Surveillance of antibiotic resistance in the Netherlands (SARIN) by quantitative susceptibility testing

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 is, the higher the cure rate. Strains with MICs around and just below 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. Therefore we tested our strains quantitatively over a large range and studied MIC distributions over time to discover changes, drifts and moving "to the right" (also called MIC creep), the latter being indicative for development of real resistance in the following years. Reporting these strains susceptible by using only the breakpoint (qualitative methods) may hide upcoming resistance.

Quantitative data from the community - SERIN (Susceptibility of Extramural Resistance in the Netherlands)

General Practice

Escherichia coli

The prevalence of antibiotic resistance among *E. coli* was determined for strains collected from male 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 catheterised, had urological or nephrological problems, diabetes mellitus or other immune compromising diseases (see also table 1).

A dip slide 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 dip slides were considered positive when bacterial growth was observed of $>10^2$ cfu/ml. Dip slides 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 $5x10^5$ cfu/ml and overnight incubation at 37*C.* 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 antibiotics (range in mg/ml) tested were: amoxicillin (0.06-128), co-amoxiclav (0.06-128), trimethoprim (0.03-64), co-trimoxazole (0.03-64), gentamicin (0.06-64), norfloxacin (0.03-64), ciprofloxacin (0.003-16) and nitrofurantoin (0.5-512). *Escherichia coli* ATCC25922 and ATCC35218 were used as control strains. The breakpoints for resistance were according to the EUCAST guidelines. Co-amoxiclav resistant E. coli were assessed for the presence of ESBL production using the combination disc diffusion test with ceftazidime, cefepime and cefotaxime alone and in combination with clavulanic acid according to the guidelines of the NVMM. Confirmation was by PCR.

The data were compared with those of the 2004 study, using the same group of general practitioners in the same setting and with those of a similar study performed among female patients (table 1).

Table 1. Demographic data of patients from the community participating in the SERIN studies

Study	**Year**				
	2002	**2004**	**2007/2008**	**2009/2010**	**2011**
Escherichia coli - Uncomplicated UTI					
GP patients	1697	2146		1034	
Nursing homes				308	
Staphylococcus aureus - carriership					
Healthy individuals			4000		
GP patients			2691		
Nursing homes			260	308	
Streptococcus pneumoniae					
healthy adults			593		
healthy children 0-4 y of age			620		
healhty children 9 y of age			698		
GP patients with RTI			451		
S. haemolyticus - carriership					
GP patients without infection					1925

Streptococcus haemolyticus

The prevalence and its resistance to penicillin of *S. haemolyticus* in the normal nasopharyngeal flora were investigated by taking nasal swabs from patients visiting the general practitioner with non-infectious- and non-throat complaints. A total of 41 GPs from the NIVEL network participated in this study and sent swabs from 1925 patients from which 33 strains of *S. haemolyticus* serogroup A were isolated (1.7%). This study is still ongoing (table 1).

Nursing homes

Escherichia coli

Nursing homes in the province of Limburg were approached for participation in the project as follows: (1) a letter explaining the background and aim of the project was sent to the board of directors of the nursing homes. (2) After agreement of the directors a letter was sent to the managers and the physicians for the elderly explaining the aim and the relevance for the daily practice as well as the work load for the health care workers. (3)Then a letter for the residents describing the aim of the project and the potential risk for the residents was sent to the residents or their legal representatives. In total 6 nursing homes agreed to participate and only residents which gave their informed consent were included in the study. Ethical approval was obtained from the METC of the AZM. Freshly voided urine or pressed in incontinence materials from 308 residents without infection from six nursing homes were cultured by taking dip slides in 2010-2011 (table 1). Susceptibility testing was performed according to the procedure described above.

Nursing homes

S. aureus

Nasal swabs from 308 residents having somatic disabilities without infection and living in six nursing homes in the province of Limburg were cultured for carriership of S. aureus in the commensal flora (table 1). A total of 99 strains were isolated; susceptibility was tested quantitatively using a microbroth dilution method as described above; antibiotics tested were penicillin, methicillin, erythromycin, clindamycin, doxycycline, co-trimoxazole, fusidic acid, mupirocin and ciprofloxacin. Breakpoints for resistance were according the EUCAST guidelines.

Quantitative data from Specific Hospital Departments - SIRIN (Susceptibility of Intramural Resistance in the Netherlands)

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 -20^{0}C 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 34,254 strains were collected from 1996-2010, the results of 30,715 indicator strains, obtained from 1998-2010 (table 2) are presented in this report.

Table 2. Number of indicator strains (N=30715) isolated from patients in specified hospital wards, tested for their susceptibility to antibiotics in the period 1996-2010.

Species	Intensive Care Units	Urology Services	Pulmonology Services	
Escherichia coli	2849	8149		
Klebsiella pneumoniae	889	1082		
Enterobacter Cloacae	723	256		
Proteus mirabilis	549	1104		
Pseudomonas aeruginosa	1581	610		
Enterococcus faecalis	1091	1654		
Staphylococcus aureus	1470	494		
Staphylococcus epidermidis	694	314		
Streptococcus pneumoniae	151	3		2077
Haemophilus influenzae	191	1		3358
Moraxella catarrhalis	78			1347
Total	10266	13667		6782

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. Antibiotics

and range (mg/l) tested are presented in table 3. 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.

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. Antibiotics (range in mg/l) used for quantitative susceptibility testing for strains of Intensive Care Units, Urology Services and Pulmonology Services of centres participating in the SIRIN studies (1995-2010).

Antibiotic	***E. coli***	***K. pneumoniae***	***E. cloacae***	***P. mirabilis***	***P. aeruginosa***
Penicillin					
Oxacillin					
Amoxicillin	0.06-128	0.06-128	0.06-128	0.06-128	
co-amoxiclav	0.06-128	0.06-128	0.06-128	0.06-128	
Piperacillin	0.25-512	0.25-512	0.25-512	0.25-512	0.25-512
piperacillin-tazobactam	0.25/4-512/4	0.25/4-512/4	0.25/4-512/4	0.25/4-512/4	0.25/4-512/4
Imipenem	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
Meropenem	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
Cefuroxime	0.06-128	0.06-128	0.06-128	0.06-128	
cefotaxime/ceftriaxone	0.06-128	0.06-128	0.06-128	0.06-128	
Ceftazidime	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
Ceftibuten	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
Cefixime	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
cefepime	0.12-128	0.12-128	0.12-128	0.12-128	0.12-128
gentamicin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
tobramycin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
amikacin	0.12-256	0.12-256	0.12-256	0.12-256	0.12-256
doxycycline	0.06-128	0.06-128	0.06-128	0.06-128	
clarithromycin					
clindamycin					
chloramphenicol					
trimethoprim	0.03-64	0.03-64	0.03-64	0.03-64	
co-trimoxazole	0.03/0.6-64/1216	0.03/0.6-64/1216	0.03/0.6-64/1216	0.03/0.6-64/1216	
norfloxacin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
ciprofloxacin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
levofloxacin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
moxifloxacin	0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
nitrofurantoin	0.12-256	0.12-256	0.12-256	0.12-256	
vancomycin					
teicoplanin					
linezolid					
synercid					
rifampicin					

Antibiotic	***E. faecalis***	***S. aureus***	***S. epidermidis***	***S. pneumoniae***	***H. influenzae***	***M. catarrhalis***
Penicillin		0.004-8	0.004-8	0.015-64		
Oxacillin		0.03-64	0.03-64			
Amoxicillin	0.06-128			0.06-128	0.06-128	0.06-128
co-amoxiclav					0.06-128	0.06-128
Piperacillin						
piperacillin-tazobactam						
Imipenem	0.06-128	0.03-64	0.03-64	0.06-128	0.06-128	0.06-128
Meropenem	0.06-128	0.06-64	0.06-64	0.06-128	0.06-128	0.06-128
Cefuroxime		0.06-128	0.06-128	0.06-128	0.06-128	0.06-128
cefotaxime/ceftriaxone				0.06-128	0.06-128	0.06-128
Ceftazidime				0.06-128	0.06-128	0.06-128
Ceftibuten						
Cefixime				0.06-128	0.06-128	0.06-128
Cefepime				0.06-128	0.06-128	0.06-128
Gentamicin	0.06-64	0.06-64	0.06-64		0.06-64	0.06-64
Tobramycin						
Amikacin						
Doxycycline	0.06-128	0.03-64	0.03-64	0.03-64	0.03-64	0.03-64
clarithromycin	0.06-128	0.03-64	0.03-64	0.06-128	0.06-128	0.06-128
Clindamycin		0.03-64	0.03-64	0.03-64		
chloramphenicol	0.12-256					
trimethoprim						
co-trimoxazole		0.015/29-32/608	0.015/29-32/608	0.015/29-32/608	0.015/29-32/608	0.015/29-32/608
norfloxacin	0.06-128					
ciprofloxacin	0.06-128	0.25-8	0.25-8	0.03-64	0.03-64	0.03-64
levofloxacin	0.06-128			0.03-64	0.03-64	0.03-64
moxifloxacin	0.06-128			0.03-64	0.03-64	0.03-64
nitrofurantoin	0.12-256					
vancomycin	0.12-256	0.06-128	0.06-128	0.06-128		
teicoplanin	0.12-256	0.06-128	0.06-128	0.06-128		
linezolid	0.03-64	0.03-64	0.03-64	0.03-64		
synercid	0.06-64					
rifampicin	0.008-16	0.008-16	0.008-16	0.008-16	0.008-16	0.008-16