, oseltamivir was used mainly for treatment of severe cases (figure). However, a substantial amount of prescriptions as precaution cannot be excluded (5). After the pandemic prescriptions dropped to levels seen before the A(H1N1) 2009 pandemic (figure). For the 2010/11 season prescription data were not available at the time this report was written. In Europe the number of prescriptions of NAI by country is in general low, but the Netherlands is among the lowest (6).

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 Netherlands Institute for Health Services Research (NIVEL) 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.

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 (7-10). For NAIs the IC50 can also be determined using an enzyme inhibition assay (11, 12). 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

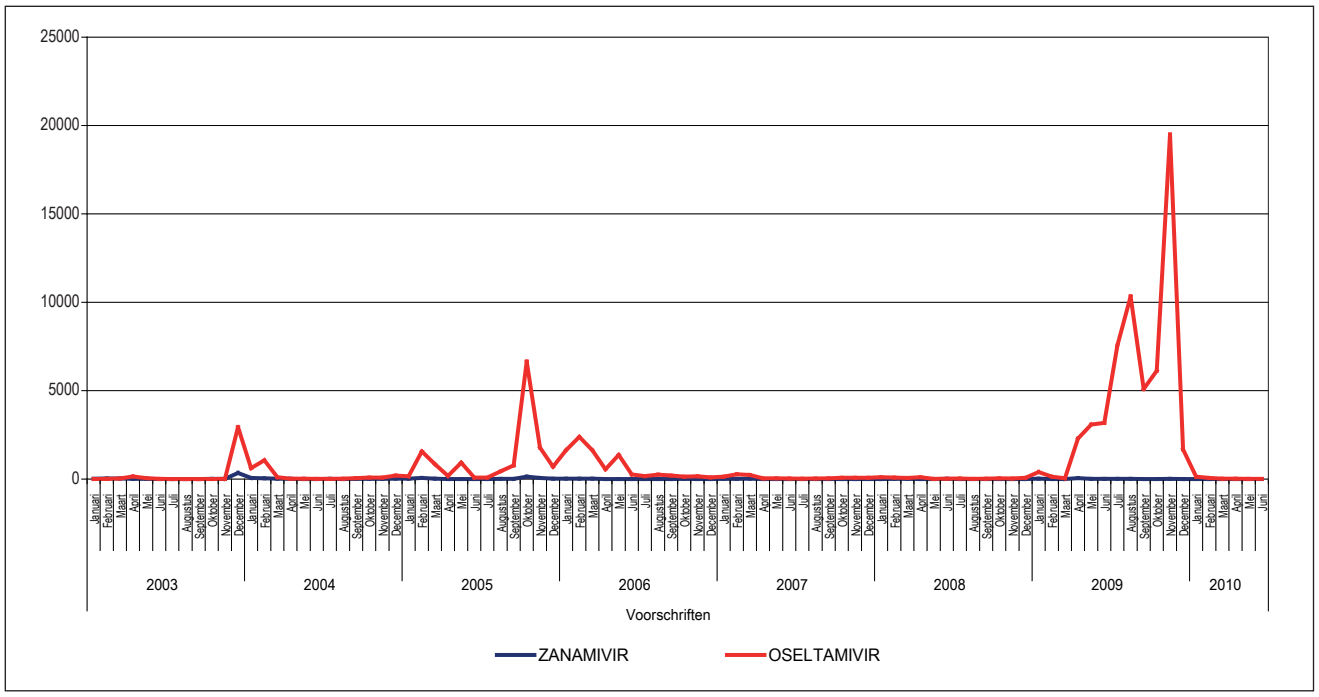

Figure. Monthy prescription data for zanamavir and oseltamivir for The Netherlands 2003-2010. Source: Stichting Farmaceutische Kengetallen, Den Haag, the Netherlands for commercial prescriptions and the Dutch Vaccine Institute, Bilthoven. The Netherlands.

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 (4,13). In addition, the emergence of oseltamivir resistant A(H1N1) viruses during the 2007/2008 season was described. Preliminary data for the 2009/2010 season in the Netherlands in our 2010 SWAB report have been supplemented with additional data (14). However, the overall pattern did not change (table). During the A(H1N1) 2009 influenza pandemic in the Netherlands enhanced molecular influenza surveillance was implemented in which a total of 1219 clinical specimens were sequenced, yielding 1096 neuraminidase (NA) sequences, 941 hemagglutinin (HA) sequences and 1026 PB2 sequences. The success of direct sequencing from the clinical specimens was directly correlated with the viral load. The sequence success rate decreased dramatically with viral load of < 3000 viral particles per ml, corresponding with ct-values of >35 in the matrix gene real-time RT-PCR. At higher viral loads, >77% of all clinical samples could be fully sequenced, while at lower viral loads only 10% could be sequenced directly. A total of 19 cases with the H275Y resistance amino acid substitution in the neuraminidase were detected, 18 of them associated with oseltamivir therapy. By phylogenetic analysis of combined HA, NA and PB2 sequences using a Maximum Parsimony planar network, no (person-to-person) spread of antiviral resistant virus variants with potentially increased public health risk was detected. Moreover, the absence of detection of resistant variants neither in community specimens nor in specimens from contacts of cases with resistant virus suggested that the transmissibility of the resistant virus was affected by the H275Y mutation. In addition to molecular data, the neuraminidase inhibition assay was performed on A(H1N1) 2009 virus representatives for the whole pandemic period and compared with seasonal A(H1N1) virus isolates from the latest season in which seasonal A(H1N1) viruses were dominant in the Netherlands (2007/2008) (13). A(H1N1) 2009 viruses appeared to be 1.4-fold more susceptible for oseltamivir and 2.0-fold more susceptible for zanamivir than the seasonal viruses. Although only one resistant A(H1N1) 2009 virus isolate was available for phenotypic testing, this resistant virus was 2.9-fold more susceptible, when compared to the mean IC50 of oseltamivir resistant seasonal A(H1N1) viruses harboring the same H275Y amino acid substitution. The A(H1N1) 2009 influenza viruses thus far analysed from the 2010/2011 season were all susceptible for the NAI (table).

All the A(H1N1) 2009 influenza viruses from the 2008/2009, 2009/2010 and the 2010/2011 season of which the M2 gene was sequenced had the S31N amino acid substitution characteristic for resistance against the M2 blockers (table).

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) 2009 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 (15).

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

Season	A(H3N2)		A(H1N1) seasonal		A(H1N1) 2009		B
	NAI	M2B	NAI	M2B	NAI	M2B	NAI
2005/2006	1/39 (3%) (2)	29/39 (74%)	NA	NA	NA	NA	2/48 (4%) (3)
2006/2007	0/50	38/51 (75%)	0/5	0/6	NA	NA	0/3
2007/2008	0/10	12/12 (100%)	47/172 (27%) (4)	0/49	NA	NA	1/81 (1%) (2)
2008/2009	5/74 (7%) (5)	8/8 (100%)	5/5 (100%)	ND	0/492	8/8 (100%)	0/19
2009/2010	ND	1/1 (100%)	NA	NA	20/627 (3%) (6)	54/54 (100%)	NA
2010/2011 (7)	NA	NA	NA	NA	0/54	24/24 (100%)	0/8

(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 IC50 for oseltamivir and mild outlier IC50 for zanamivir.

(3) Both resistant viruses had outlier IC50 values for oseltamivir as well as zanamivir.

(4) Viruses resistant to oseltamivir only. Viruses were sensitive to zanamivir and M2Bs.

(5) The 5 viruses had mild outlier IC50 values for oseltamivir but normal IC50 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 IC50 for oseltamivir and a 5-fold increased IC50 for zanamivir.

(7) Preliminary data; analysis of the viruses from the 2010/2011 season is ongoing.

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) (13).

Conclusion

Emergence of oseltamivir resistance in A(H1N1) 2009 influenza virus, which circulated predominantly since April 2009 in the Netherlands, is still 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.

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8 Materials and Methods

8.1 Surveillance of antibiotic use in humans

Data on the consumption of antibiotics were collected by a pre-established protocol, using the ATC/DDD classification that is developed by WHO Collaborating Centre for Drug Statistics Methodology (http://www.whocc.no). The Defined Daily Dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. The DDD is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. It enables however comparison of drug consumption statistics at international and other levels (1). The 2009 update of the ATC/DDD classification system is used to calculate the number of DDDs in this report.

8.1.1 Primary health care

All antibiotics for human use are prescription-only medicines in The Netherlands. The majority of antibiotics are delivered to patients by community pharmacies. Direct delivery of medicines by general practitioners from their own pharmacy reaches approximately 8.4% of the Dutch population, mainly in rural areas (2).

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.
Sales data from approximately 90% of all community pharmacies are transferred monthly to SFK in an electronically format. The data are subsequently weighted statistically and extrapolated to cover 100% of the deliveries by community pharmacies. The total number of DDDs is divided by the total number of inhabitants that is registered by a community pharmacy (approximately 91.6% of the total number of inhabitants in The Netherlands). Data on the number of inhabitants in The Netherlands are obtained from Statistics Netherlands (CBS; http://www.cbs.nl).
SFK data on antibiotic use do not include the use of antibiotics in hospitals. Antibiotics prescribed by hospital based medical specialists to their outpatients are however included. Deliveries from community pharmacies to nursing-homes as an institute are not covered.

8.1.2 Hospitals

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. The number of admissions and the number of days spent in the hospital (bed-days) are also registered in the questionnaire. The use of antibiotics is expressed as DDD/100 patient-days and in DDD/100 admissions (3). The number of patient-days is calculated by subtracting the number of admissions from the number of bed-days to compensate for the fact that in the bed-days statistics both the day of admission and the day of discharge are counted as full days.

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8.2 Surveillance of antibiotic resistance and susceptibility testing

8.2.1 Community

8.2.1.1 Escherichia coli

The prevalence of antibiotic resistance among *E. coli* was determined for strains collected from patients visiting their general practitioner in communities of The Netherlands from January 2009 to December 2010. General Practitioners (N-42) from the NIVEL Sentinel Stations Network participated in the study for the recruitment of patients. The Network is nationally representative for age, gender, regional distribution and population density. The GPs collected urinary samples from male patients (>11 years) with symptoms indicative for a UTI in the absence of fever. Patients were excluded when they were catheterized, had urological or nephrological problems, diabetes mellitus or other immune compromising diseases.
A dipslide from a fresh urine sample was prepared according to the manufacturer's instructions and send by mail to the laboratory of Medical Microbiology of the Maastricht University Medical Centre (MUMC). The dipslides were considered positive when bacterial growth was observed of >102 cfu/ml. Dipslides showing growth of 2 or more bacterial species were excluded from the final analysis

Uropathogens were identified and the antimicrobial susceptibility of the *E. coli* isolates was determined quantitatively using a microbroth dilution method with Mueller Hinton II cation adjusted broth (Becton – Dickinson, Sparks, USA), an inoculum size of 5x105 cfu/ml and overnight incubation at 37C. The MIC plates were custom made and contained freeze dried antibiotics and had a guaranteed shelf live of > 1 year. (MCS Diagnostics, Swalmen , the Netherlands).
The following antibiotics (range in mg/ml) were tested: amoxicillin (0.06-128), co-amoxiclav (0.06-128),), trimethoprim (0.03-64), co-trimoxazole (0.03-64), norfloxacin (0.03-64), ciprofloxacin (0.003-16) and nitrofurantoin (0.5-512).
Escherichia coli ATCC25922 and 35218 were used as control strains. The breakpoints for resistance were according to the EUCAST guidelines. The susceptibility to fosfomycin was tested with Neo-Sensitabs (Rosco, Diagnostics, Denmark) and read according to the CLSI guidelines.
Co-amoxiclav resistant *E. coli* were assessed for the presence of ESBL production using the combination disc diffusion test with ceftazidime and cefotaxime with and without clavulanic acid according to the guidelines of the NVMM. Confirmation was by PCR.

8.2.1.2 Neisseria gonorrhoeae
In 2006 a project called Gonococcal Resistance to Antimicrobials Surveillance (GRAS) was implemented in The Netherlands. This surveillance project consists of systematically collecting data on gonorrhoea and standardised measurement of resistance patterns by using an E-test, linked with epidemiological data. Participants are STI clinics and associated laboratories that identify the majority of STI in high risk populations. Isolates are sent to the RIVM for further analysis. From July 2006 through December 2010, the susceptibility of *N. gonorrhoeae* from 4362 patients was determined by E-test. Resistance levels were calculated using both the breakpoints for resistance according to the EUCAST guidelines.

8.2.1.3 Neisseria meningitidis
From 1993-2010 The Netherlands Reference Laboratory for Bacterial Meningitis received isolates from CSF and / or blood of patients with meningococcal disease. These strains were submitted by 75 bacteriological laboratories distributed over the country. The susceptibility to penicillin was determined by the E-test method. Strains with MIC < 0.64 mg/l were recorded susceptible, with an MIC 0.125-0.25 mg/l intermediate and with an MIC >0.25 mg/l resistant.

8.2.1.4 Mycobacterium tuberculosis
The first isolate of *M. tuberculosis* of each patient with tuberculosis in The Netherlands is routinely sent to the RIVM for susceptibility testing and confirmation of identification. Isolates obtained after more than six months from the same patient, are judged a new isolate. The susceptibility of the strains is tested quantitatively with a standard agar dilution assay according to the recommendations of the CLSI. The antibiotics chosen for reporting are INH, rifampicin, streptomycin and ethambutol. Resistance rates represent the proportion of moderately and fully resistant strains.
The susceptibility data of 11705 strains, isolated from 1998-2010 are presented in this report.

8.2.2 Data reported to ISIS-AR
The overall prevalence of antibiotic resistance in hospitals was estimated from the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) dataset, based on routine antimicrobial susceptibility data obtained from laboratories in the Netherlands. ISIS-AR is coordinated by the Centre for Infectious Disease Control, at the National Institute for Public Health and the Environment (RIVM) in Bilthoven, the Netherlands, and collaborates with the Society of Medical Microbiology (NVMM). In 2007, the new surveillance system ISIS-AR replaced the old ISIS system that started in 1998 with collecting data. The new ISIS-AR collects next to antibiotic resistance data all epidemiological data present in the laboratory information systems. Furthermore, there is strong focus on the quality of data by national standardisation, structural quality control, and confirmation of unusual resistance data. The change to the new system also resulted in a change of the participating laboratories. In 2010, 21 laboratories reported results to ISIS-AR, two laboratories in academic hospitals and 19 laboratories serving non-academic hospitals and public health institutions. These laboratories provided ISIS-AR with data from 2008 onwards, which was used for the trend analysis.
The susceptibility of the isolates reported to ISIS-AR was routinely determined according to the standard techniques used in the individual laboratories. The majority of participating laboratories used automated systems for susceptibility testing, and used CLSI breakpoints, except for two laboratories using CRG (Dutch) breakpoints and two laboratories using EUCAST breakpoints. The S-I-R interpretation as reported by the local laboratory was used for calculating resistance percentages. We took the number of intermediate and resistant (I+R) isolates for *E. coli*, *P. mirabilis*, *K. pneumoniae*, *E. cloacae*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*, as these are identical to the R breakpoint of EUCAST for most antibiotics. This made it possible to compare the data of ISIS-AR with the results obtained from other databases, SERIN and SIRIN. Resistance percentages of *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, and *H. pylori* also included strains that showed intermediate and resistant isolates.
For analyses, the first isolate per species per patient in

Table 1. First isolates per clinical sample of patients in Unselected Hospital Departments in 2010.

	Blood	Lower respiratory tract	CSF	Urine	Wound	Total
Number	8704	9723	239	28485	17209	68621
Gram-positive cocci						
Staphylococcus aureus	997	2052	18	826	5175	9068
Coag neg Staphylococci	3201	50	127	830	1531	5739
Enterococcus spp	591	245	13	5002	1983	7834
Streptococcus pneumoniae	575	1499	39	4	141	2258
Streptococcus agalactiae	134	105	6	860	451	1556
Streptococcus pyogenes	124	47	1	20	365	557
Subtotal	5622	3998	204	7542	9646	27012
Enterobacteriaceae						
Enterobacter cloacae	155	632	1	640	719	2147
Escherichia coli	1942	1460	11	13351	3284	20048
Klebsiella oxytoca	129	421	1	725	394	1670
Klebsiella pneumoniae	385	698	2	2124	634	3843
Proteus mirabilis	141	363	0	2329	802	3635
Subtotal	2752	3574	15	19169	5833	31343
Respiratory pathogens						
Haemophilus influenzae	48	3010*	3	3	137	3201
Moraxella catarrhalis	11	1137*	1	0	27	1176
Neisseria meningitidis	20	28	9	0	4	61
Subtotal	79	28	13	3	168	4438
Non-fermentors						
Acinetobacter baumannii	19	115	1	93	127	355
Pseudomonas aeruginosa	219	1514	5	1624	1290	4652
Subtotal	238	1629	6	1717	1417	5007
Other						
Stenotrophomonas maltophilia	13	494	1	54	145	707
Helicobacter pylori						114#

*For *H. influenza* and *M. catarrhalis*, only isolates from the (higher and lower) respiratory tract were analyzed.
#Helicobacter pylori Selection of all institutions (general practice, clinic, outpatients' department, and long term care facilities)

2010 was included, selected from blood, wound, the lower respiratory tract and urine, except for *H. influenza* and *M. catarrhalis*, from which only isolates from the (higher and lower) respiratory tract were analyzed, and *H. pylori* from which also the samples of the intestine and the data from outpatient clinics and general practice were included (table 1). For the analyses of data from GP and outpatient clinics, only urine samples were included to determine the resistance rates for *E. coli*, *K. pneumoniae*, *K. oxytoca*, and P. mirabilis. Resistance rates are based on the number of I and R isolates. Only positive cultures were included. Isolates for screening and inventory purposes were excluded.

For the analyses, we included bug/drug combinations if 50% or more of first isolates was tested. Only susceptibility results were included as the bug/drug combination was tested by at least 6 laboratories. One exception was made. Fosfomycin susceptibility was structurally reported to ISIS-AR by 2 laboratories. As fosfomycin is part of the NHG guideline for treatment

Table 2. Number of indicator strains (N=27853) isolated from patients in specified hospital wards, tested for their susceptibility to antibiotics in the period 1998-2009.

Species	Intensive Care Units	Urology Services	Pulmonology Services
E. coli	2511	7453	
K. pneumoniae	795	959	
E. cloacae	650	237	
P. mirabilis	495	1027	
P. aeruginosa	1440	543	
E. faecalis	991	1518	
S. aureus	1293	441	
S. epidermidis	629	282	
S. pneumoniae			2043
H. influenzae			3221
M. catarrhalis			1325

of UTI, we included the results but emphasize that the results are based on a small part of the total number of isolates reported. For unusual susceptibility results, the laboratory was specifically asked for confirmation.
The susceptibility results in hospitals over the years are presented as graphics (chapter 4), where the change between ISIS and ISIS-AR (2007 to 2008) is displayed by a break in the trend line. For comparability over the years, results from non-ICU as well as from ICU were included. As the participating laboratories are not all identical to those participating in 2009, small differences in resistance rates as reported in Nethmap 2010 may appear. Not all laboratories tested all isolates to the indicator antibiotics formulated by SWAB especially with the Gram-positive isolates.

8.2.3 Quantitative data from Specific Hospital Departments - SIRIN

Unique unrelated consecutive isolates isolated from various clinical materials of patients admitted to Intensive Care Units, from urine of patients admitted to Urology Services and from respiratory specimens of patients admitted to Pulmonology Services were yearly collected from March 1st to October 1st. A maximum of 100 isolates per ward were collected each year. The strains were identified at the local laboratory for medical microbiology, stored at -200C and then sent to a single laboratory (department of Medical Microbiology of the UMC St Radboud, Nijmegen from 1995-2001, and the department of Medical Microbiology of the University Hospital Maastricht from 2002 on) for quantitative susceptibility testing. A total of 31.250 strains were collected from 1996-2009, the results of 27.853 indicator strains, obtained from 1998-2009 (table 2) are presented in this report.

The susceptibility of the strains from the specific wards was determined quantitatively, i.e. by MIC determinations by broth micro-dilution assays using breakpoints for resistance according to the recommendations of EUCAST (January 2011) for *E. coli*, *P. mirabilis*, *K. pneumoniae*, *E. cloacae*, P. aeruginosa, *E. faecalis*, *S. aureus*, *S. epidermidis*, *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *H. influenzae* ATCC 49247 and *S. aureus* ATCC 25923 were used as control strains in the MIC tests performed in the central laboratory.
The antibiotics chosen for reporting were the antibiotics indicated by the Resistance Surveillance Standard of the SWAB published in 1999. This SWAB Resistance Surveillance Standard was also the guideline used for the presentation of these data. The guideline provides criteria for indicator-organisms, indicator-antibiotics, methods and breakpoints to be used.

8.2.4 (I+R) and (R) values of ISIS-AR, comparison of CLSI and EUCAST breakpoints and impact on resistance levels

The resistance levels obtained by ISIS-AR are calculated from the I+R values reported by the participating laboratories. When taken only the R values for resistance, the resistance levels changed significantly for some antibiotics and some micro-organisms. Table 3 summarizes the findings for the indicator strains and antibiotics used. These differences may be also representative for the differences to be found when comparing breakpoints for resistance according to CLSI and EUCAST.
Clear patterns were not found. The use of two breakpoints had impact on the resistant rates for co-amoxiclav, cefuroxime, ciprofloxacin, nitrofurantoin and aminoglycosides for most *Enterobacteriaceae*. Co-trimoxazole resistance changed for the three respiratory pathogens tested, resistance to macrolides changed for *S. pneumoniae* and *M. catarrhalis*. Such changes are understandable when most strains of a population have MICs around the lower breakpoint. They will be judged resistant by use of the lower breakpoint and susceptible by use of the higher breakpoint.
Effective treatment from an infectious disease depends on many factors, but one is the inverse relationship between the MIC of an organism and the antibiotic concentration reached in the patient. The lower the MIC, the higher the cure rate. Strains with MICs around the breakpoints are potentially less susceptible to an antibiotic because of the low ratio between MIC and antibiotic concentration and they may therefore contribute to failure. From the studies on MIC distributions over time, we concluded that strains in this area are often moving “to the right” (also called MIC creep) in the following years, becoming really resistant. Reporting these strains susceptible by using high breakpoints may hide upcoming resistance as well.

Table 3. Impact on resistance rate when using R or I+R breakpoints for resistance for indicator strains from Unselected Hospital Departments reported to ISIS-AR. x = difference between R and I+R; 0 = no difference. .

Antibiotic	Micro-organisms										
	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*	*N. gonorrhoeae*
penicillin								x			0
methicillin						0	0				
amoxicillin	0			0					x	0	
co-amoxiclav	x	x		x						0	
piperacillin	x				0						
piperacillin-tazobactam	0	0	x	0	0						
cefaclor											
cefuroxime	x	x		0							
ceftriaxone	0	0	0	0						0	x
ceftazidime	0	0		0	x						
doxycycline						0	x	x		0	0
trimethoprim	0	0									
cotrimoxazole	0	0	0	0		0	0	x	x	x	
nitrofurantoin	x	x		0							
ciprofloxacin	0	0	x	x	x	0	x	0			x
gentamicin	x	0	0	x	x	0	x				
tobramycin	x	x	x	0	0	0	x				
amikacin	0	0	0	0	x			0			
macrolides						0	0		x	x	
fusidic acid						x	x				
mupirocin						0	0				
metronidazole											
rifampicin						0	0				

8.2.5 EUCAST criteria

EUCAST criteria for both MIC testing as well as disk diffusion can be found on the website of EUCAST, www. eucast.org. The criteria are freely available as a downloadable pdf file for printing and reference as well as an excel file to be adapted for personal use. The excel file contains active links to rational documents that describe the rationale behind the breakpoints.

8.3 Demographics and numerator data

Table A. Trend in the number of inhabitants in the Netherlands (Source: CBS).

Year	Number of inhabitants (January 1st)
1997	15 567 107
1998	15 654 192
1999	15 760 225
2000	15 863 950
2001	15 987 075
2002	16 105 285
2003	16 192 572
2004	16 258 032
2005	16 305 526
2006	16 334 210
2007	16 357 992
2008	16 405 399
2009	16 485 787
2010	16 574 989

Table B. Trend in the number of inhabitants in the Netherlands (Source: CBS).

Year	Hospitals	Admissions (x 1000)	Bed-days (x 1000)	Length of stay (mean in days)
1998	115	1551	14790	9.0
1999	109	1522	13940	8.7
2000	104	1485	13332	8.4
2001	101	1479	12778	8.2
2002	98	1544	12946	7.8
2003	97	1602	12651	7.5
2004	97	1681	12557	7.0
2005	96	1711	12396	6.8
2006	90	1749	11564	6.6
2007	89	1780	11271	6.3
2008	87	1873	11172	6.1

Table C. Resource indicators of University Hospital care in the Netherlands (Source: CBS).

Year	Hospitals	Admissions (x 1000)	Bed-days (x 1000)	Length of stay (mean in days)
1998	8	200	2032	10.2
1999	8	201	1914	9.5
2000	8	197	1842	9.4
2001	8	193	1805	9.4
2002	8	193	1820	9.4
2003	8	200	1837	9.2
2004	8	210	1830	8.7
2005	8	214	1825	8.5
2006	8	218	1806	8.3
2007	8	na	na	na
2008	8	na	na	na

Table D. Resource indicators of General Hospital care in the Netherlands (Source: CBS).

Year	Hospitals	Admissions (x 1000)	Bed-days (x 1000)	Length of stay (mean in days)
1998	107	1323	11768	8.9
1999	101	1300	11071	8.5
2000	96	1263	10544	8.3
2001	93	1265	10107	8.0
2002	90	1308	10266	7.8
2003	89	1374	9963	7.3
2004	89	1446	9929	6.9
2005	88	1467	9690	6.6
2006	82	1507	9641	6.4
2007	81	na	na	na
2008	79	na	na	na

na: not available

8.4 List of abbreviations

APUA	Alliance for the Prudent Use of Antibiotics
ATC	Anatomical Therapeutic Chemical Classification System
ATCC	American Type Culture Collection
CBS	Statistics Netherlands
CIb	Centre for Infectious Disease Control Netherlands
CLSI	Clinical and Laboratory Standards Institute
CRG	Committe on Guidelines for Susceptibility Testing
CSF	cerebrospinal fluid
CT	Computed tomography
DDD	Defined daily dosage
EARSS	European Antimicrobial Resistance Surveillance System
EARS-net	European Antimicrobial Resistance Surveillance network
ECDC	European Centre for Disease Prevention and Control
ESBL	Extended-spectrum beta lactamase
EUCAST	European Committee on Antiicrobial Susceptibility Testing
GGD	Municipal Health Services
GP	General practice
GRAS	Gonococcal Resistance to Antimicrobials Surveillance
HA	Hemagglutinin
I	Intermediate
ICAAC	Interscience Conference on Antimicrobial Agents and Chemotherapy
ICM	Intersectoral Coordinating Mechanisms
ICU	Intensive Care Unit
IC50	50 percent inhibitory concentration
INH	Isoniazid
ISIS-AR	Infectious Disease Surveillance Information System on Antibiotic Resistance
M2B	M2 ion-channel blocker
MARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
MSM	Men who have sex with men
NAI	Neuraminidase inhibitor
NHG	Dutch College of General Practitioners
NIVEL	Netherlands Institute for Health Services research
NVMM	Netherlands Society for Medical Microbiology
OPD	Outpatient department
PCR	Polymerase chain reaction
R	Resistant
RIVM	National Institute for Public Health and the Environment
RTI	Respiratory tract infection
S	Sensitive
SARIN	Surveillance of Antimicrobial Resistance in the Netherlands
SERIN	Surveillance of Extramural Resistance in the Netherlands
SFK	Foundation for Pharmaceutical Statistics
SIRIN	Surveillance of Intramural Resistance in the Netherlands
STD	Sexually transmitted disease
STI	Sexually transmitted infection
SWAB	Dutch Working Party on Antibiotic Policy
UTI	Urinary tract infection
VANTURES	Antibiotic Usage and Resistance Surveillance Working Group
VIZ	Netherlands Society for Infectious Diseases
WHO	World Health Organization
WIP	Dutch Working Party on Infection Prevention
UMC	University Medical Center

UTI	urinary tract infection
VANTURES	Antibiotic Usage and Resistance Surveillance Working Group
VIZ	Netherlands Society for Infectious Diseases
WHO	World Health Organization
WIP	Dutch Working Party on Infection Prevention

Notes

Notes