HOPITAL ERASME

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Microbiological Surveillance of Methicillin Resistant Staphylococcus aureus (MRSA) in Belgian Hospitals in 2003

Final report

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Introduction

Staphylococcus aureus is a leading cause of skin and soft tissue infection, surgical site and catheter infection, pneumonia, bacteremia and osteo-articular infections (1). In the past two decades, methicillin-resistant S. aureus (MRSA) has increased in incidence in many parts of the world as agent of nosocomial infections. More recently, community-acquired infections caused by MRSA have been reported in the USA, Australia and Europe (2). In Europe, the proportion of methicillin resistant strains of S. aureus ranged in hospitalised patients from more than 30% in Southern countries like Italy, Spain and Portugal, to less than 2% in Northern countries such as the Netherlands and Scandinavia. (3). Epidemic MRSA strains disseminate within and between healthcare facilities over large geographic areas (4).

Since 1992, the ULB Reference Laboratory for Staphylococci organises epidemiological surveillance of MRSA by means of biannual surveys in collaboration with the scientific Public Health Institute (ISP-WIV) and the GDEPIH/GOSPIZ (5). The objectives are to follow the evolution of genotype and of antimicrobial resistance profile of MRSA isolates from patients admitted to Belgian acute-care hospitals. In the last survey conducted in 2001, we observed the diversification of MRSA clones disseminated in Belgian hospitals. Seven major clones were identified that belong to the five pandemic MRSA lineages (CC 5, 8, 22, 30 and 45) that are associated with nosocomial infections worldwide (5). The changes in the prevalence of epidemic MRSA genotypes led to a shift in resistance patterns with a decreased proportion of multi-drug and gentamicin-resistant MRSA strains as compared to previous surveys.

Until now, glycopeptides have been considered as the treatment of choice for MRSA infections. In 1997, the first infection with MRSA isolate with intermediate susceptibility to vancomycin was reported in Japan. Since then, at least 20 cases of infection caused by MRSA with intermediate susceptibility to both vancomycin and teicoplanin (GISA) have been reported worldwide(6)7;8). In addition to GISA, strains named hetero-GISA, that are borderline susceptible to glycopeptides but exhibit resistance to glycopeptides at low frequency ($\sim 10^{-6}$ sub-population) have been described more frequently in Europe, Brazil and Asia (8). In Belgium, we found a low prevalence of hetero-GISA among MRSA strains collected from hospitals in 2001 (9).

In this report, we describe the results of molecular typing and antimicrobial susceptibility of MRSA strains from the national survey conducted in 2003 in 112 Belgian hospitals.

Materials and methods

Survey methods and collection of bacterial strains

From January to December 2003, microbiological laboratories serving all Belgian acute-care hospitals (n = 180 sites) were invited to collect up to 5 non-duplicate MRSA isolates per hospital sites, which recovered consecutively in hospitalised patients from any body site. These strains were sent to the Reference Laboratory with a case report form describing the following demographic data : patient age, sex, type of specimen, category of hospital unit, MRSA acquisition (nosocomial or imported). Nosocomial acquisition was defined as a MRSA strain firstly isolated from a patient who had been hospitalised for more than 48 hours. Strains were stored at –80°C until testing.

Identification and characterisation of oxacillin resistance

MRSA were confirmed genotypically by PCR for detection of *mec*A and *nuc* genes as previously described (10).

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MIC) with a test dilution range from 0.06 to 128 mg/l were determined by the agar dilution method according to NCCLS guidelines for oxacillin, cefoxitin, ceftobiprole, vancomycin, teicoplanin, erythromycin, clindamycin, quinupristin-dalfopristin, ciprofloxacin, gentamicin, amikacin, kanamycin, tobramycin, minocycline, tetracycline, tigecycline, rifampin, trimethoprim-sulfamethoxazole, fusidic acid, linezolid, daptomycin and mupirocin. MICs for mupirocin resistant strains were further tested by the E-test method (AB Biodisk, Solna, Sweden) to determine high-level resistance. NCCLS breakpoints were used for MIC interpretation except for fusidic acid and mupirocin (11). Fusidic acid breakpoints were interpreted according to the criteria of the Committee for Antimicrobial Testing of the French Society of Microbiology (CA-SFM) (12). Mupirocin resistant strains were classified into two categories according to the British Society for Antimicrobial Chemotherapy (BSAC): low level resistance (MIC = 8 – 256 mg/l) and high-level resistance (MIC > 256 mg/l) (13).

Glycopeptide susceptibility testing

All strains were tested on vancomycin agar screen (VAS) (Becton Dickinson, Heidelberg, Germany) and teicoplanin agar screen (TAS). For VAS, 10 µl of a McFarland 0.5 inoculum was spotted onto Brain Heart Infusion agar (BHI) supplemented with 6 µg/ml vancomycin and incubated for a full 24h at 35°C. For TAS, 10 µl of a McFarland 0.5 inoculum was spotted onto BHI agar supplemented with 5 µg/ml teicoplanin and incubated for a full 48h at 35°C. Strains showing MIC ≥ 4 µg/ml for vancomycin and/or teicoplanin by agar dilution or growing on VAS or TAS agar were further characterised by the "E-test macromethod" (AB Biodisk, Solna, Sweden) for vancomycin and teicoplanin (14). Briefly, 100 µl of a McFarland 2.0 suspension was inoculated onto BHI agar and incubated for 48h at 35°C. Results of glycopeptide inhibition concentration were interpreted according to criteria provided by the manufacturer: strains inhibited by both vancomycin and teicoplanin at ≥ 8 µg/ml or by teicoplanin alone at ≥ 12 µg/ml were considered as putative hetero-glycopeptide intermediate *S. aureus* (hetero-GISA) (14).

Molecular typing

Pulsed-field gel electrophoresis (PFGE)

All MRSA strains were genotyped by SmaI macrorestriction analysis of genomic DNA resolved by PFGE. The digitised PFGE patterns were analysed using BioNumerics software version 2.0 (Applied Maths, Belgium). Dendrograms were constructed using the Dice coefficient of similarity of PFGE patterns grouped with the UPGMA clustering method. PFGE patterns were classified according to the following nomenclature (8): (a) PFGE Group included patterns showing ≤ 6 DNA fragments difference, equivalent to ≥ 65% similarity. These groups were designated by a capital letter (e.g. A).; (b) PFGE Type included PFGE patterns showing ≤ 3 DNA fragment difference equivalent to ≥ 80 % similarity. Types were designated by the group letter followed by a Roman numeral suffix (e.g. A1); (c) PFGE Subtype described any pattern profile within a type. Each subtype was designated by lower case letter suffix (e.g. A1a).

A representative set of MRSA strains belonging to major epidemic groups was further analysed by Multilocus Sequence Typing (MLST) and by determination of their Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type.

Multi-locus sequence typing (MLST)

MLST was performed on selected MRSA strains (n = 20) belonging to the major epidemic types as previously described (15). In brief, alleles at seven housekeeping genes were amplified by PCR

(thermocycler ABI 9700) and sequenced on both strands over a ~450 bp region (ABI Sequencer 3100). Electropherograms were imported to BioNumerics (Applied Maths, Belgium) for the quality control and trimming of the 5' and 3' non discriminatory regions. Allelic profiles were determined by comparison with those recorded in the MLST database (http://www.mlst.net).

Staphylococcal cassette chromosome *mec* typing (SCC*mec*)

SCC*mec* type was determined by multiplex PCR as described by Oliveira et al for representative MRSA strains (n = 90) of every PFGE type (16).

Results

Hospital participation and bacterial strains

One hundred twelve hospitals (62% of all sites) participated. They were located in Brussels (n = 16), Flanders (n =57) and Wallonia (n = 39) (Table1). Among 547 isolates sent as MRSA to the Reference Laboratory, 511 MRSA strains (93%) were confirmed as such whereas 26 strains were identified as coagulase negative staphylococci (n = 10) or as oxacillin susceptible *S. aureus* (n = 16). Ten isolates did not grow.

Demographic data

The majority of case patients with MRSA infection or colonisation were elderly (> 60 years old) (Table 1). The median age of patients was 76 years old. Patients were mainly hospitalised in medical wards (32%), geriatric wards (25%), surgical wards (17%) or intensive care units (10%). MRSA strains were recovered from respiratory tract (24%), skin or soft tissue infections (21%), MRSA screening at muco-cutaneous sites (24%), blood (8%), urine (8%) and other specimens (15.3%).The proportion of MRSA "imported acquisition", defined as MRSA isolates detected on the first 48h after admission, was 35.4%.

Table 1 : Age distribution of patients with MRSA, 2003

Age group (years)	No patients with MRSA (% total by category)		
	Male	Female	Total
<1	2 (1)	1 (<1)	3
1-19	0	1 (<1)	1
20-49	20 (9)	17 (6)	37
50-59	19 (9)	16 (6)	35
60-69	36 (16)	24 (8)	60
70-79	70 (31)	92 (32)	162
≥ 80	76 (34)	136 (47)	212
Unknown	0	1 (<1)	1
Total	223	288	511

Antimicrobial susceptibility data

MIC of 20 antimicrobials for 511 MRSA strains determined by agar dilution are shown in Table 6 and 7 and Figure 1.

All isolates were susceptible to glycopeptides (but see below results for hGISA), quinupristin-dalfopristin, linezolid and cotrimoxazole.

More than 90% of strains were susceptible to fusidic acid (99%), rifampin (97%) and mupirocin (94%). Resistance to tetracycline (12%) was higher than for minocycline (5%).

Resistance to ML was frequent, ranging from 63% for erythromycin to 40% for clindamycin. For aminoglycosides, resistance was more frequent to kanamycin (37%) and tobramycin (44%) than to gentamicin (5%) and amikacin (1%). Ninety-eight percent of the strains of the strains were resistant to ciprofloxacin.

In comparison with previous surveys conducted in the early 1990s, MRSA isolates were more susceptible to non beta-lactam antimicrobial agents (Figure 2) (8). This phenomenon reflects the change of the prevalence of epidemic MRSA genotypes with shift in the resistance genes distribution (6).

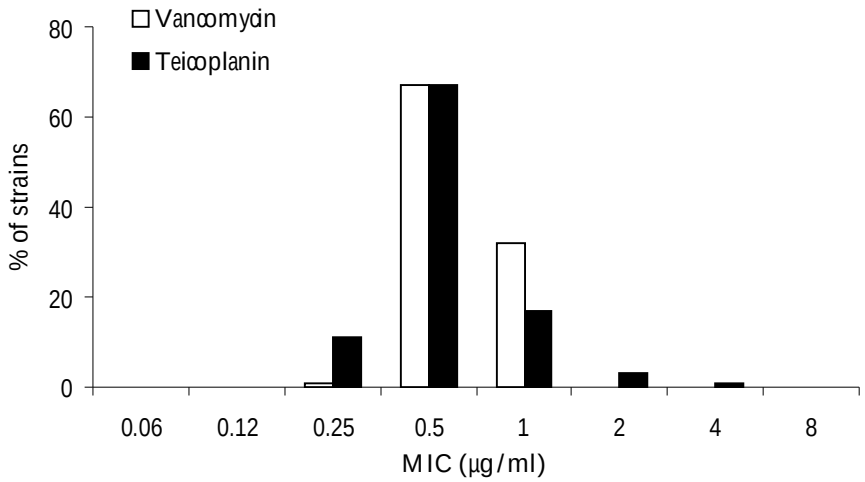
Table 2 : Cumulative proportions of MRSA isolates (n = 511) inhibited by increasing concentrations of antimicrobial agents

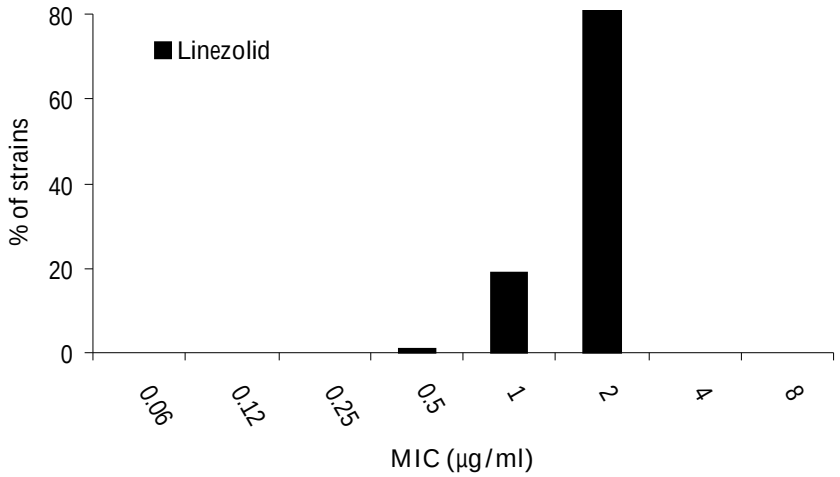
Antimicrobial agent	% of strains resistant at MIC (mg/l) of :													
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	>1024
Oxacillin	0	0	0	0	0	0	0	4	9	41	75	86	100	-
Cefoxitin	0	0	0	0	0	0	0	4	24	49	69	79	100	-
Vancomycin	0	0	1	68	100	100	100	100	100	100	100	100	100	-
Teicoplanin	0	0	12	79	96	99	100	100	100	100	100	100	100	-
Erythromycin	0	8	36	37	37	37	37	37	38	41	41	41	100	-
Clindamycin	31	56	59	60	60	60	60	60	60	60	60	60	100	-
Quinupristin-dalfoprisitin	0	3	44	94	100	100	100	100	100	100	100	100	100	-
Ciprofloxacin	0	0	0	1	2	2	3	5	13	20	29	45	100	-
Linezolid	0	0	0	1	19	100	100	100	100	100	100	100	100	-
Gentamicin	0	23	91	95	95	95	95	95	95	96	97	99	100	-
Tobramycin	0	26	54	54	55	55	55	56	58	61	62	68	100	-
Kanamycin	0	0.2	1	5.7	43.2	55.2	56.9	58.3	59.9	62.8	72.2	83.6	100	-
Amikacin	0	0	0	0	33	56	59	84	98	99	100	100	100	-
Minocycline	47	82	90	90	91	92	94	95	100	100	100	100	100	-
Tetracycline	0	31	75	86	87	87	87	88	89	91	96	98	100	-
Rifampin	96	97	97	97	97	99	99	99	99	99	99	99	100	-
Cotrimoxazole	99	99	100	100	100	100	100	100	100	100	100	100	100	-
Fusidic acid	46	83	96	96	97	99	99	99	99	99	99	100	100	-
Mupirocin	15	51	91	93	93	94	94	95	96	96	96	96	96	100

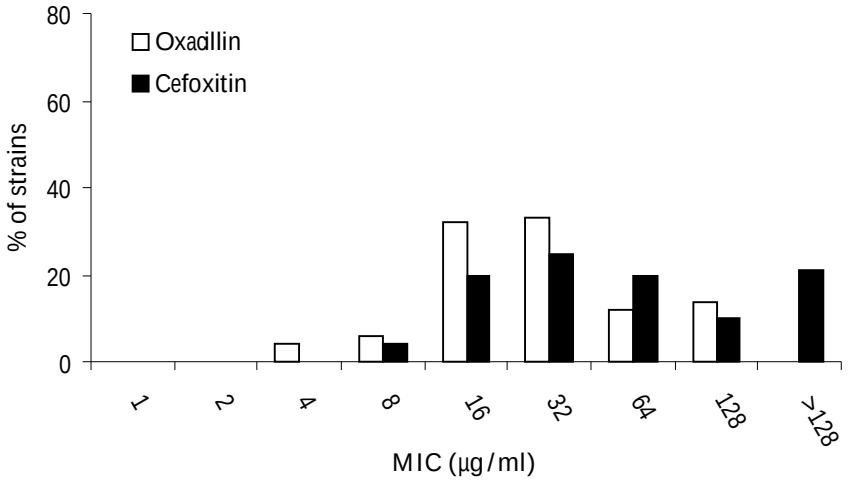
Table 3 : Range of MIC, MIC_{50}, MIC_{90} and proportion by susceptibility category of 511 MRSA isolates by antimicrobial agents

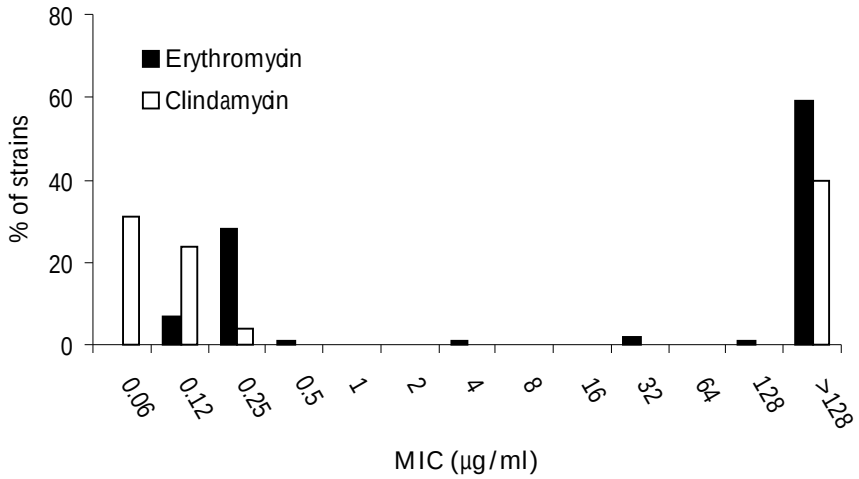
Antimicrobial agent	Range (mg/l)	MIC50 (mg/l)	MIC90 (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	8 - >128	64	>128	0	0	100
Cefoxitin	8 - >128	64	>128	3.7	20.4	75.9
Vancomycin	0.25 – 2	0.5	1	100	0	0
Teicoplanin	0.12 – 4	0.5	1	100	0	0
Erythromycin	0.06 - >128	>128	>128	36.6	0.4	63.0
Clindamycin	0.06 - >128	0.12	>128	59.7	0	40.3
Quinupristin-Dalfopristin	0.12 – 1	0.5	0.5	100	0	0
Ciprofloxacin	0.5 - >128	>128	>128	1.6	0.6	97.8
Linezolid	0.5 – 2	2	2	100	0	0
Gentamicin	0.06 - >128	0.25	0.25	94.9	0	5.1
Tobramycin	0.12 - >128	0.25	>128	55.5	0.4	44.1
Kanamycin	0.12 - >128	2	>128	59.9	2.9	37.2
Amikacin	0.5 - 128	2	16	97.7	1.4	1.0
Minocycline	0.06 – 16	0.12	0.12	93.7	1.8	4.5
Tetracycline	0.06 - >128	0.25	32	87.5	0.8	11.7
Rifampin	0.06 - >128	0.06	0.06	97.1	1.6	1.4
Cotrimoxazole	0.06 – 1	0.06	0.06	100	0	0
Fusidic acid	0.06 - >128	0.12	0.25	98.6	0.6	0.8
Mupirocin	0.06 - >1024	0.12	0.25	93.5	2.9	3.5

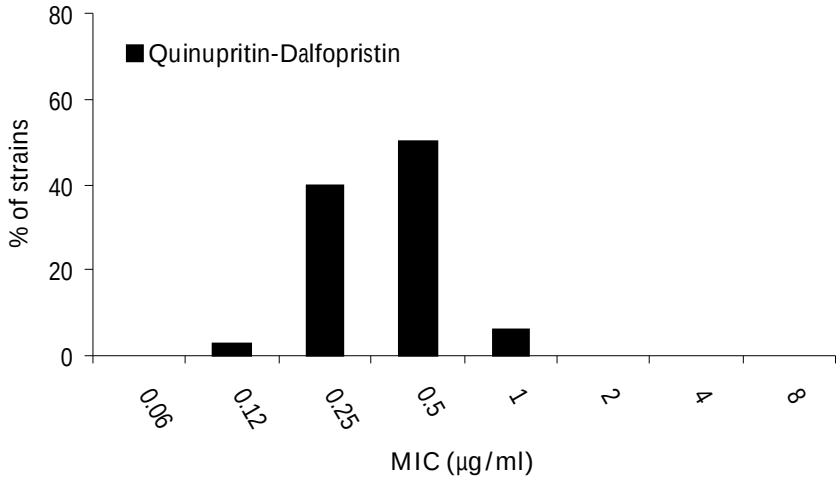
Figure 1 : MIC distribution by antimicrobial for 511 MRSA isolates, Belgian hospital survey, 2003

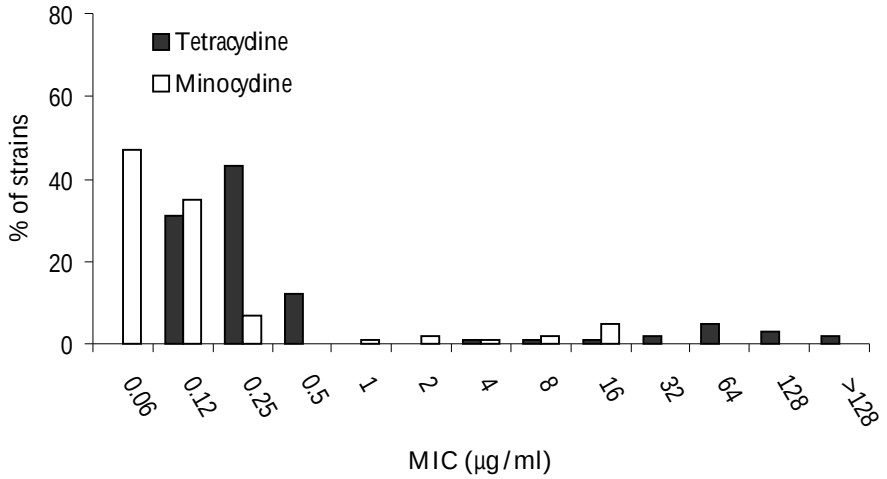


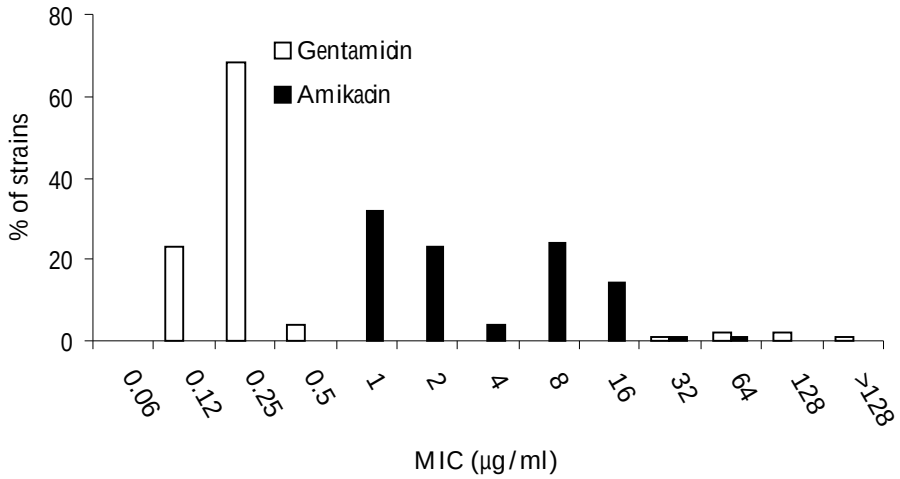


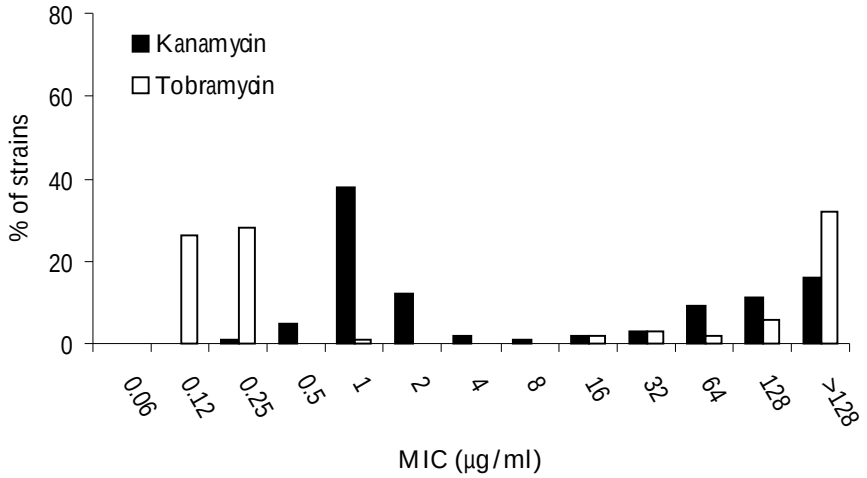

Oxacillin
Cefoxitin
% of strains
80
60
40
20
0
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)

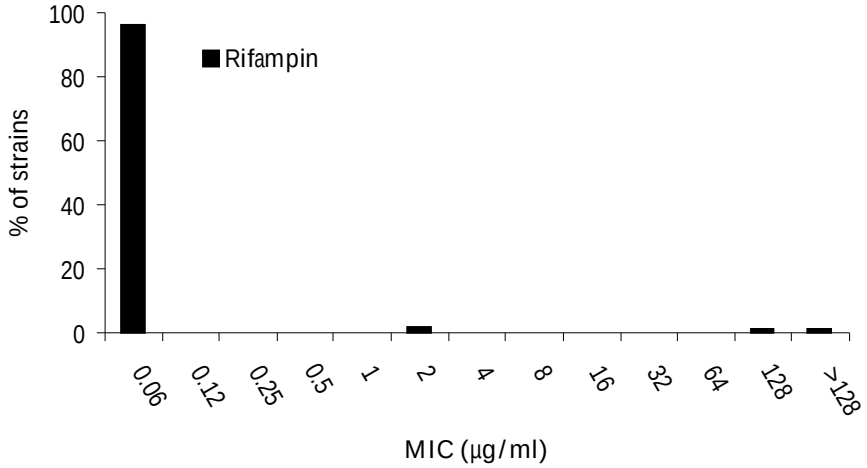

Erythromycin
Clindamycin
% of strains
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)

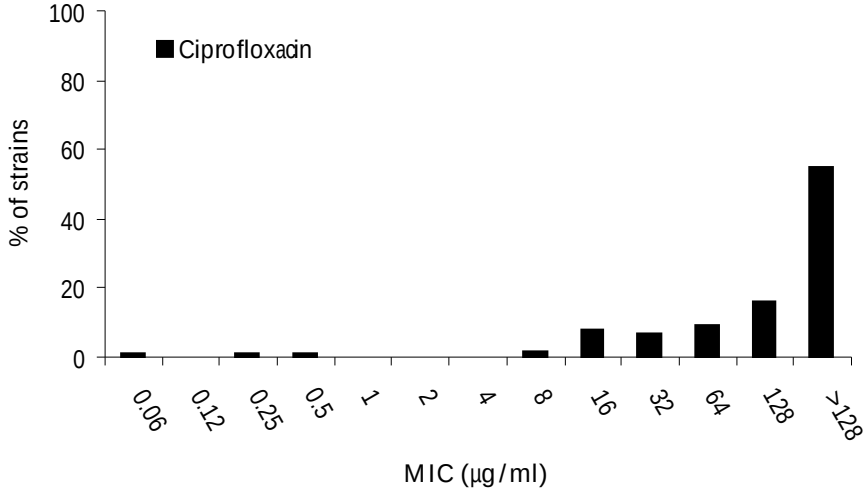

Quinupritin-Dalfopristin
% of strains
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
MIC (µg/ml)

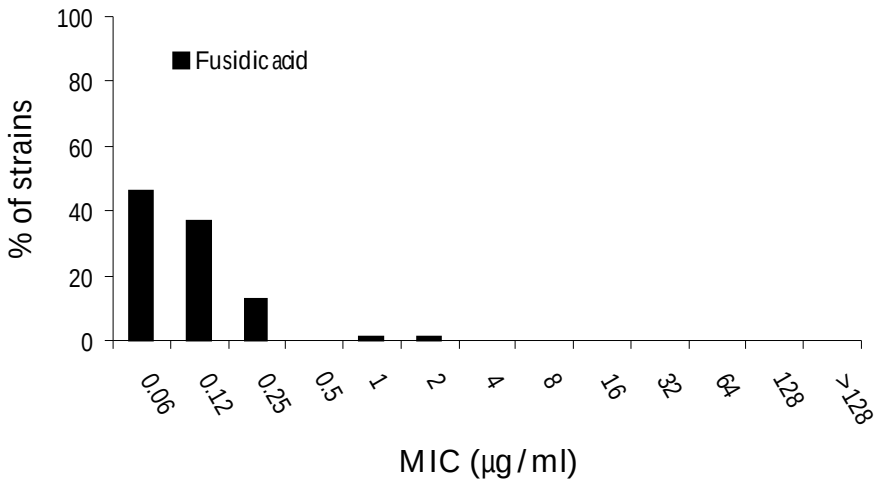

80
60
40
20
0
% of strains
Tetracydine
Minocydine
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)

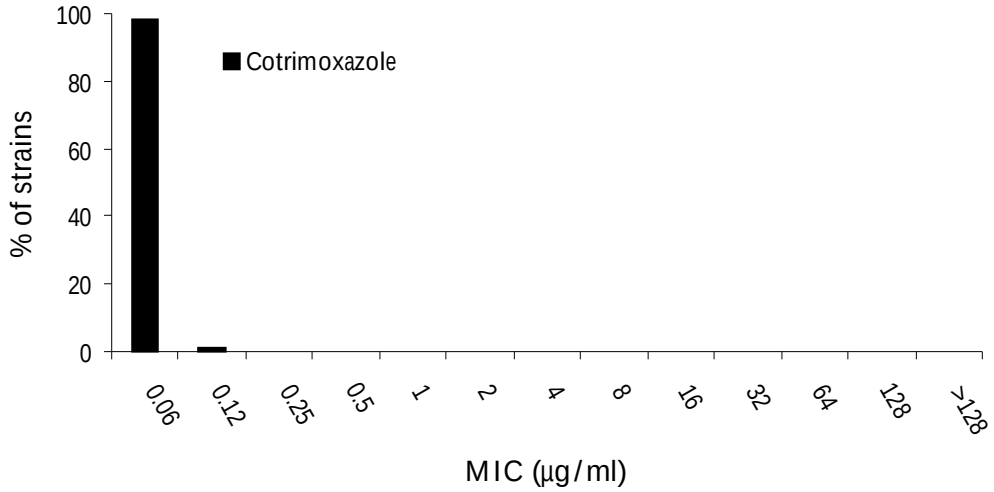

80
60
40
20
0
% of strains
Gentamicin
Amikacin
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)


80
60
40
20
0
% of strains
Kanamycin
Tobramycin
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)


Rifampin
% of strains
100
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)


Ciprofloxacin
% of strains
100
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)


Fusidic acid
% of strains
100
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (µg/ml)



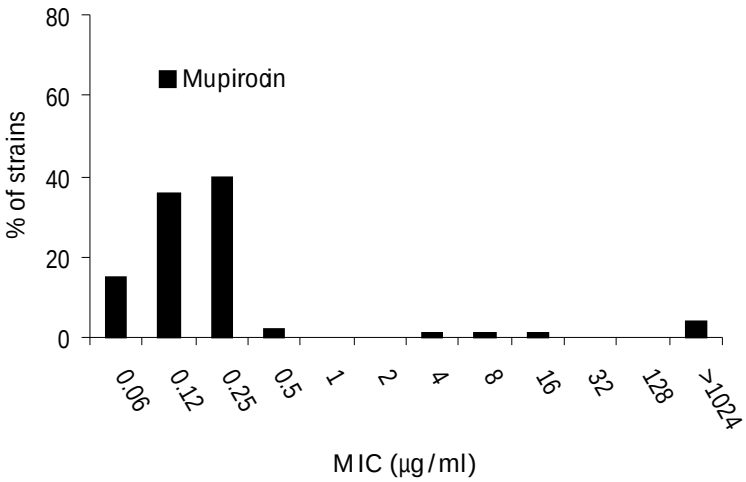
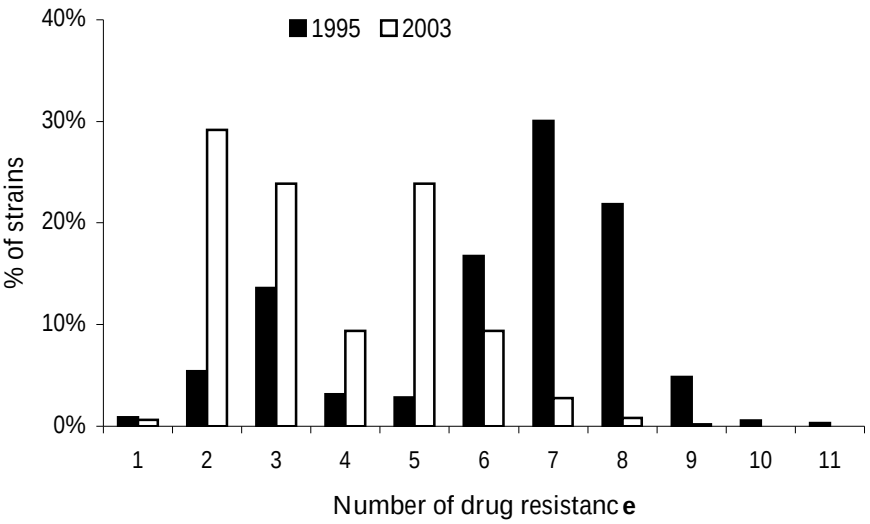


Figure 2: Evolution of co-resistance to non beta-lactam drugs in MRSA isolates from hospitalised patients, Belgium, 1995 to 2003



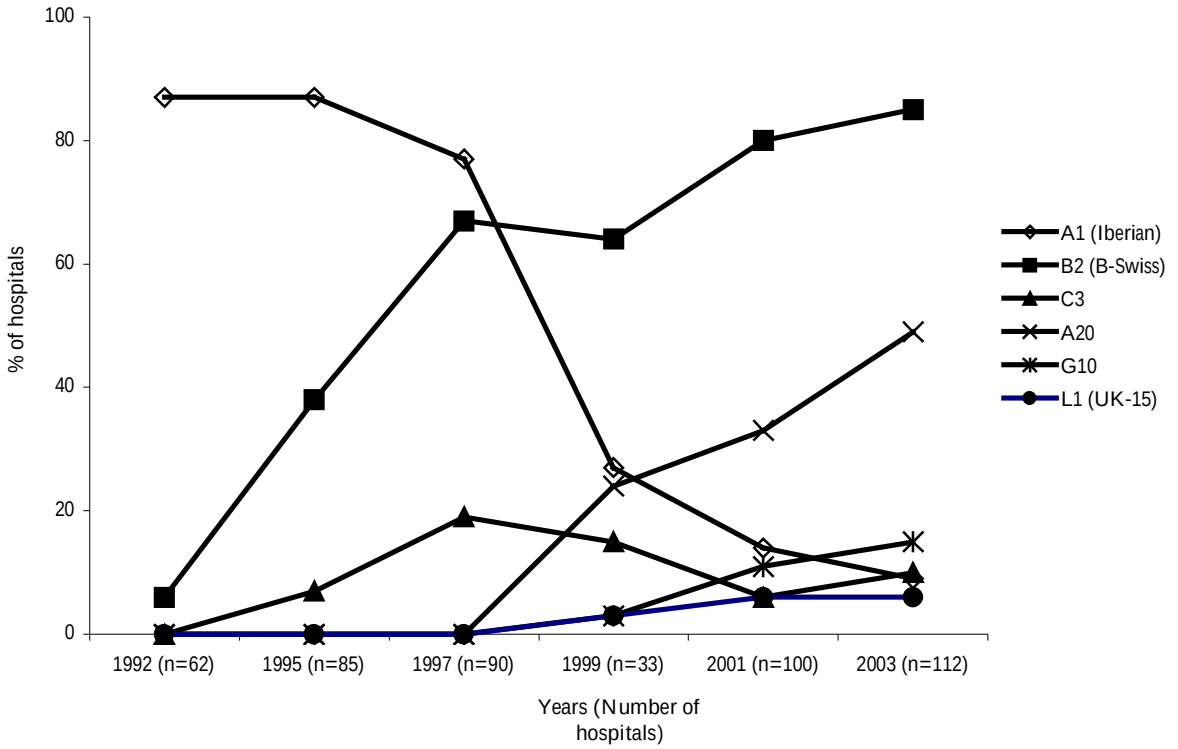
Glycopeptide susceptibility

Forty-two (8.4%) MRSA isolates grew on TAS after 48h and only one isolate (0.2%) on VAS. By E-test macromethod, no strain had MIC ≥ 8 mg/l for vancomycin and teicoplanin. Six strains (1.1%) had MIC ≥ 12 mg/l for teicoplanin. This low prevalence of hetero-GISA (1.1%) is similar to that (2.6%) observed in a previous collection of nosocomial MRSA strains collected from a large survey of Belgian hospitals in 2001 (22). The presence of hetero-resistant population for glycopeptides should be confirmed by population analysis.

Genotype distribution

PFGE patterns of 511 MRSA strains were classified into 15 groups and 36 types. Ninety percent of the strains belonged to 10 PFGE types including B2 (n = 251), A20 (n = 97), A22 (n = 27), G10 (n = 27), C1 (n = 13), A1 (n = 12), C3 (n = 12), D8 (n = 11), L1 (n = 10). Those epidemic MRSA types were found in 95 (85%), 55 (49%), 24 (21%), 17 (15%), 10 (9%), 10 (9%), 11 (10%), 9 (8%) and 7 (6%) hospitals, respectively. The SCCmec type was determined for 90 MRSA stains by multiplex PCR. The type distribution of SCCmec was as follow : type IV (n = 64), type II (n = 16), type I (n = 10.By MLST and SCCmec, PFGE type B2 strains belonged to ST45-SCCmec IV clone (CC45). Group A strains belong to the CC8. Type A1 strains belonged to ST247-SCCmec I clone while A20 and A21 belonged to ST8-SCCmec IV clone. Type C1 and C3 strains belonged to ST5-SCCmec II and ST5-SCCmec IV clone. Type G10 strains belonged to ST5-SCCmec II. Type L1 strains were identical by MLST and SCCmec type analysis to the "UK epidemic MRSA (EMRSA) 15 clone" (ST22-SCCmec IV). Type D8 strains belonged to ST228-SCCmec I. The evolution of epidemic MRSA clone in Belgian hospitals since 1992 is shown in Figure 3.

Figure 3: Distribution of Epidemic MRSA PFGE Types National Surveillance, Belgium, 1992-2003



Conclusions

- Hetero-GISA were found at low frequency (1.1%).
- A high proportion of MRSA strains was resistant to ciprofloxacin and ML.
- Epidemic MRSA clones were more susceptible to antimicrobial agents than clones which were disseminated in the early 1990s.
- Most epidemic strains belonged to the four pandemic MRSA lineages CC 5, 8, 22 and 45 associated with nosocomial infections.

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Ref Type: Electronic Citation

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