Microbiological Surveillance of *Staphylococcus aureus* in Belgian Hospitals in 2008

Final report

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Introduction

Staphylococcus aureus is a leading cause of skin and soft tissue infections, surgical site and catheter infections, 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 2005, we observed the predominance of 7 major clones belonging to the five pandemic MRSA lineages (CC 5, 8, 22, 30 and 45) that are causing nosocomial infections worldwide.

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 2008 in 108 Belgian hospitals.

Materials and methods

Survey methods and collection of bacterial strains

From January to December 2008, laboratories of all Belgian acute-care hospital (n = 155) were invited to collect up to non-duplicate MRSA (n = 3) and MSSA (n =2) isolates recovered consecutively in hospitalised patients from any body site. These strains were sent to the Reference Laboratory and stored at –80°C until testing.

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.

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 testing

Minimal inhibitory concentrations (MIC) were determined by the agar dilution method (with a test dilution ranging from 0.06 to 128 mg/l) according to CLSI guidelines for oxacillin, clindamycin, ciprofloxacin, gentamicin, kanamycin, tobramycin, minocycline, tetracycline, rifampin, trimethoprim-sulfamethoxazole, fusidic acid, linezolid and mupirocin. MICs to vancomycin and teicoplanin were determined by the broth microdilution method ranging from 0.06 to 64 mg/l, according to CLSI guidelines.

MICs for mupirocin resistant strains were further tested by the E-test method (AB Biodisk, Solna, Sweden) to determine high-level resistance.

CLSI breakpoints (CLSI M100-S19, 2009) 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).

Glycopeptide hetero-resistance testing: all MRSA isolates were tested by teicoplanin screen agar method. The Etest macromethod was performed on isolates showing growth after 24 or 48 h incubation. Strains with vancomycin and teicoplanin MICs ≥ 8 mg/l or teicoplanin MIC ≥ 12 mg/l were considered to be putative hetero-GISA.

Molecular typing

(i) Surface protein A (spa) typing

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 four.

(ii) SCCmec typing

SCC*mec* type was tested by multiplex PCR for *ccr* and *mec* complex as described by Kondo *et al.*

(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). Allelic profiles were determined by comparison with those recorded in the MLST database (http://www.mlst.net).

(iv) Resistance and toxin gene profile

Resistance genes to aminoglycosides, tetracyclines, macrolides-lincosamides-streptogramins and mupirocin, and genes encoding PVL and TSST-1 were determined by multiplex PCR.

Results

Hospital participation

One hundred nine hospitals (70% of all sites) participated. They were located in Brussels (n = 18), Flanders (n = 51) and Wallonia (n = 39).

Bacterial strains

Among 323 isolates sent as MRSA to the Reference Laboratory, 314 strains (97%) were confirmed as MRSA by multiplex PCR whereas 7 strains were identified as coagulase negative staphylococci (CoNS) (n = 4), as methicillin susceptible *S. aureus* (MSSA) (n = 2) and borderline oxacillin susceptible *S. aureus* (n = 1). Two isolates did not grow after subculture. Among 218 MSSA isolates, the identification of 211 isolates (97%) was confirmed genotypically. Six isolates were identified as MRSA (n = 2) or as CoNS (n = 4).

MRSA isolates were recovered from MRSA screening at muco-cutaneous sites (45%), skin or soft tissue (23%), respiratory tract (13%), blood (4%), urine (5%) and other specimens (10%). MSSA isolates were recovered from skin and soft tissues (36%), respiratory tract (21%), blood (11%), urine (1%) and other specimens (23%).(Table 1).

Table 1 Distribution of S.aureus strains by methicillin resistance and specimen category

Type of specimen	MRSA Number (%)	MSSA Number (%)
Blood	11 (3)	23 (11)
Skin and soft tissue	74 (23)	75 (36)
Respiratory tract	40 (13)	45 (21)
Nares	141 (45)	14 (7)
Urinary tract	17 (5)	3 (1)
Other	31 (10)	50 (24)
Unknown	0 (0)	1 (0)
Total	314 (100)	211 (100)

Demographic data

The proportion of MRSA "imported acquisition" defined as MRSA isolates detected on the first 48 h after admission was 52% .

The majority of patients with MRSA infection or colonisation were elderly (> 60 years old) (Table 2). The median age of patients was 79 years old (range: <1-101 years). Patients were mainly hospitalised in medical wards (33%), geriatric wards (23%), surgical wards (16%) or intensive care units (ICU) (13%).

The median age of patients with MSSA infection or colonisation was 61 years old (range: <1-95) (Table 2). Patients were mainly hospitalised in medical wards (24%), surgery wards (20%), ICU (11%) and geriatric wards (9%).

Table 2: Age and sex distribution of patients with *S. aureus*, 2008

Age group (years)	No patients with MRSA (% total by category)		No patients with MSSA (% of total by category)	
	Female	Male	Female	Male
<1	1 (1)	1 (1)	6 (7)	6 (5)
1-19	1 (1)	1 (1)	7 (8)	10 (8)
20-49	19 (9)	11 (7)	24 (26)	23 (19)
50-59	7 (4)	19 (13)	10 (11)	15 (12)
60-69	12 (7)	17 (11)	11 (12)	27 (22)
70-79	41 (25)	44 (29)	17 (19)	24 (20)
≥80	87 (53)	58 (38)	16 (18)	17 (14)
Total	163	151	91	122

Antimicrobial susceptibility

MICs for 19 antimicrobials were determined for 314 MRSA strains (Table 3 and 4, Figure 1 and 2).

All MRSA isolates were susceptible to glycopeptides, cotrimoxazole, rifampin, tigecycline and linezolid. More than 90% of strains were susceptible to gentamicin (99%), minocycline (99%), mupirocin (94%), tetracycline (93%) and fusidic acid (92%). Resistance to MLS was frequent, ranging from 51% for erythromycin to 35% for clindamycin. For aminoglycosides, resistance was more frequent to kanamycin (44%) and tobramycin (40%) than to gentamicin (1%). Ninety-five percent of the strains of the strains were resistant to ciprofloxacin and 92% to moxifloxacin.

Two hundred and twelve MSSA strains were tested for their susceptibility to 19 antimicrobials (Tables 5 and 6, Figures 1 and 2).

All MSSA were susceptible to gentamicin, cotrimoxazole, minocycline, linezolid, rifampin and glycopeptides. Most of the strains were susceptible to clindamycin, fusidic acid, aminoglycosides, tetracyclines and mupirocin. Resistance to MLS was frequent ranging from 22% to erythromycin to 3% to clindamycin. For quinolones, resistance rate was higher for ciprofloxacin (9%) than for moxifloxacin (8%). MSSA isolates were more frequently susceptible to these antimicrobials than MRSA.

Table 3 : Cumulative proportions of MRSA isolates (n = 314) inhibited by increasing concentrations of antimicrobial agents, Belgium, 2008

Antimicrobial agents	% of strains inhibited 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	0.3	0.3	0.3	0.3	0.3	3.5	9.2	34.7	73.2	82.8	100	-
Vancomycin	0	0	1.3	79.3	100	100	100	100	100	100	100	100	100	-
Teicoplanin	0	0.3	8.6	80.9	99.0	100	100	100	100	100	100	100	100	-
Erythromycin	2.2	13.4	37.6	49.0	49.7	49.7	50.3	51.6	51.9	52.9	53.5	59.6	100	-
Clindamycin	37.9	61.1	65.0	65.0	65.0	65.0	65.0	65.0	65.3	65.3	65.3	66.2	100	-
Ciprofloxacin	0	0.3	2.2	4.8	5.1	5.1	5.7	8.3	19.1	22.9	37.9	59.6	100	-
Moxifloxacin	3.8	5.7	6.7	8.0	17.8	47.1	90.1	100	100	100	100	100	100	-
Linezolid	0	0	0	0.3	41.7	100	100	100	100	100	100	100	100	-
Gentamicin	1.0	42.4	99.0	99.4	99.4	99.4	99.4	99.4	99.7	100	100	100	100	-
Tobramycin	3.2	47.1	56.4	57.3	57.6	57.6	60.5	61.1	62.1	62.4	66.9	89.5	100	-
Kanamycin	0	0	0	0.3	45.5	51.6	55.1	55.4	56.1	56.1	75.5	95.5	100	-
Minocycline	63.4	71.0	95.5	96.5	96.8	97.8	98.7	100	100	100	100	100	100	-
Tetracycline	1.6	38.9	56.4	85.0	88.5	89.8	93.0	94.6	95.2	95.9	98.1	99.7	100	-
Tigecycline	0.3	2.9	82.5	100	100	100	100	100	100	100	100	100	100	-
Rifampin	99.7	100	100	100	100	100	100	100	100	100	100	100	100	-
Cotrimoxazole	94.9	100	100	100	100	100	100	100	100	100	100	100	100	-
Fusidic acid	13.1	54.8	89.2	90.4	91.1	92.4	93.9	99.4	99.7	100	100	100	100	-
Mupirocin	8.9	66.9	91.7	93.0	93.3	93.3	93.6	95.5	96.8	96.8	96.8	96.8	97.1	100

Table 4 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 314 MRSA isolates by susceptibility category to 18 antimicrobial agents, Belgium, 2008

Antimicrobial agents	Range (mg/l)	MIC50 (mg/l)	MIC90 (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	0.25 – >128	64	>128	0.3	0	99.7
Vancomycin	0.25 – 1	0.5	1	100	0	0
Teicoplanin	0.12 – 2	0.5	1	100	0	0
Erythromycin	0.06 – >128	4	>128	49	1.3	49.7
Clindamycin	0.06 – >128	0.12	>128	65	0	35
Ciprofloxacin	0.12 – >128	128	>128	5.1	0	94.9
Moxifloxacin	0.06 – 8	4	4	8	9.9	82.1
Linezolid	0.5 – 2	2	2	100	0	0
Gentamicin	0.06 – 32	0.25	0.25	99.4	0	0.6
Tobramycin	0.06 – >128	0.25	>128	60.5	0.6	38.9
Kanamycin	0.5 – >128	2	128	56.1	0	43.9
Minocycline	0.06 – 8	0.06	0.25	98.7	1.3	0
Tetracycline	0.06 – >128	0.25	4	93	1.6	5.4
Tigecycline	0.06 – 0.5	0.25	0.25	100	0	0
Rifampin	0.06 – 0.12	0.06	0.06	100	0	0
Cotrimoxazole	0.06 – 0.12	0.06	0.06	100	0	0
Fusidic acid	0.06 – 32	0.12	0.5	92.4	7.3	0.3
Mupirocin	0.06 – >1024	0.12	0.25	93.6	3.5	2.9

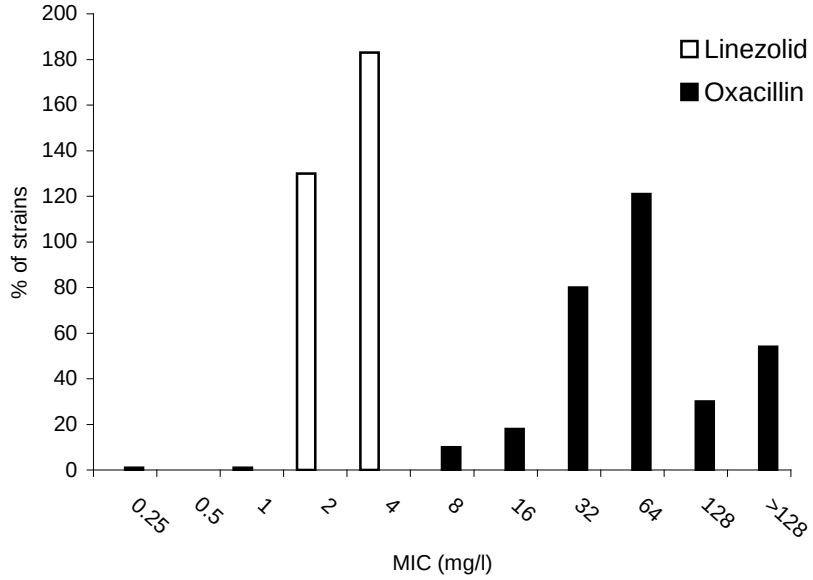
Table 5 : Cumulative proportions of MSSA isolates (n = 212) inhibited by increasing concentrations of antimicrobial agents, Belgium, 2008

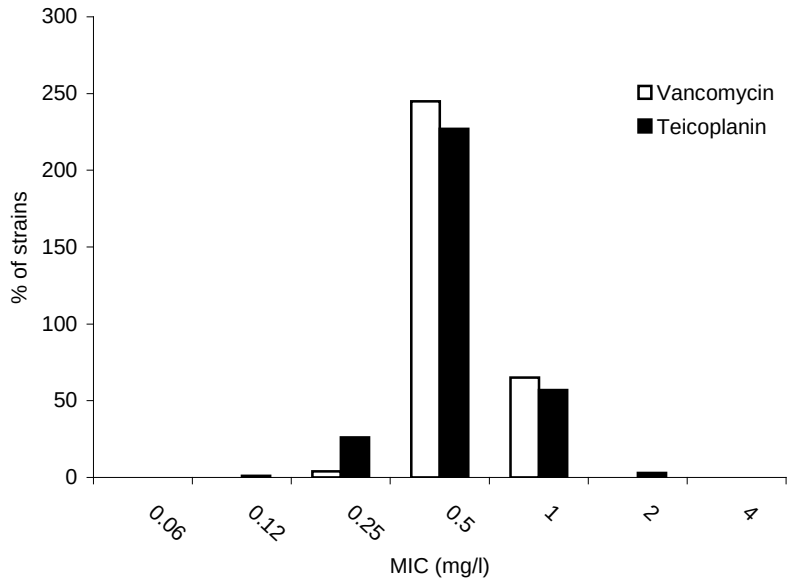
Antimicrobial agents	% of strains inhibited at MIC (mg/l) of :													
	0.06	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**>1024**
Oxacillin	0.9	9.9	74.1	99.1	100	100	100	100	100	100	100	100	100	-
Vancomycin	0.5	0.5	23.1	91.5	100	100	100	100	100	100	100	100	100	-
Teicoplanin	0	0.5	7.5	74.1	98.6	100	100	100	100	100	100	100	100	-
Erythromycin	0.5	19.8	62.3	78.3	79.7	80.7	82.5	84.4	85.4	86.9	88.2	93.4	100	-
Clindamycin	54.7	80.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7	97.6	100	-
Ciprofloxacin	0.5	2.4	34.9	89.6	90.6	91.0	91.5	92.0	92.5	92.9	95.8	98.6	100	-
Moxifloxacin	80.7	91.5	92.0	92.0	93.4	97.2	99.5	100	100	100	100	100	100	-
Linezolid	0	0	0	0	27.4	100	100	100	100	100	100	100	100	-
Gentamicin	0.9	41.0	99.1	100	100	100	100	100	100	100	100	100	100	-
Tobramycin	4.2	33.5	95.8	97.2	97.6	97.6	99.1	99.5	99.5	99.5	99.5	100	100	-
Kanamycin	0	0	0	1.9	74.5	96.7	98.6	98.6	99.1	99.1	99.5	99.5	100	-
Minocycline	90.1	96.2	99.1	99.5	99.5	100	100	100	100	100	100	100	100	-
Tetracycline	0.9	23.1	54.2	94.8	94.8	94.8	95.8	96.7	97.2	98.1	99.5	100	100	-
Tigecycline	16.0	42.9	96.7	99.1	100	100	100	100	100	100	100	100	100	-
Rifampin	100	100	100	100	100	100	100	100	100	100	100	100	100	-
Cotrimoxazole	97.6	98.6	98.6	99.5	100	100	100	100	100	100	100	100	100	-
Fusidic acid	45.3	64.2	80.7	96.2	96.2	96.2	97.2	98.1	98.1	98.6	99.1	99.5	100	-
Mupirocin	1.9	55.2	92.9	98.6	98.6	98.6	98.6	98.6	98.6	98.6	98.6	98.6	98.6	100

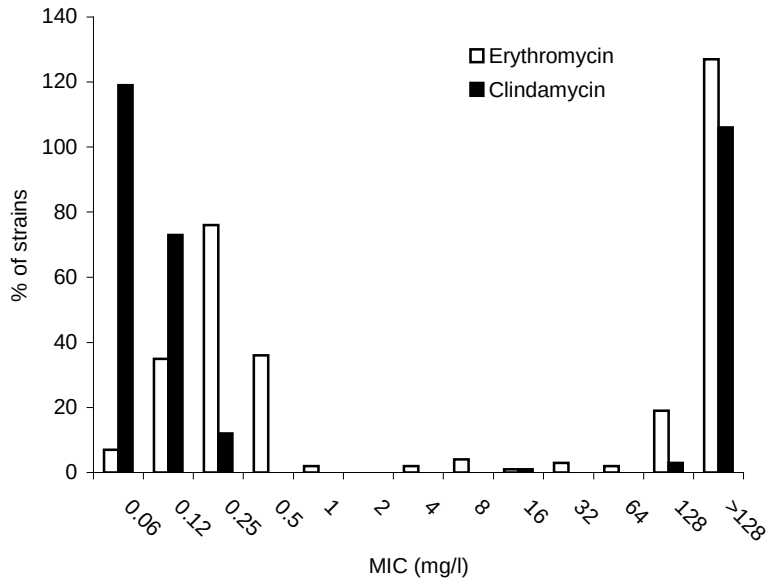
Table 6 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 212 MSSA isolates by susceptibility category to 18 antimicrobial agents, Belgium, 2008

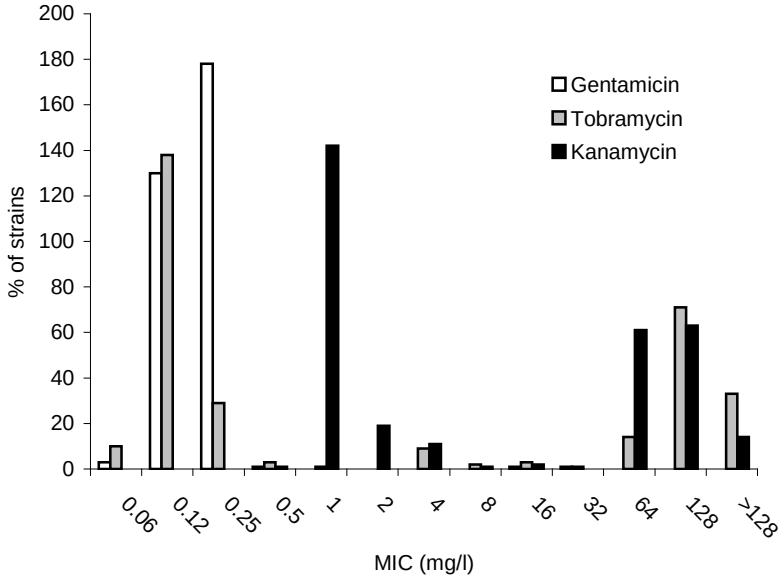
Antimicrobial agents	Range (mg/l)	MIC50 (mg/l)	MIC90 (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	0.06 – 1	0.25	0.5	100	0	0
Vancomycin	0.06 – 1	0.5	0.5	100	0	0
Teicoplanin	0.12 – 2	0.5	1	100	0	0
Erythromycin	0.06 – >128	0.25	128	78.3	4.2	17.5
Clindamycin	0.06 – >128	0.06	0.25	96.7	0	3.3
Ciprofloxacin	0.06 – >128	0.5	1	90.6	0.4	9.0
Moxifloxacin	0.06 – 8	0.06	0.12	92.0	1.4	6.6
Linezolid	1 – 2	2	2	100	0	0
Gentamicin	0.06 – 0.5	0.25	0.25	100	0	0
Tobramycin	0.06 – 128	0.25	0.25	99.1	0.4	0.4
Kanamycin	0.5 – >128	1	2	99.1	0	0.9
Minocycline	0.06 – 2	0.06	0.06	100	0	0
Tetracycline	0.06 – 128	0.25	0.5	95.8	0.9	3.3
Tigecycline	0.06 – 1	0.25	0.25	99.1	0	0.9
Rifampin	0.06 – 0.06	0.06	0.06	100	0	0
Cotrimoxazole	0.06 – 1	0.06	0.06	100	0	0
Fusidic acid	0.06 – >128	0.12	0.5	96.2	1.9	1.9
Mupirocin	0.06 – >1024	0.12	0.25	98.6	0	1.4

Figure 1 : MIC distribution by antimicrobial for 314 MRSA isolates, Belgian hospital survey, 2008





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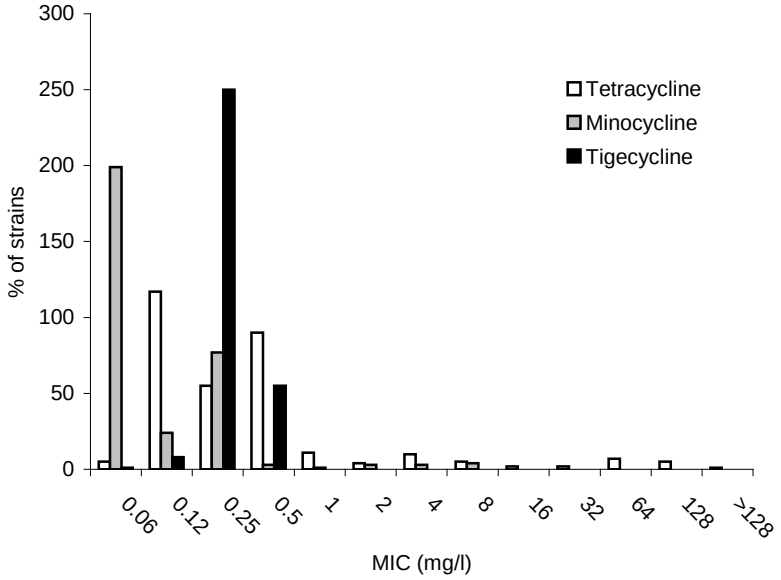
180
160
140
120
100
80
60
40
20
0
% of strains
Gentamicin
Tobramycin
Kanamycin
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)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250
200
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100
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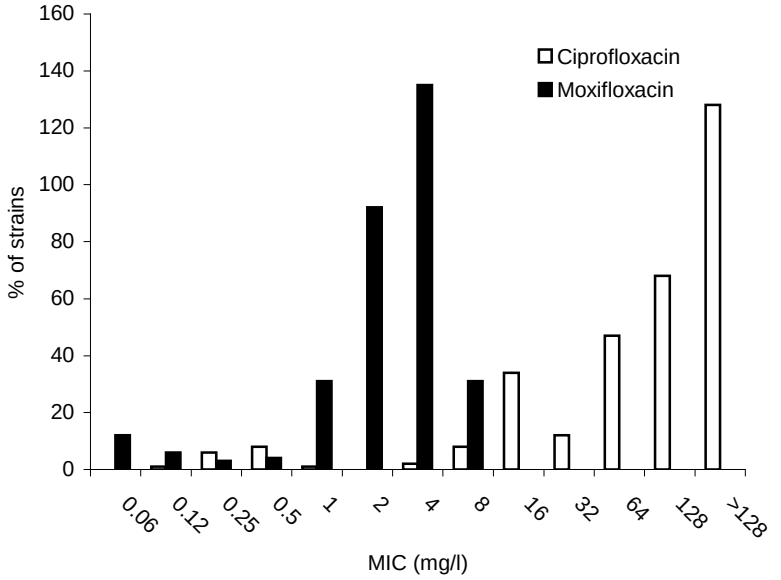
0
% of strains
Tetracycline
Minocycline
Tigecycline
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)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140
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20
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% of strains
Ciprofloxacin
Moxifloxacin
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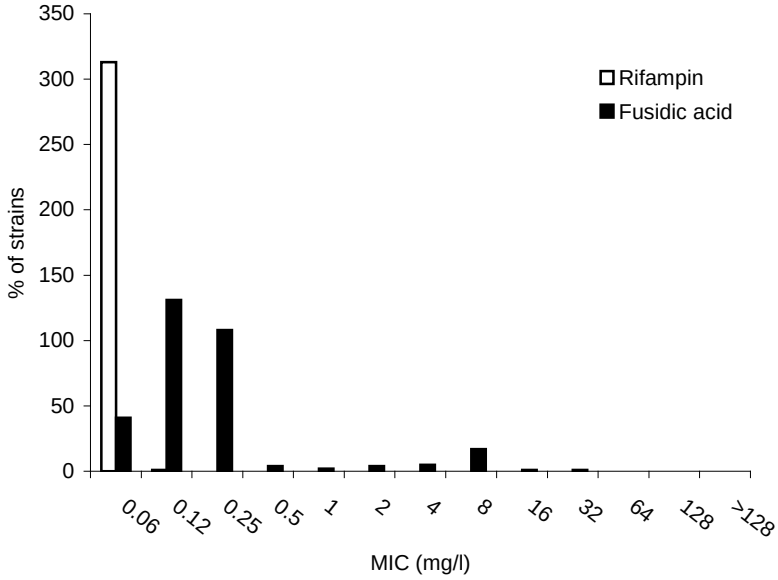
64
128
>128
MIC (mg/l)


350
300
250
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50
0
% of strains
Rifampin
Fusidic acid
0.06
0.12
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1
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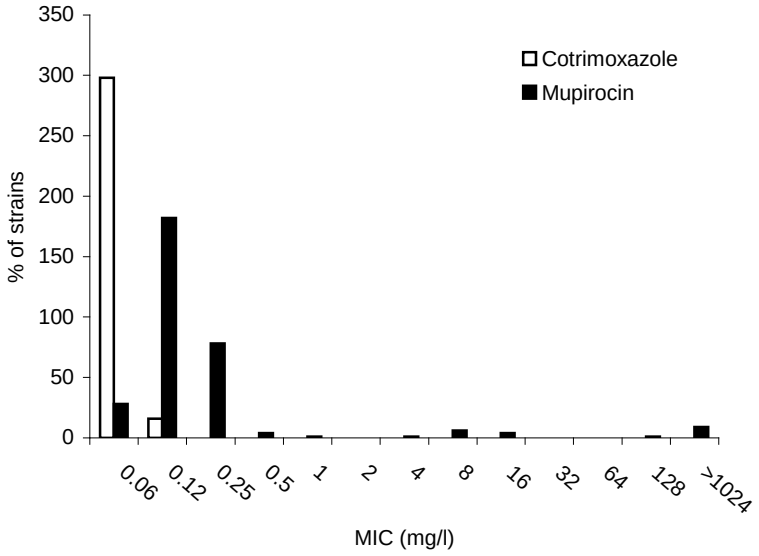
64
128
>128
MIC (mg/l)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50
0
% of strains
Cotrimoxazole
Mupirocin
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0.12
0.25
0.5
1
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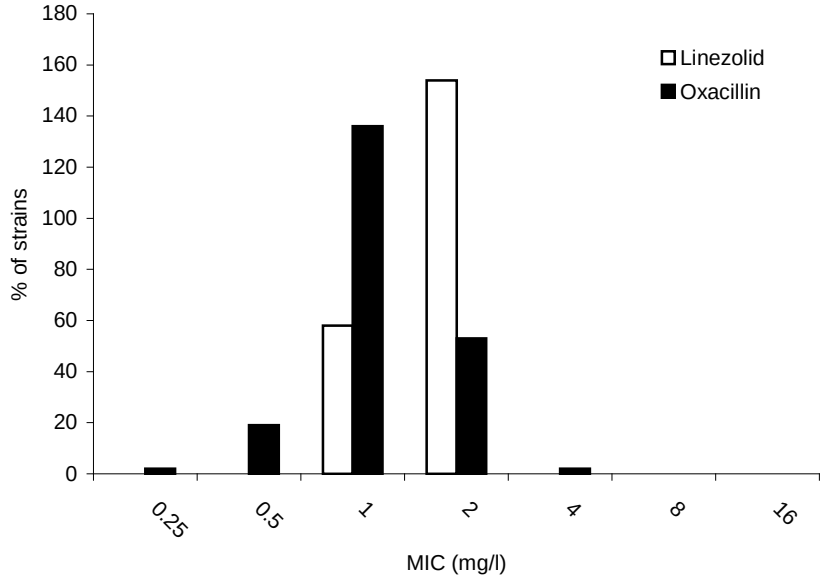
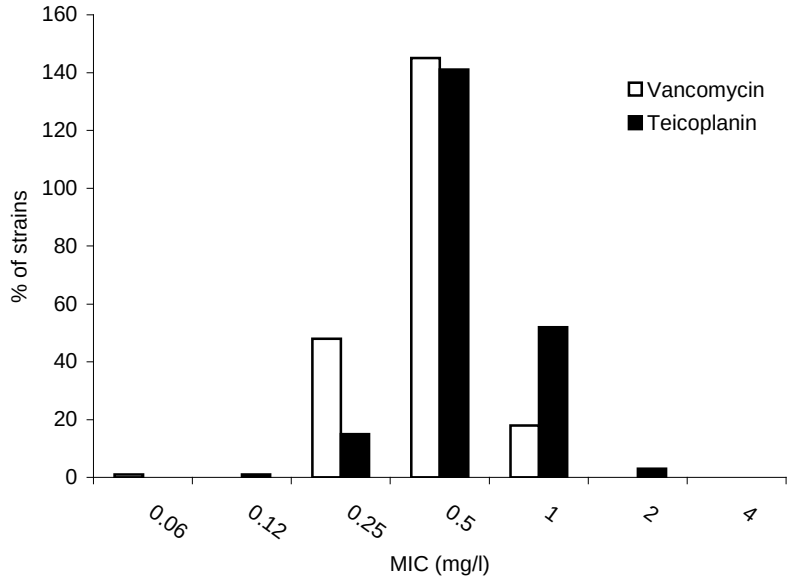
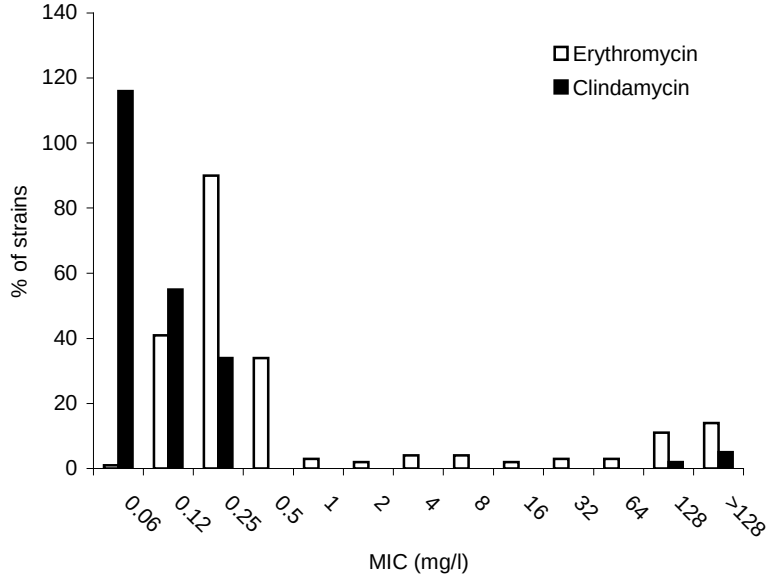
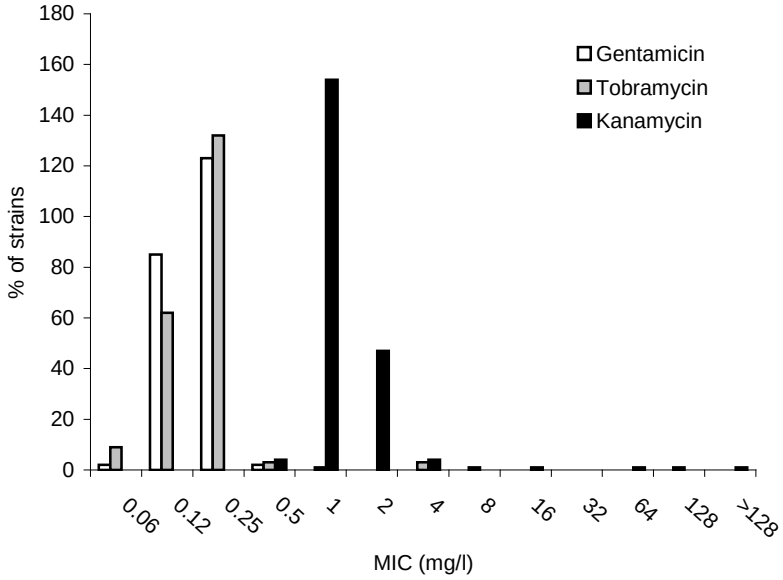
4
8
16
32
64
128
>1024
MIC (mg/l)

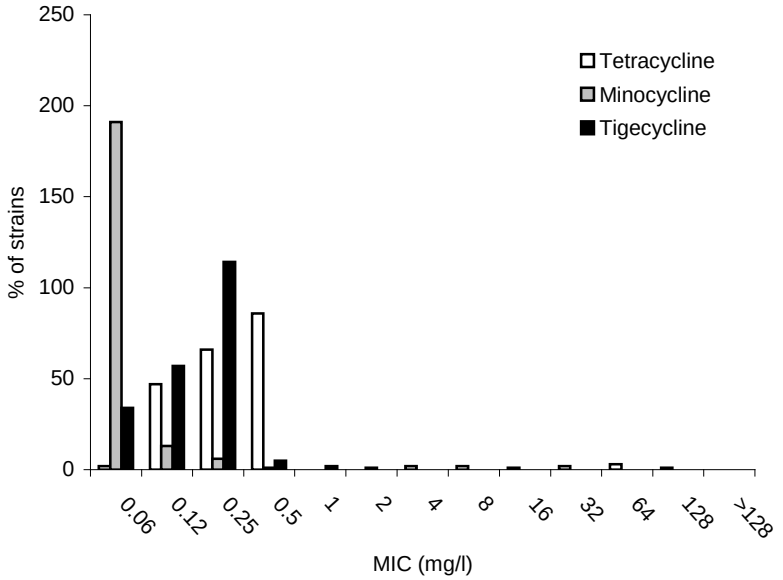
Figure 2: MIC distribution by antimicrobial for 212 MSSA isolates, Belgian hospital survey, 2008








180
160
140
120
100
80
60
40
20
0
% of strains
Gentamicin
Tobramycin
Kanamycin
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


250
200
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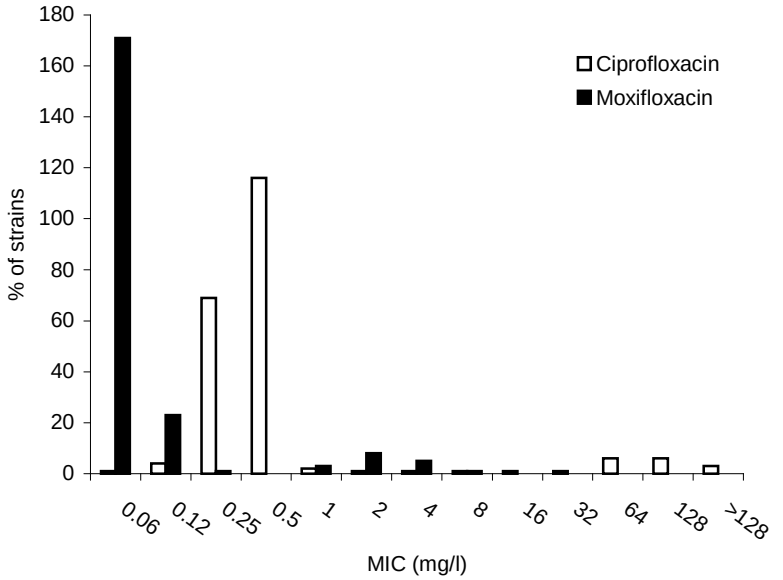
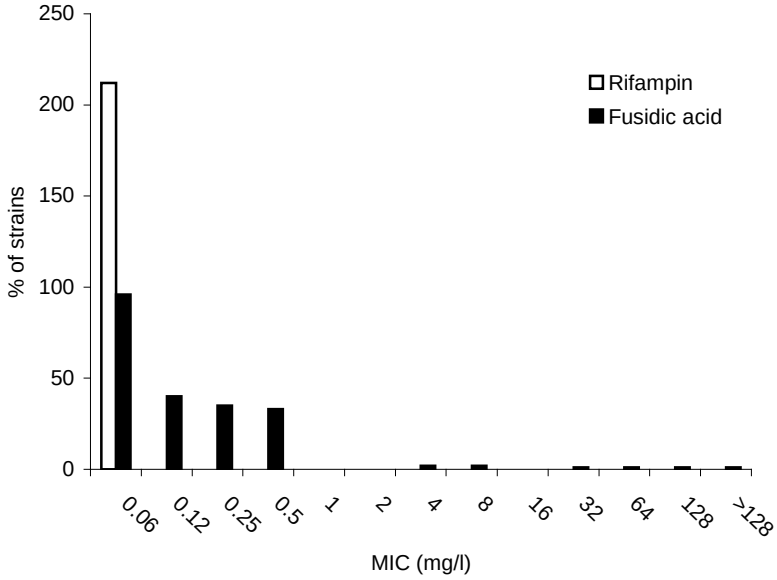
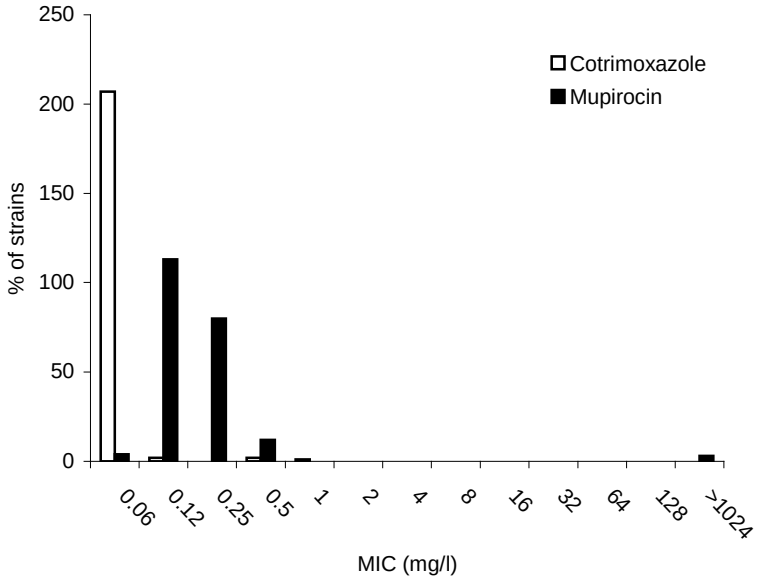
100
50
0
% of strains
Tetracycline
Minocycline
Tigecycline
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


180
160
140
120
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)


250
200
150
100
50
0
% of strains
Rifampin
Fusidic acid
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)

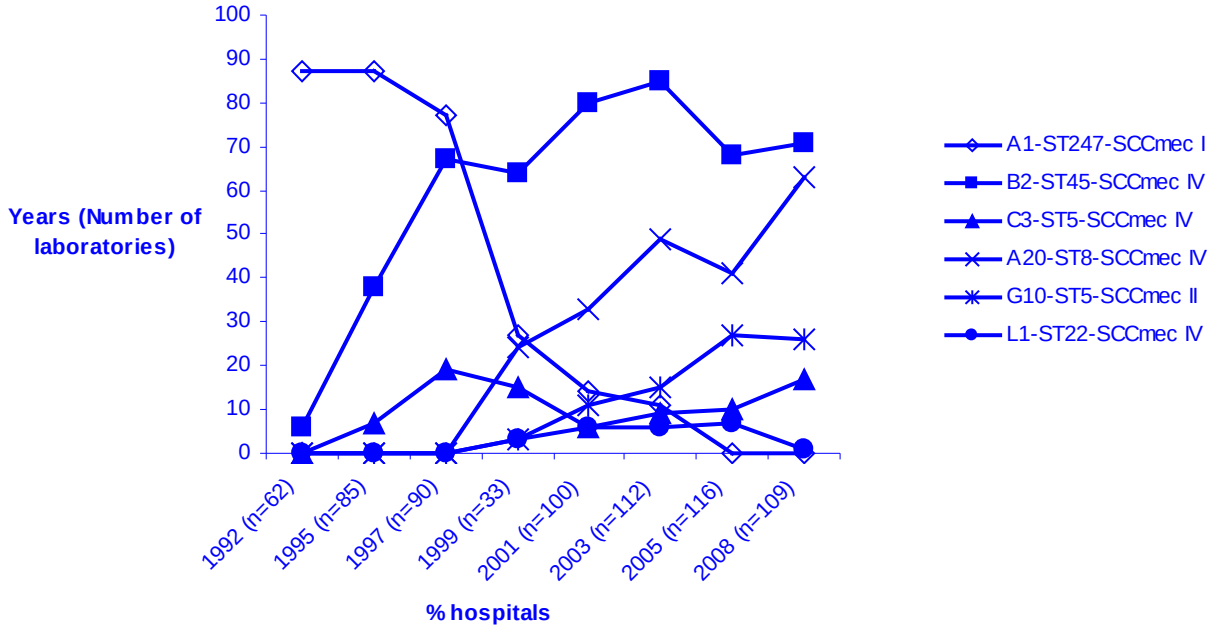

250
200
150
100
50
0
% of strains
Cotrimoxazole
Mupirocin
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
>1024
MIC (mg/l)

Genotype distribution

SCC*mec* typing showed that a majority of strains harboured methicillin resistance cassettes of type IV (79.0%) or type II (14%). By *spa* typing combined with SCC*mec* type, 86% of MRSA strains belonged to 4 epidemic clones: *spa* CC38 (formerly PFGE type B2) ST45-SCC*mec* IV (40%); *spa* CC8 (formerly PFGE type A20) ST8-SCC*mec* IV (30%), *spa* CC2 (formerly PFGE type G10) ST5-SCC*mec* II (12%) and *spa* CC2 (formerly PFGE type C3) ST5-SCC*mec* IV (6.3%).

These major MRSA clones were found in 71 (65%), 63 (58%), 26 (24%) and 17 (16%) hospitals, respectively (Figure 4).

Figure 4: Distribution of Epidemic MRSA PFGE Types National Surveillance, Belgium, 1992-2008



Five isolates were PVL-positive and belonged to community-acquired MRSA (CAMRSA) clone ST80-SCC*mec* IV (n=4) or ST5-SCC*mec* IV (n=1). MRSA strains displaying genotypic characteristics (t011-ST398) similar to animal-associated MRSA strains were detected in two (0.6%) patients hospitalized in Flanders.

Conclusions

1. **As in previous surveys, no *S. aureus* isolate resistant to linezolid was found in 2008.**

2. **Contrary to previous surveys, no MRSA isolates with decreased susceptibility to glycopeptides were found in this survey. This may be related to the disappearance of MRSA strains of ST247-SCCmecI, a genotype previously shown to be significantly associated with the GISA phenotype.**

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

4. **MSSA isolates were susceptible to more antimicrobials than MRSA isolates. However, the frequency of MSSA isolates resistance to MLS (22%) and ciprofloxacin (9%) was also clinically relevant.**

5. **Successful HA-MRSA clones of ST 8 and ST 45 lineages harbouring SCCmec IV are widespread in Belgian acute care hospitals.**

6. **In 2008, hospitalized patients infrequently showed (<3%) MRSA isolates belonging to PVL-positive community-associated clones or ST 398 livestock associated clones.**

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