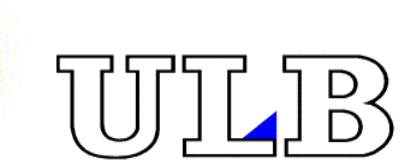

Microbiological Surveillance of *Staphylococcus aureus* in Belgian Hospitals in 2005

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, bactaeremia and osteo-articular infections . 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, Asia and Europe . 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 . Some epidemic MRSA strains disseminate within and between healthcare facilities over large geographic areas .

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 Belgian Infection Control Society (BICS). 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 2003, 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 cause nosocomial infections worldwide. The changes in the prevalence of epidemic MRSA genotypes led to shifts in resistance patterns with a decreased proportion of multi-drug and gentamicin-resistant MRSA strains as compared to previous surveys.

In this report, we describe the results of molecular typing and antimicrobial susceptibility of MRSA and methicillin susceptible *S. aureus* (MSSA) strains from the national survey conducted in 2005 in 116 Belgian hospitals.

Materials and methods

Survey methods and collection of bacterial strains

From January to December 2005, laboratories of all Belgian acute-care hospital (n = 180) were invited to collect up to 3 non-duplicate MRSA and 2 non duplicate MSSA isolates 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

S. aureus isolates were confirmed genotypically by PCR for detection of *mecA* and *nuc* genes as previously described.

Antimicrobial susceptibility

Minimal inhibitory concentrations (MIC) (with a test dilution ranged from 0.06 to 128 μg/ml) were determined by the agar dilution method according to CLSI guidelines for oxacillin, cefoxitin, ceftobiprole, vancomycin, teicoplanin, dalbavancin, clindamycin, ciprofloxacin, gentamicin, amikacin, kanamycin, tobramycin, minocycline, tetracycline, tigecycline, rifampin, trimethoprim-sulfamethoxazole, fusidic acid, linezolid and mupirocin. MICs for daptomycin were determined by E-test method. MICs for mupirocin resistant strains were further tested by the E-test method (AB Biodisk, Solna, Sweden) to determine high-level resistance. CLSI breakpoints were used for MIC interpretation except for fusidic acid and mupirocin . Fusidic acid breakpoints were interpreted according to the criteria of the Committee for Antimicrobial Testing of the French Society of Microbiology (CASFM) . 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).

Molecular typing

(i) Surface protein A (spa) typing

The *spa* typing was performed as previously described by Hallin M. *et al. spa* types were determined with Ridom StaphType software (www.ridom.de/staphtype/) and analysed by the Burp algorithm. . *Spa* types shorter than 5 repeats were considered as non-groupable and *spa* types were assigned to the same clonal complex (CC) if the cost is less than or equal to six.

(ii) SCCmec typing

The SCC*mec* type was determined by multiplex PCR as described by Oliveira *et al. U*ntypeable SCC*mec* type strains were further characterized for their *ccr* and *mec* complex by PCR.

(iii) Multi-locus sequence typing (MLST)

MLST was performed on selected MRSA strains (n = 20) belonging to the major epidemic types as previously described . 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).

Results

Hospital participation and bacterial strains

One hundred sixteen hospitals (65% of all sites) participated. They were located in Brussels (n = 13), Flanders (n =65) and Wallonia (n = 38). Among 347 isolates sent as MRSA to the Reference Laboratory, 337 MRSA strains (93%) were confirmed as such by multiplex PCR whereas 7 strains were identified as enterococci (n = 3) or as oxacillin susceptible *S. aureus* (n = 4). Three isolates did not grow after subculture. Among 227 MSSA isolates, the identification of 220 isolates (97%) was confirmed genotypically. Four isolates were identified as MRSA (n = 1) or as CoNS (n = 3). Three isolates did not grow after subculture. MRSA strains were recovered from screening, respiratory tract and wounds whereas MSSA strains were mainly isolated from wounds and respiratory tract (Table 1).

Table 1 Distribution of strains by sample category

Type of sample	MRSA	MSSA
	Number (%)	Number (%)
Blood	21 (6)	23 (10)
Wound	95 (27)	105 (46)
Respiratory tract	84 (24)	48 (21)
Nares	114 (33)	21 (9)
Urinary tract	19 (5)	9 (4)
Other	13 (4)	19 (8)
Unknown	1	2 (1)
Total	348 (100)	227(100)

Demographic data

The majority of case patients with MRSA infection or colonisation were elderly (> 60 years old) (Table 2). The median age of patients was 78 years old (range: 6-96 years). Patients were mainly

hospitalised in medical wards (31%), geriatric wards (22%), surgical wards (16%) or intensive care units (ICU) (14%). MRSA strains were recovered from MRSA screening at muco-cutaneous sites (33%), skin or soft tissue infections (27%), respiratory tract (24%), blood (6%), urine (5%) and other specimens (4%). The proportion of MRSA "imported acquisition" defined as MRSA isolates detected on the first 48h after admission was 36% in 2005.
The median age of patients with MSSA infection or colonisation was 69 years old (range: 1-98) (Table 2). Patients were mainly hospitalised in medical wards (25%), surgery wards (20%), ICU (13%) and geriatric wards (13%). MSSA were recovered from skin and soft tissue infections (46%), respiratory tract (21%), blood (10%), urine (4%) and other specimen (19%).

Table 2: Age and sex distribution of patients with *S. aureus*, 2005

Age group (years)	No patients with MRSA (% total by category)		No patients with MSSA (% of total by category)	
	Female	Male	Female	Male
<1	0	0	0	0
1-19	1	0	5	8
20-49	11	7	18	25
50-59	8	11	7	15
60-59	19	33	12	23
70-79	45	55	29	25
≥80	95	59	34	23
Total	179	165	105	119

Antimicrobial susceptibility

MICs for 21 antimicrobials were determined for 337 MRSA strains (Table 3 and 4, Figure 1 and 2). All MRSA isolates were susceptible to ceftobiprole, dalbavancin, daptomycin and tigecycline (Figure 1) with MIC_{50} and MIC_{90} of 1 and 2 mg/l for ceftobiprole; 0.12 and 0.25 mg/l for dalbavancin; 0.25 and 0.5 mg/ml for daptomycin; and 0.25 /l and 0.5 mg/l for tigecycline, respectively. All MRSA isolates were susceptible to glycopeptides, linezolid. More than 90% of strains were susceptible to cotrimoxazole (99%), rifampin (98%), fusidic acid (96%) and mupirocin (93%). Resistance to tetracycline (16%) was higher than for minocycline (10%). Resistance to MLS was frequent, ranging from 54% for erythromycin to 39% for clindamycin. For aminoglycosides, resistance was more frequent to kanamycin (39%) and tobramycin (41%) than to gentamicin (2%). Ninety-six percent of the strains of the strains were resistant to ciprofloxacin and 92% to moxifloxacin.

Two hundred and twenty three MSSA strains were tested for their susceptibility to 21 antimicrobials (Table 5 and 6, Figure 1 and 3). All MSSA strains were susceptible to ceftobiprole, dalbavancin, daptomycin and tigecycline with MIC50 and MIC90 of 0.5 and 0.5 mg/l for ceftobiprole, 0.12 and 0.12 mg/l for dalbavancin, 0.25 and 0.5 mg/l for daptomycin, 0.12 and 0.25 mg/l for tigecycline, respectively. All MSSA were susceptible to linezolid, rifampin and glycopeptides. Most of the strains were susceptible to cotrimoxazole, fusidic acid, aminoglycosides, ,tetracyclines and mupirocin. Resistance to MLS was frequent ranging from 22% to erythromycin to 5% to clindamycin. For quinolones, resistance was higher (13%) for ciprofloxacin than for moxifloxacin (6%). MSSA isolates were more susceptible to antimicrobials than MRSA. Most of MRSA strains were co-resistant to at least 2 antibiotics (Figure 4).

Table 3 : Cumulative proportions of MRSA isolates (n = 337) inhibited by increasing concentrations of antimicrobial agents

Antimicrobial agents	% 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	0.6	2.1	12.5	39.5	72.1	78.5	100	-
Ceftobiprole	0	0	6.8	29.4	86.1	100	100	100	100	100	100	100	100	-
Vancomycin	0	0	7.4	85.8	100	100	100	100	100	100	100	100	100	-
Teicoplanin	0	0.3	6.8	63.5	96.1	99.7	100	100	100	100	100	100	100	-
Dalbavancin	24.9	82.8	100	100	100	100	100	100	100	100	100	100	100	
Daptomycin	1.2	32.9	87.8	99.4	100	100	100	100	100	100	100	100	100	
Erythromycin	0.3	2.7	32.3	44.5	44.8	44.8	45.4	45.7	46.6	46.6	46.9	48.4	100	-
Clindamycin	32.6	59.9	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.8	60.8	100	-
Ciprofloxacin	0	0	0.90	3.0	3.3	3.6	3.9	7.4	20.2	23.7	36.8	55.2	100	-
Moxifloxacin	0.6	3.3	4.2	7.4	14.5	29.4	84.3	98.5	99.4	100	100	100	100	
Linezolid	0	0	0.3	0.3	34.1	100	100	100	100	100	100	100	100	-
Gentamicin	0.6	37.4	91.7	96.4	97.3	97.3	97.3	97.3	97.9	99.7	98.5	99.4	100	-
Tobramycin	3.3	44.8	56.7	57.9	58.2	58.2	58.2	59.3	61.7	64.1	74.5	90.8	100	-
Kanamycin	0	0.3	1.8	13.9	49.6	56.4	57.0	59.3	60.5	62.6	83.7	95.0	100	-
Minocycline	34.1	85.8	86.6	88.1	88.7	89.0	89.9	97.3	100	100	100	100	100	-
Tetracycline	0	7.4	77.7	78.0	82.8	83.4	84.0	85.5	86.6	88.1	96.4	96.4	100	-
Tigecycline	8.0	30.0	87.5	100	100	100	100	100	100	100	100	100	100	-
Rifampin	96.1	97.3	97.6	97.9	98.2	99.1	99.1	99.1	99.1	99.1	99.4	99.4	100	-
Cotrimoxazole	91.7	98.2	98.8	99.7	99.7	99.7	100	100	100	100	100	100	100	-
Fusidic acid	31.8	58.8	91.7	93.5	94.7	96.1	97.9	99.4	99.7	100	100	100	100	-
Mupirocin	0.9	19.6	83.4	92.3	92.6	92.6	93.5	93.8	94.7	94.7	94.7	95.0	94.9	100

Table 4 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 337 MRSA isolates by susceptibility category to 21 antimicrobial agents from Belgian Hospitals, 2005

Antimicrobial agents	Range (mg/l)	MIC50 (mg/l)	MIC90 (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	4 - >128	64	>128	0	0	100
Ceftobiprole	0.06 - 2	1	2	100	0	0
Vancomycin	0.25 – 1	0.5	1	100	0	0
Teicoplanin	0.12 – 4	0.5	1	100	0	0
Dalbavancin	0.06-0.25	0.12	0.25	100	0	0
Daptomycin	0.06-1	0.25	0.5	100	0	0
Erythromycin	0.06 - >128	>128	>128	44.5	0.9	54.6
Clindamycin	0.06 - >128	0.12	>128	60.5	0	39.5
Ciprofloxacin	0.25 - >128	128	>128	3.3	0.3	96.4
Moxifloxacin	0.06 - 32	4	8	7.4	7.1	85.5
Linezolid	0.5 – 2	2	2	100	0	0
Gentamicin	0.06 - >128	0.25	0.25	97.3	0	2.7
Tobramycin	0.06 - >128	0.25	128	58.2	1.2	40.7
Kanamycin	0.12 - >128	2	128	60.5	2.1	37.4
Minocycline	0.06 – 16	0.12	0.12	89.9	7.4	2.7
Tetracycline	0.12 - 128	0.25	64	84.0	1.5	14.5
Tigecycline	0.06 – 0.5	0.25	0.5	100	0	0
Rifampin	0.06 - >128	0.06	0.06	97.1	1.6	1.4
Cotrimoxazole	0.06 – 4	0.06	0.06	99.7	0	0.3
Fusidic acid	0.06 - 32	0.12	0.25	96.1	3.6	0.3
Mupirocin	0.06 - >1024	0.25	0.5	93.5	1.5	5.0

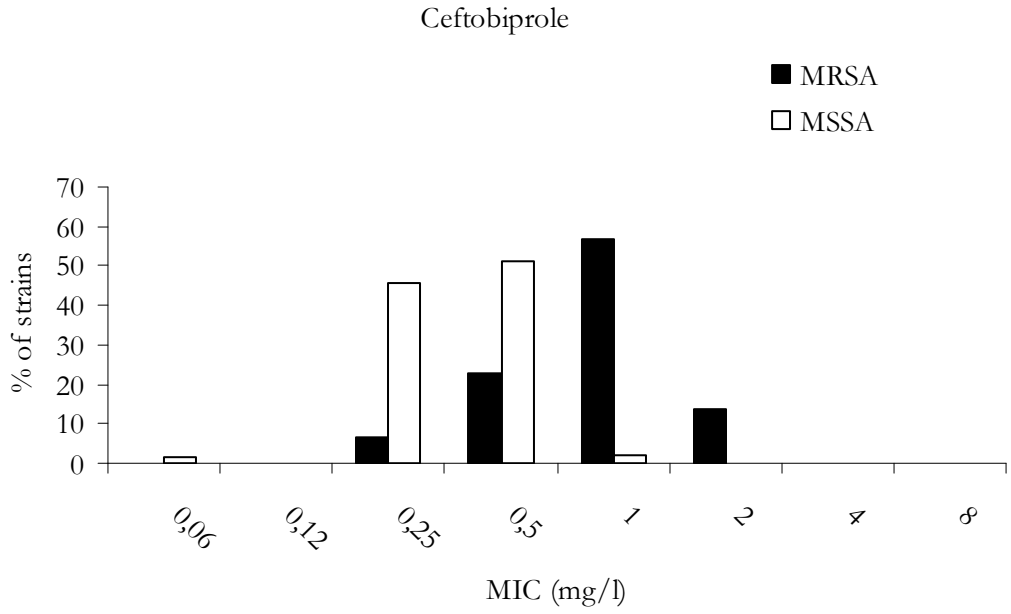
Table 5 : Cumulative proportions of MSSA isolates (n = 223) inhibited by increasing concentrations of antimicrobial agents

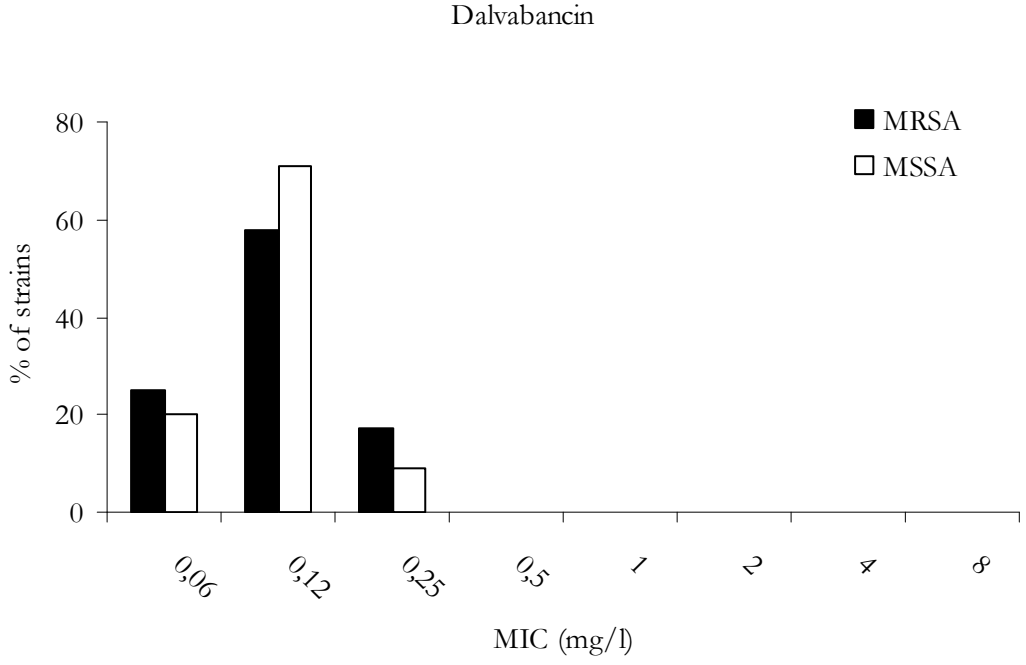
Antimicrobial agents	% of strains resistant at MIC (mg/l) of :													
	0.06	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**>1024**
Oxacillin	0	1.8	47.5	94.6	99.6	100	100	100	100	100	100	100	100	-
Ceftobiprole	1.3	1.3	47.1	98.2	100	100	100	100	100	100	100	100	100	-
Vancomycin	0	0	0	70.9	97.8	100	100	100	100	100	100	100	100	-
Teicoplanin	0	0.4	2.2	26.9	97.3	100	100	100	100	100	100	100	100	-
Dalbavancin	20.2	91.0	100	100	100	100	100	100	100	100	100	100	100	
Daptomycin	0.4	23.3	77.6	100	100	100	100	100	100	100	100	100	100	
Erythromycin	0.4	1.8	54.3	78.5	78.5	78.5	79.8	80.7	81.6	81.6	82.5	83.4	100	-
Clindamycin	55.6	95.5	95.5	95.5	95.5	95.5	95.5	95.5	95.5	95.5	95.5	95.5	100	-
Ciprofloxacin	0.5	1.4	12.0	64.5	87.6	92.6	93.5	93.5	94.9	95.4	96.8	99.1	100	-
Moxifloxacin	87.9	91.0	94.6	94.6	95.5	98.2	99.6	100	100	100	100	100	100	
Linezolid	0.5	0.5	0.5	2.7	48.6	100	100	100	100	100	100	100	100	-
Gentamicin	0.9	9.0	80.7	99.1	100	100	100	100	100	100	100	100	100	-
Tobramycin	0.4	12.6	94.2	98.2	99.1	99.1	99.1	99.6	99.6	99.6	99.6	99.6	100	-
Kanamycin	0	0	0.4	1.3	23.8	95.5	98.2	99.1	99.1	99.1	99.1	99.6	100	-
Minocycline	75.8	97.8	98.7	98.7	98.7	98.7	99.1	100	100	100	100	100	100	-
Tetracycline	14.8	38.1	75.3	92.8	96.4	96.4	96.4	96.4	96.9	98.2	99.6	100	100	-
Tigecycline	3.6	65.9	97.8	100	100	100	100	100	100	100	100	100	100	-
Rifampin	100	100	100	100	100	100	100	100	100	100	100	100	100	-
Cotrimoxazole	87.4	95.5	98.2	98.7	98.7	98.7	98.7	99.1	99.6	99.6	100	100	100	-
Fusidic acid	69.1	81.6	90.6	95.1	95.5	96.9	98.2	99.6	99.6	100	100	100	100	-
Mupirocin	0.4	8.1	73.1	97.8	97.8	97.8	98.2	98.2	98.2	98.2	98.2	98.2	98.2	100

Table 6 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 223 MSSA isolates by susceptibility category to 21 antimicrobial agents from Belgian Hospitals, 2005

Antimicrobial agents	Range (mg/l)	MIC50 (mg/l)	MIC90 (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	0.12 - 2	0.5	1	100	0	0
Ceftobiprole	0.06 - 1	0.5	0.5	100	0	0
Vancomycin	0.5 - 2	0.5	1	100	0	0
Teicoplanin	0.12 - 2	1	1	100	0	0
Dalbavancin	0.06 - 0.25	0.12	0.12	100	0	0
Daptomycin	0.06 - 0.5	0.25	0.5	100	0	0
Erythromycin	0.06 - >128	0.25	>128	78.1	1.3	20.5
Clindamycin	0.06 - >128	0.06	0.12	95.5	0	4.5
Ciprofloxacin	0.06 - >128	0.5	2	87.2	5.0	7.8
Moxifloxacin	0.06 - 8	0.06	0.12	94.2	0.9	4.9
Linezolid	0.06 - 2	2	2	100	0	0
Gentamicin	0.06 - 1	0.25	0.5	100	0	0
Tobramycin	0.06 - 8	0.25	0.25	99.1	0.4	0.4
Kanamycin	0.25 - >128	2	2	99.1	0	0.9
Minocycline	0.06 - 4	0.06	0.12	99.1	0	0.9
Tetracycline	0.06 - 128	0.25	0.5	96.4	0	3.6
Tigecycline	0.06 - 0.5	0.12	0.25	100	0	0
Rifampin	0.06 - 0.06	0.06	0.06	100	0	0
Cotrimoxazole	0.06 - 64	0.06	0.12	98.7	0	1.3
Fusidic acid	0.06 - 32	0.06	0.25	96.4	3.1	0.4
Mupirocin	0.06 - >1024	0.25	0.5	98.2	0	1.8

Figure 1: MIC distribution by antimicrobial for 337 MRSA and 223 MSSA isolates, Belgian hospital survey, 2005





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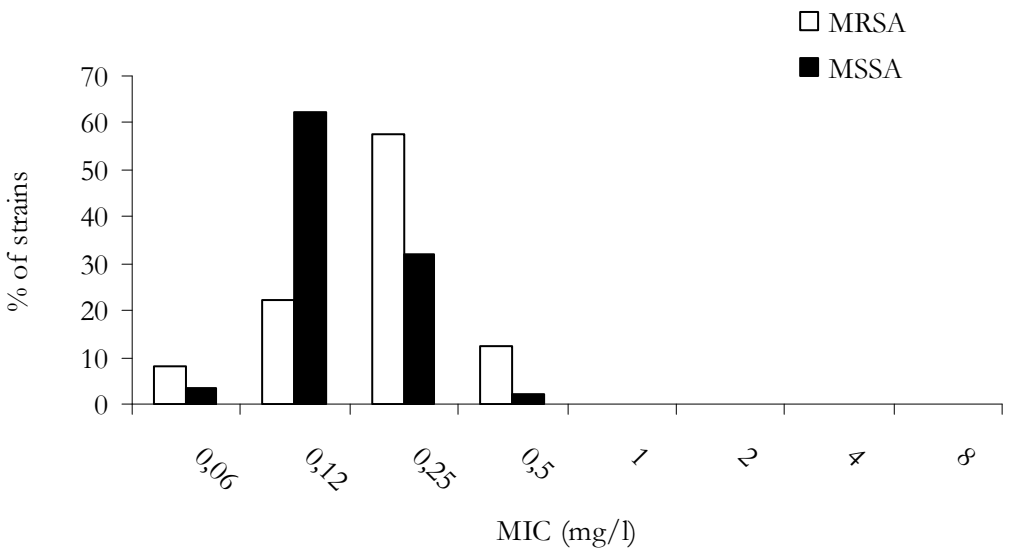


Daptomycine

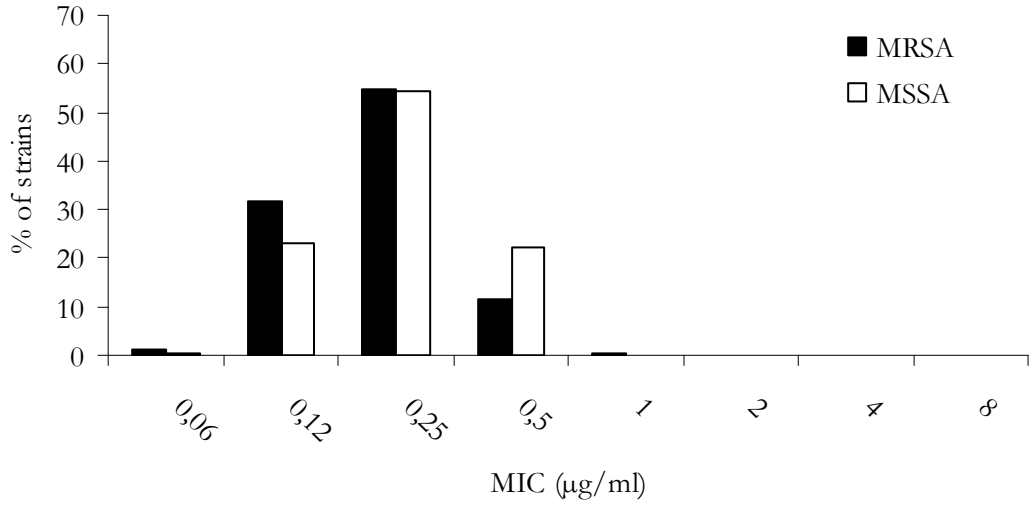
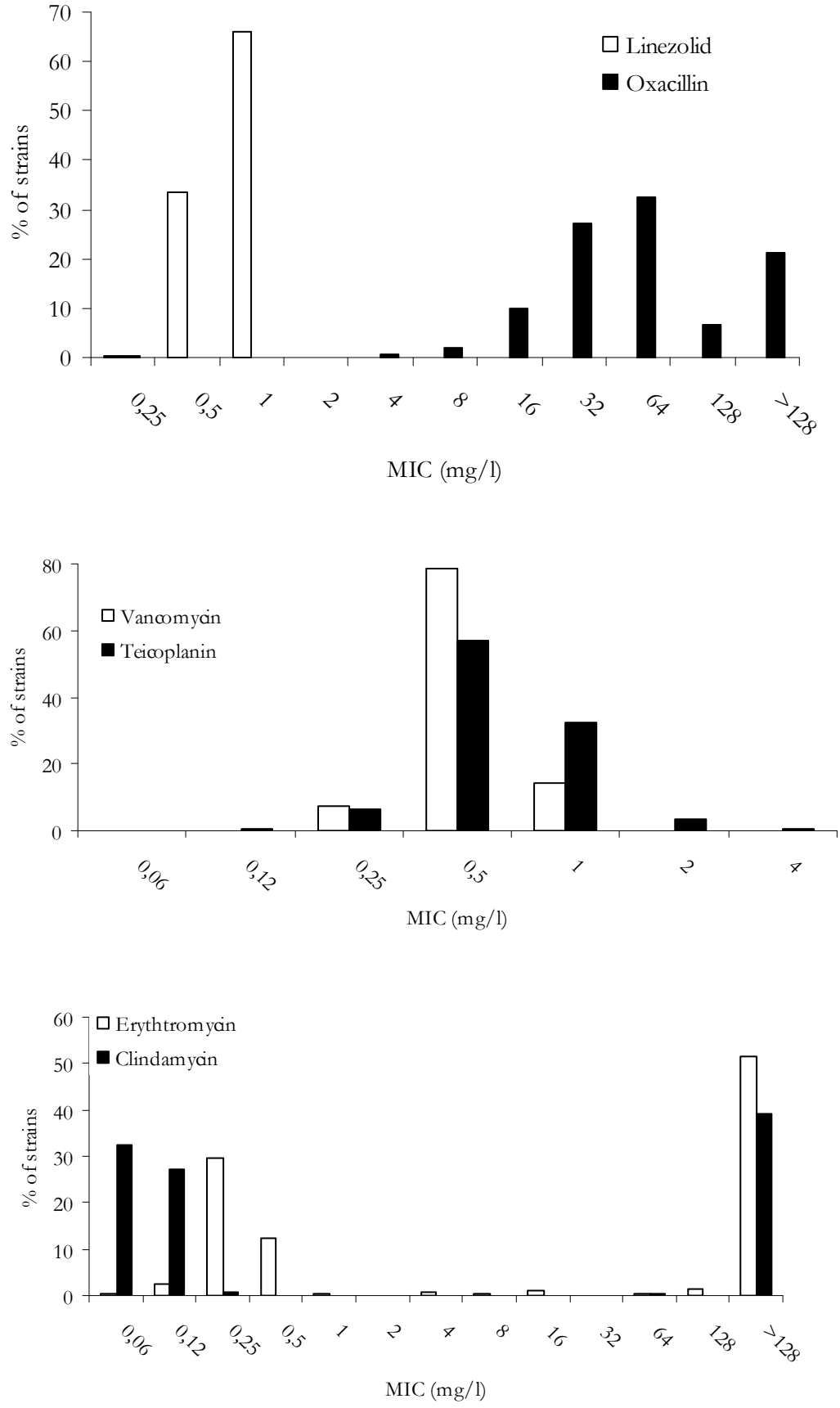
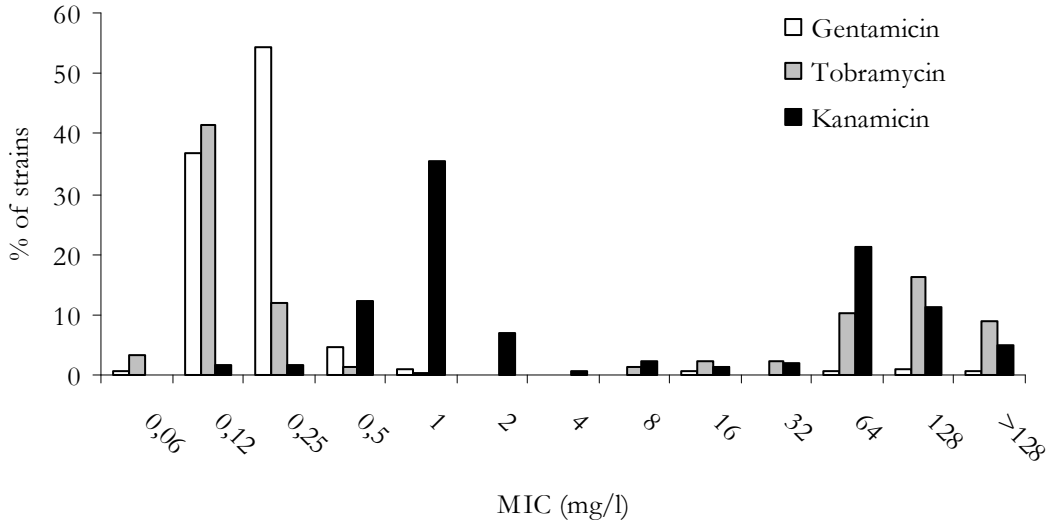
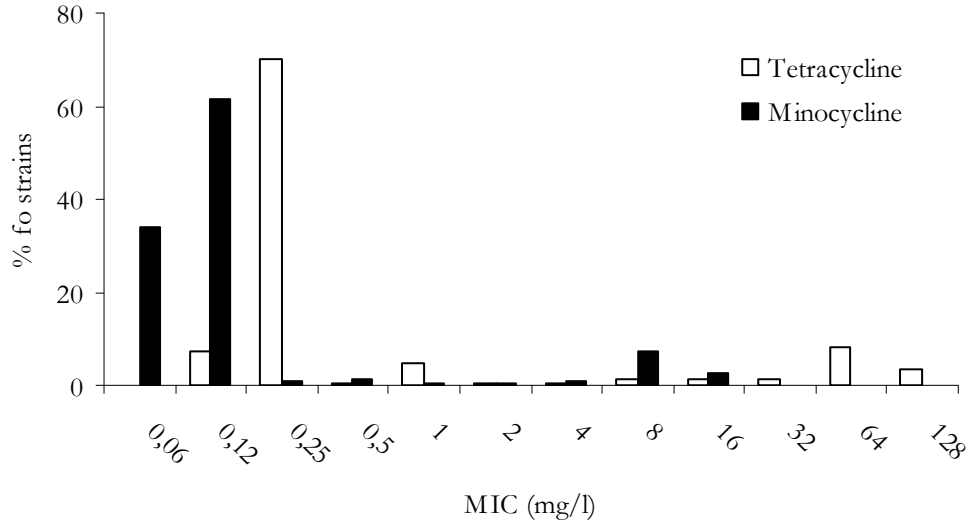
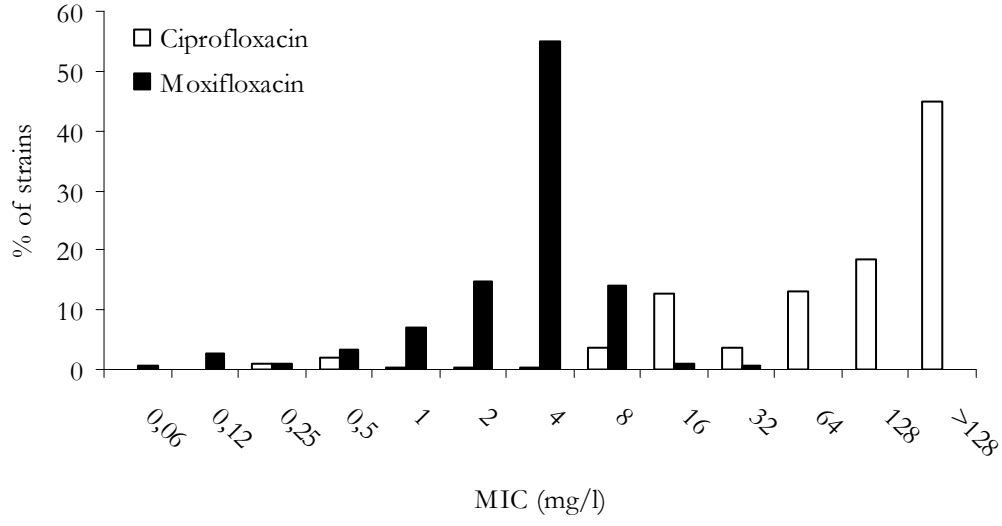


Figure 2: MIC distribution by antimicrobial for 337 MRSA isolates, Belgian hospital survey, 2005




60
50
40
30
20
10
0
% of strains
Gentamicin
Tobramycin
Kanamicin
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


80
60
40
20
0
% fo strains
Tetracycline
Minocycline
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
MIC (mg/l)


60
50
40
30
20
10
0
% of strains
Ciprofloxacin
Moxifloxacin
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)

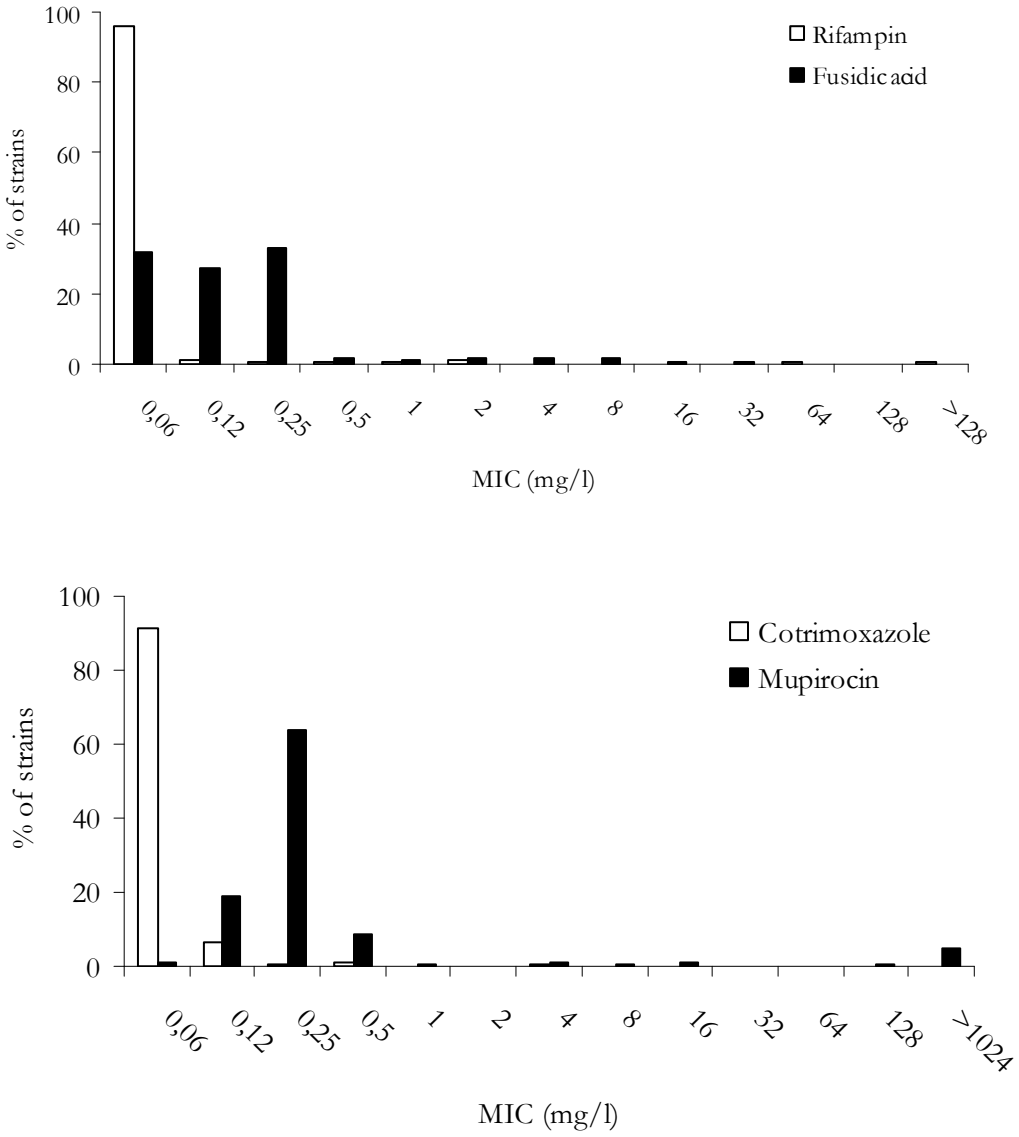
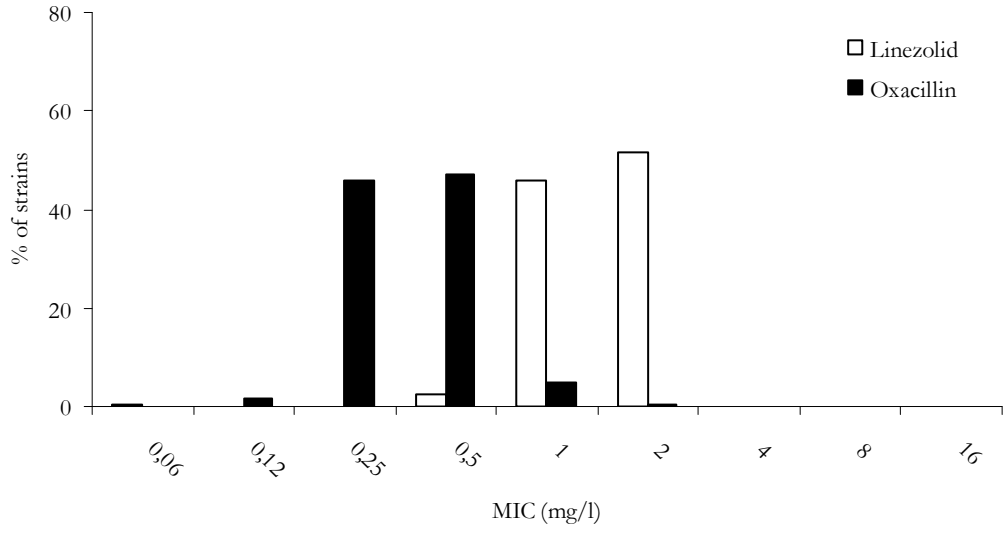
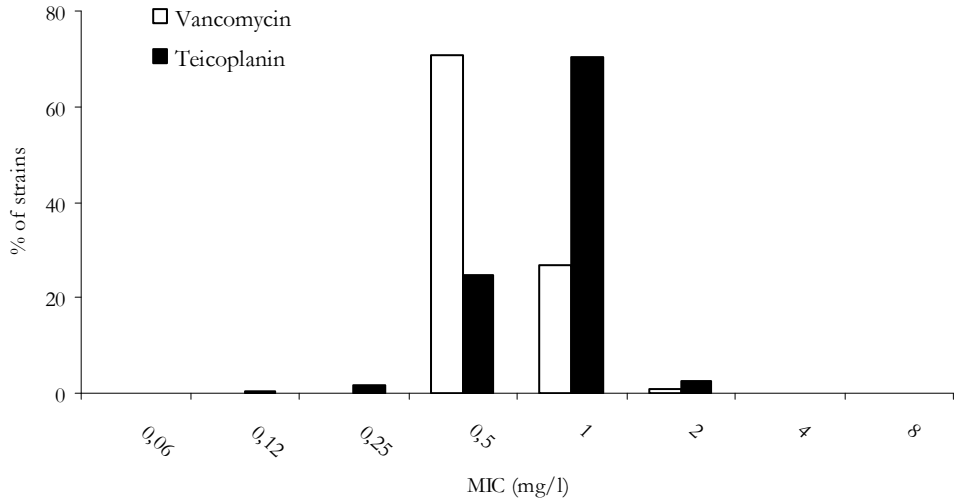
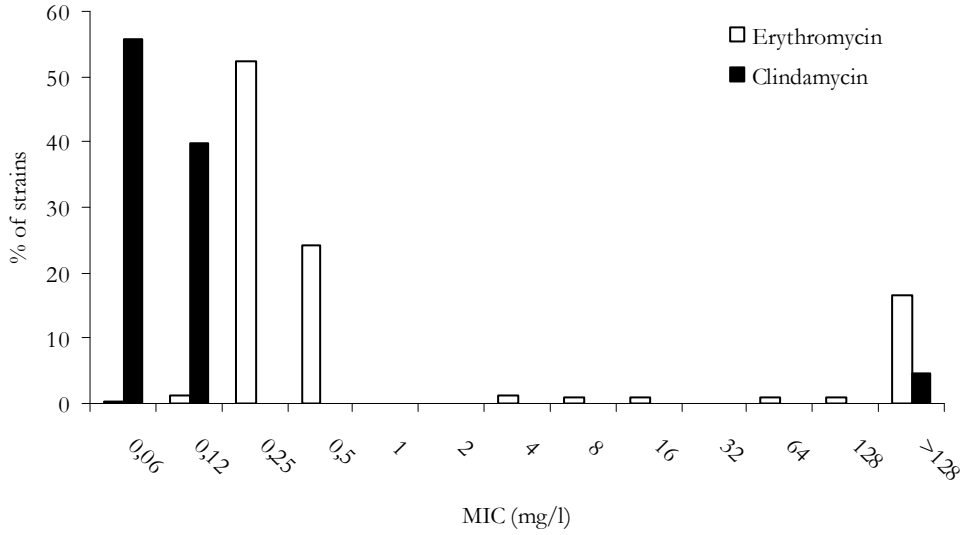
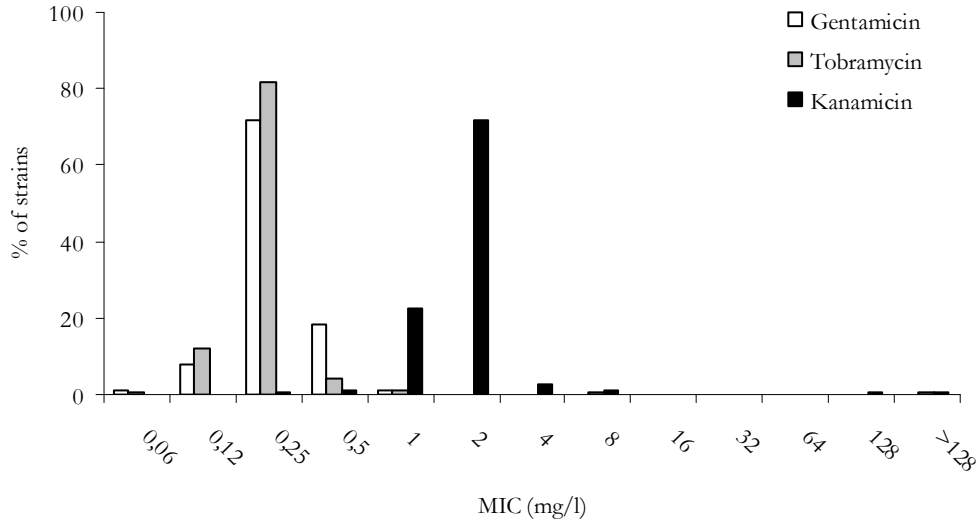


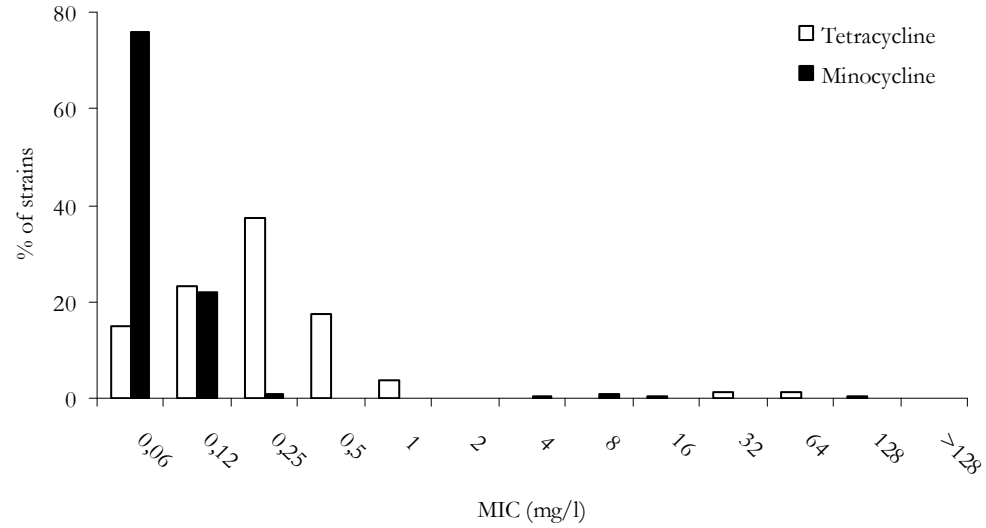
Figure 3 : MIC distribution by antimicrobial for 223 MSSA isolates, Belgian hospital survey, 2005

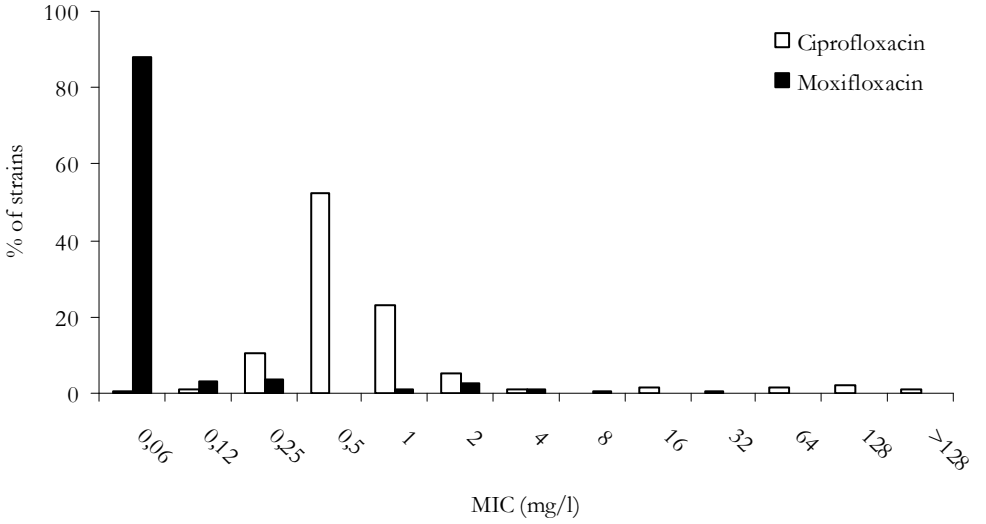


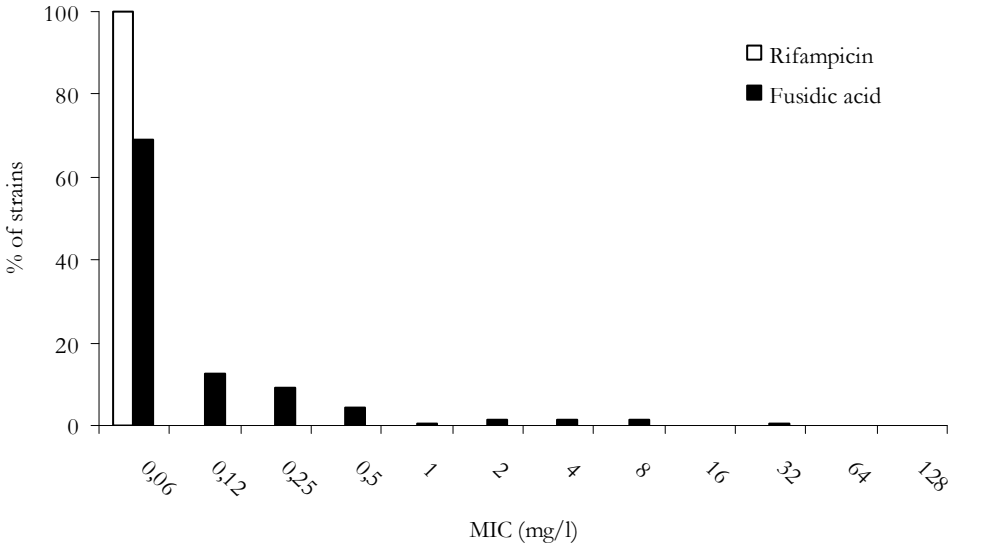

Vancomycin
Teicoplanin
% of strains
80
60
40
20
0
0,06
0,12
0,25
0,5
1
2
4
8
MIC (mg/l)


Erythromycin
Clindamycin
% of strains
60
50
40
30
20
10
0
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


Gentamicin
Tobramycin
Kanamicin
% 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 (mg/l)


80
60
40
20
0
% of strains
Tetracycline
Minocycline
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


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


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

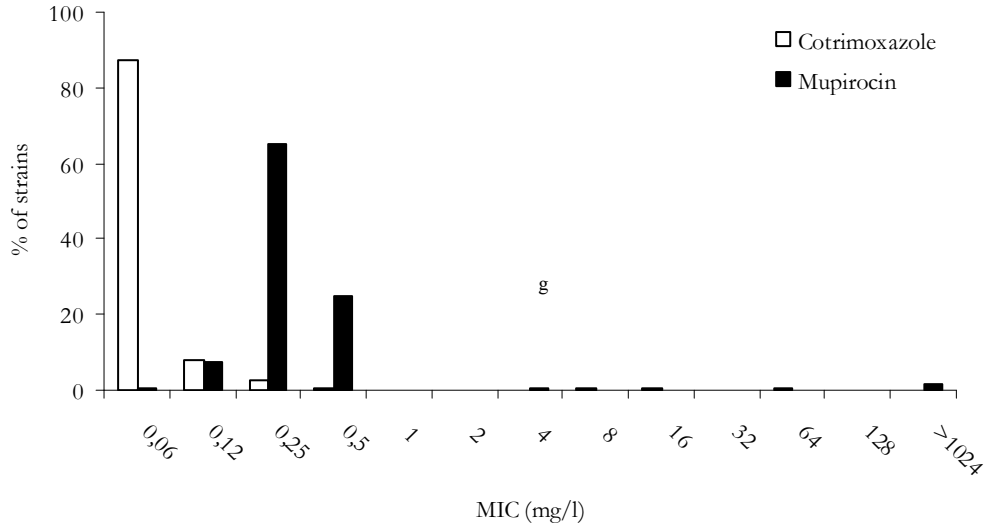
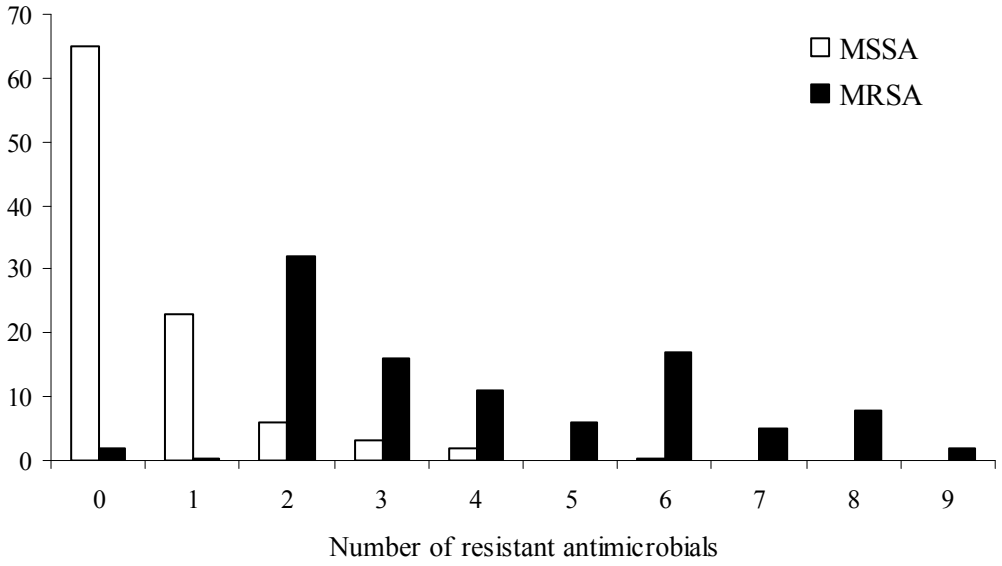


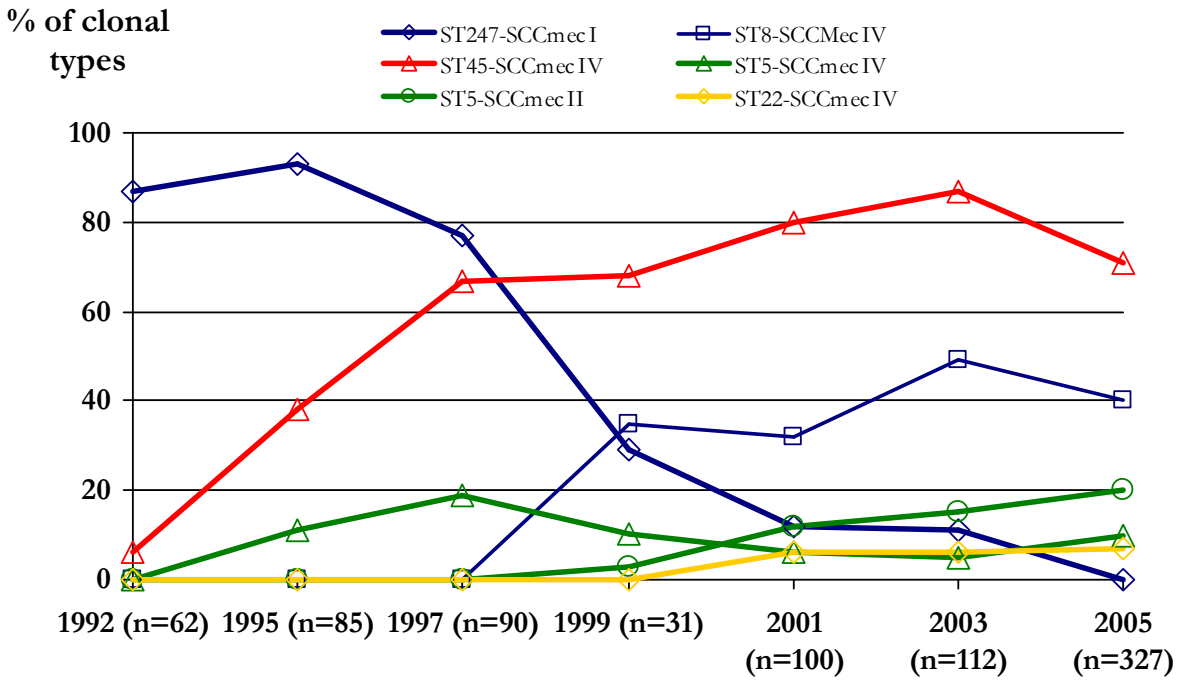
Figure 4: Comparison of co-resistance to non beta-lactam drugs MSSA versus MRSA isolates from hospitalised patients, Belgium, 2005



Genotype distribution

SCC*mec* were classified into type IV (82.0%), type II (13.9%), type I (2.2%) and type V (0.3%). By *spa* typing, 86% of MRSA strains belonged to 5 epidemic clones: *spa* CC38 (formerly PFGE type B2) ST45-SCC*mec* IV (44.6%); *spa* CC8 (formerly PFGE type A20) ST8-SCC*mec* IV (21.1%), *spa* CC2 (formerly PFGE type G10) ST5-SCC*mec* II (9.2%), *spa* CC2 (formerly PFGE type C3) ST5-SCC*mec* IV (4.3%) et *spa* CC790 (formerly PFGE type L1) ST22-SCC*mec* IV (3.7%). They were found in 79 (68%), 49 (42%), 31 (27%), 12 (10%) et 8 (7%) hospitals, respectively (Figure 5). Two isolates were PVL-positive and belonged to community-acquired MRSA (CAMRSA) clone ST80-SCC*mec* IV.

Figure 5: Distribution of Epidemic MRSA PFGE Types National Surveillance, Belgium, 1992-2005



Conclusions

1. The new antimicrobial drugs ceftobiprole, dalbavancin and tigecycline showed excellent activities against S. aureus isolates recovered from hospitalised patients in Belgian hospitals regardless methicillin resistance.

2. No *S. aureus* isolate resistant to linezolid or glycopeptides was found.

3. A high proportion of MRSA isolates were resistant to quinolones (>90%) and to MLS (>50%).

4. Resistance to MLS (>20%) and quinolones (>10%) was frequent in MSSA isolates

5. MSSA isolates were more susceptible to antimicrobials than MRSA isolates

6. MRSA strains belonged to international MRSA clones with a predominance of ST45-SCC*mec* IV and ST8-SCC*mec* IV.

7. We observed an increasing proportion of MRSA strains belonging to ST5-SCC*mec* II clone closely related to the "New-York/Japan clone"

8. Few MRSA isolates belonged to European CA-MRSA clone PVL-positive ST80-SCC*mec* IV

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