Microbiological Surveillance of *Staphylococcus aureus* in Belgian Hospitals in 2011

Final report

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Introduction

Staphylococcus aureus is a leading cause of skin and soft tissue infections (SSTI), surgical site and catheter infections, pneumonia, bactaeremia and osteo-articular infections. Methicillin-resistant *S. aureus* (MRSA) is a major cause of hospital infections (hospital-associated MRSA, HA-MRSA) worldwide but it also affects people lacking previous contact with acute and chronic care institutions (community-associated MRSA, CA-MRSA). CA-MRSA strains belong to genetically distinct clones and frequently produce the Panton-Valentine leukocidin (PVL). More recently a third MRSA reservoir was reported in livestock animals (livestock-associated MRSA, LA-MRSA) and in persons in frequent contact with them. LA-MRSA strains mainly belong to sequence type (ST)398, a genotype that differs from HA- and CA-MRSA found in the general human population.

Since 1992, the ULB Reference Laboratory for Staphylococci, in collaboration with the Scientific Institute of Public Health (ISP-WIV) and the Belgian Infection Control Society (BICS), organises epidemiological MRSA surveillances by means of biannual surveys. The objectives are to follow the evolution of genotypes and of antimicrobial resistance profiles of MRSA isolates from patients admitted to Belgian acute-care hospitals. In the last survey conducted in 2008, we observed the diversification of MRSA clones disseminated in Belgian hospitals. Over 90% of isolates belonged to five healthcare-associated clones (clonal complex CC 5, 8, 22, 30 and 45) that 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 profiling of MRSA and methicillin susceptible *S. aureus* (MSSA) strains from the national survey conducted in 2011 in 107 Belgian hospitals.

Materials and methods

Survey methods and collection of bacterial strains

From January to June 2011, laboratories of all Belgian acute-care hospital (n = 156) 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 for Staphylococci 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. Community-associated MRSA (CA-MRSA) strains were defined as MRSA isolated within 48h upon hospital admission. Strains were stored at –80°C until further analysis.

Identification and characterisation of oxacillin resistance

S. aureus isolates were confirmed genotypically by PCR for detection of *16S*, *mecA* and *nuc* genes as previously described.

Antimicrobial susceptibility

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 Clinical and Laboratory Standards Institute (CLSI) guidelines for oxacillin, cefoxitin, vancomycin, teicoplanin, erythromycin, clindamycin, ciprofloxacin, gentamicin, tobramycin, kanamycin, tetracycline, minocycline, tigecycline, rifampin, cotrimoxazole, fusidic acid, linezolid, chloramphenicol and mupirocin. MICs for mupirocin resistant strains (MIC >128 µg/ml) 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, tigecycline and mupirocin. Fusidic acid and tigecylcine breakpoints were interpreted according to the criteria of the EUCAST. Mupirocin resistant strains were classified into two categories according to the British Society for Antimicrobial Chemotherapy (BSAC): low level resistance (MIC = 2 – 256 mg/l) and high-level resistance (MIC > 256 mg/l). Reference strains S. aureus ATCC29213 and ATCC 43300 were included in each run as internal quality control.

Toxin gene detection

The presence of the genes encoding PVL was tested by PCR.

Molecular typing

(i) Surface protein A (spa) typing

For all isolates, *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 Based Upon Repeated Patterns (BURP) algorithm using default parameters: *spa* types shorter than five repeats were considered as non-groupable and *spa* types were assigned to the same CC if the cost is less than or equal to four.

(ii) SCCmec typing

The SCC*mec* type was determined by two multiplex PCR for determination of *ccr* and *mec* complex.

(iii) Multi-locus sequence typing (MLST)

MLST was performed as previously described on selected MRSA strains belonging to the major epidemic clones. In brief, alleles at seven housekeeping genes were amplified by PCR and sequenced on both strands over a ~450 bp region. 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 of the sequences with those recorded in the MLST database (http://www.mlst.net).

Results

Hospital participation and bacterial strains

One hundred seven hospitals (68% of all sites) participated. They were located in Brussels (n = 16), Flanders (n =55) and Wallonia (n = 36). Among 323 isolates sent as MRSA to the Reference Laboratory, 314 MRSA strains (98%) were confirmed as such by multiplex PCR whereas three strains were identified as MSSA. Six isolates did not grow after subculture. Among 213 MSSA isolates, the identification of 210 isolates (98%) was confirmed genotypically. One isolate was identified as CoNS; two isolates did not grow after subculture. MRSA strains were mainly recovered from screening swabs at muco-cutaneous sites and SSTI, whereas MSSA strains were mainly isolated from SSTI and respiratory tract (Table 1).

Table 1 Distribution of *S. aureus* strains by sample category, Belgian Hospitals, 2011

Type of sample	MRSA Number (%)	MSSA Number (%)
Blood	8 (3)	25 (12)
SSTI	72 (23)	77 (37)
Respiratory tract	34 (11)	46 (22)
Screening swabs	167 (53)	7 (3)
Pus	16 (5)	33 (16)
Urine	8 (3)	8 (4)
Other	7 (2)	13 (6)
Unknown	2 (1)	1 (0)
Total	314 (100)	210 (100)

SSTI, skin and soft tissue infections

Demographic data

The majority of case patients with MRSA infection or colonisation were elderly with a median age of 79 years old (range: <1-98 years) (Table 2). Patients were mainly hospitalised in geriatric wards (30%), medical wards (24%), surgical wards (13%) or intensive care units (ICU) (10%). MRSA strains were recovered from MRSA screening at muco-cutaneous sites (53%), SSTIs (23%), respiratory tract (11%), pus (5%), urine (3%), blood (3%) and other specimens (2%). The proportion of total MRSA detected within 48h of admission was 39% (versus 52% in 2008).

The median age of patients with MSSA infection or colonisation was 69 years old (range: <1-92 years) (Table 2). Patients were mainly hospitalised in medical wards (21%), surgical wards (18%), ICU (16%) and geriatric wards (12%). MSSA were recovered from SSTIs (37%), respiratory tract (22%), pus (16%), blood (12%), urine (4%) and other specimen (6%).

Table 2: Age and sex distribution of patients with *S. aureus* carriage/infection, Belgian Hospitals, 2011

Age group (years)	No patients with MRSA (% total by category)		No patients with MSSA [b] (% of total by category)	
	Female [a]	Male [a]	Female	Male
<1	0	3 (2)	1 (1)	5 (4)
1-19	3 (2)	3 (2)	6 (7)	4 (3)
20-49	9 (5)	8 (5)	12 (14)	23 (19)
50-59	5 (3)	15 (10)	5 (6)	18 (15)
60-69	15 (9)	25 (17)	14 (16)	18 (15)
70-79	44 (27)	42 (28)	19 (22)	25 (20)
≥80	87 (54)	52 (35)	30 (34)	29 (24)
Total	163 (100)	148 (100)	87 (100)	122 (100)

[a] Age category is unknown for 3 MRSA isolates (1 female, 2 male)

[b] Sex is unknown for 1 MSSA isolate

Antimicrobial susceptibility

MIC for 19 antimicrobials were determined for 314 MRSA isolates (Table 3 and Table 4). All MRSA isolates were susceptible to linezolid and tigecycline (Figure 1) with MIC_{50} and MIC_{90} of 2 and 2 mg/l for linezolid and 0.25 /l and 0.25 mg/l for tigecycline, respectively.

All MRSA isolates were susceptible to glycopeptides. More than 90% of isolates were susceptible to cotrimoxazole (99%), rifampin (99%), fusidic acid (94%) and mupirocin (94%). Resistance to tetracycline (12%) was higher than for minocycline (5%). Resistance to macrolides-lincosamides (ML) was frequent, ranging from 54% for erythromycin to 40% for clindamycin. For aminoglycosides, resistance was more frequent to kanamycin (41%) and tobramycin (40%) than to gentamicin (1%). Ninety-three percent of the isolates were resistant to ciprofloxacin. Figure 2 shows the MIC distributions by antimicrobial class for all MRSA isolates.

Two hundred and ten MSSA isolates were tested for their susceptibility to 19 antimicrobials (Table 5 and 6). All MSSA isolates were susceptible to linezolid and tigecycline with MIC_{50} and MIC_{90} of 1 and 2 mg/l for linezolid and 0.12 and 0.25 mg/l for tigecycline, respectively.

All MSSA isolates were susceptible to rifampin and glycopeptides. Most of the isolates were susceptible to cotrimoxazole (99%), fusidic acid (90%), aminoglycosides (99-100%), tetracyclines (95-100%) ciprofloxacin (94%) and mupirocin (99%). Resistance to ML was more frequent ranging from 27% for erythromycin to 6% for clindamycin. Figure 3 shows the MIC distributions by antimicrobial class for all MSSA isolates.

Co-resistance to non beta-lactam drugs is compared for MRSA and MSSA isolates in figure 4.

Table 3 : Cumulative proportions of MRSA isolates (n = 314) inhibited by increasing concentrations of 19 antimicrobial agents, Belgian hospitals, 2011

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	1	1	3	9	31	61	77	88	100	-
Cefoxitin	0	0	0	0	0	0	0	3	18	74	89	94	100	-
Vancomycin	3	4	13	72	99	100	100	100	100	100	100	100	100	-
Teicoplanin	2	7	27	75	61	100	100	100	100	100	100	100	100	-
Erythromycin	1	9	42	46	46	46	47	48	49	50	52	57	100	-
Clindamycin	23	55	58	60	60	60	60	60	60	60	60	60	100	-
Chloramphenicol	0	2	2	2	2	13	44	98	98	98	100	100	100	-
Ciprofloxacin	0	0	2	7	7	8	8	12	16	23	43	68	100	-
Linezolid	0	0	1	5	41	100	100	100	100	100	100	100	100	-
Gentamicin	3	44	76	98	98	98	98	99	100	100	100	100	100	-
Tobramycin	2	18	43	58	59	59	60	62	62	63	79	92	100	-
Kanamycin	0	0	0	1	35	55	57	58	58	65	92	99	100	-
Tetracycline	0	5	34	85	86	87	88	92	92	94	99	100	100	-
Minocycline	41	89	91	92	92	93	95	96	100	100	100	100	100	-
Tigecycline	3	32	90	100	100	100	100	100	100	100	100	100	100	-
Rifampin	89	99	99	99	99	100	100	100	100	100	100	100	100	-
Cotrimoxazole	76	89	90	99	99	99	99	99	99	99	100	100	100	-
Fusidic acid	45	80	90	92	94	96	98	99	99	100	100	100	100	-
Mupirocin	7	14	68	94	94	94	94	96	97	97	97	97	98	100

Table 4 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 314 MRSA isolates by susceptibility category to 19 antimicrobial agents, Belgian Hospitals, 2011

Antimicrobial agents	Range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	1->128	32	>128	1	0	99
Cefoxitin	8->128	32	128	0	0	100
Vancomycin	0.06-2	0.5	1	100	0	0
Teicoplanin	0.06-2	0.5	1	100	0	0
Erythromycin	0.06->128	16	>128	46	1	53
Clindamycin	0.06->128	0.12	>128	60	0	40
Chloramphenicol	0.12-64	8	8	98	0	2
Ciprofloxacin	0.25->128	128	>128	7	<1	93
Linezolid	0.25-2	2	2	100	0	0
Gentamicin	0.06->128	0.25	0.5	98	1	1
Tobramycin	0.06->128	0.5	128	60	2	38
Kanamycin	0.5->128	2	64	59	6	35
Tetracycline	0.12-128	0.5	8	88	4	8
Minocycline	0.06-32	0.12	0.25	95	4	1
Tigecycline	0.06-0.5	0.25	0.25	100	0	0
Rifampin	0.06->128	0.06	0.12	99	<1	<1
Cotrimoxazole	0.06-128	0.06	0.5	99	0	1
Fusidic acid	0.06-64	0.12	0.25	94	0	6
Mupirocin	0.06->1024	0.25	0.5	94	2	4

Table 5 : Cumulative proportions of MSSA isolates (n = 210) inhibited by increasing concentrations of 19 antimicrobial agents, Belgian hospitals, 2011

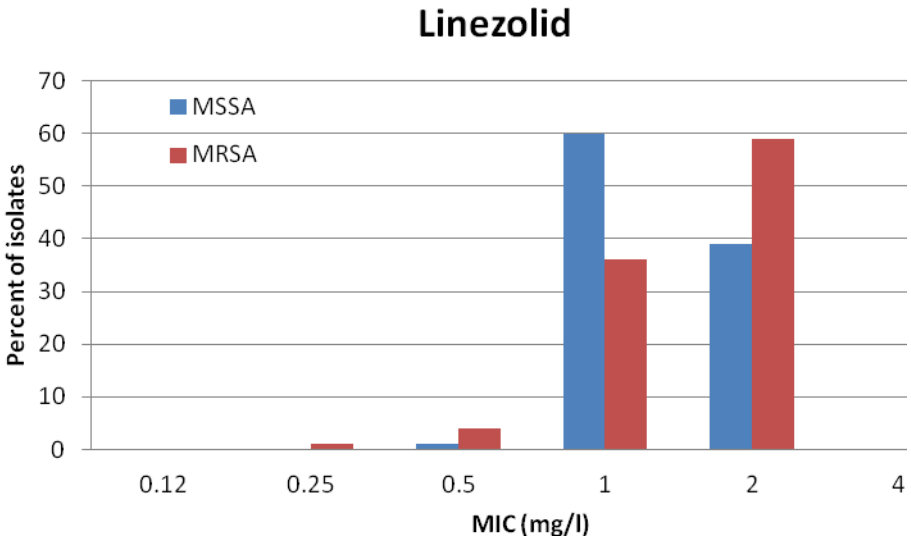
Antimicrobial agents	% of strains resistant at MIC (mg/l) of :													
	0.06	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**>1024**
Oxacillin	0	0	18	95	99	100	100	100	100	100	100	100	100	-
Cefoxitin	0	0	0	9	10	17	100	100	100	100	100	100	100	-
Vancomycin	5	10	26	95	100	100	100	100	100	100	100	100	100	-
Teicoplanin	1	7	23	72	96	100	100	100	100	100	100	100	100	-
Erythromycin	0	13	59	72	73	73	75	76	79	84	86	89	100	-
Clindamycin	39	94	94	95	95	95	95	95	95	95	95	95	100	-
Chloramphenicol	0	0	0	0	0	1	82	99	99	100	100	100	100	-
Ciprofloxacin	17	21	52	87	94	95	95	95	95	96	97	97	100	-
Linezolid	0	0	0	1	61	100	100	100	100	100	100	100	100	-
Gentamicin	10	56	99	100	100	100	100	100	100	100	100	100	100	-
Tobramycin	0	8	83	98	100	100	100	100	100	100	100	100	100	-
Kanamycin	0	0	0	0	15	94	98	99	99	99	99	99	100	-
Tetracycline	0	2	78	95	95	95	95	95	97	99	100	100	100	-
Minocycline	64	100	100	100	100	100	100	100	100	100	100	100	100	-
Tigecycline	30	71	100	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	97	99	99	99	99	99	99	100	100	100	100	100	100	-
Fusidic acid	39	66	89	89	89	91	97	99	99	99	99	99	100	-
Mupirocin	15	89	99	100	100	100	100	100	100	100	100	100	100	-

Table 6 : Range of MIC, MIC_{50}, MIC_{90} and proportion of 210 MSSA isolates by susceptibility category to 19 antimicrobial agents, Belgian Hospitals, 2011

Antimicrobial agents	Range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	% of strains per susceptibility category		
				S	I	R
Oxacillin	0.12-2	0.5	0.5	100	0	0
Cefoxitin	0.5-4	4	4	100	0	0
Vancomycin	0.06-1	0.5	0.5	100	0	0
Teicoplanin	0.06-2	0.5	1	100	0	0
Erythromycin	0.12->128	0.25	>128	73	2	25
Clindamycin	0.06->128	0.12	0.12	94	0	6
Chloramphenicol	0.5-64	4	8	99	0	1
Ciprofloxacin	0.06->128	0.25	1	94	0	6
Linezolid	0.5-2	1	2	100	0	0
Gentamicin	0.06-0.5	0.12	0.25	100	0	0
Tobramycin	0.12-16	0.25	0.5	99	0	1
Kanamycin	0.5->128	2	2	99	<1	1
Tetracycline	0.12-64	0.25	0.5	95	0	5
Minocycline	0.06-4	0.06	0.12	100	0	0
Tigecycline	0.06-0.25	0.12	0.25	100	0	0
Rifampin	0.06-1	0.06	0.06	100	0	0
Cotrimoxazole	0.06-8	0.06	0.06	99	0	1
Fusidic acid	0.06->128	0.12	2	90	0	10
Mupirocin	0.06-4	0.12	0.25	99	<1	0

Figure 1: Linezolid (A) and tigecycline (B) MIC distribution for 314 MRSA and 210 MSSA isolates, Belgian hospitals, 2011

A)



B)

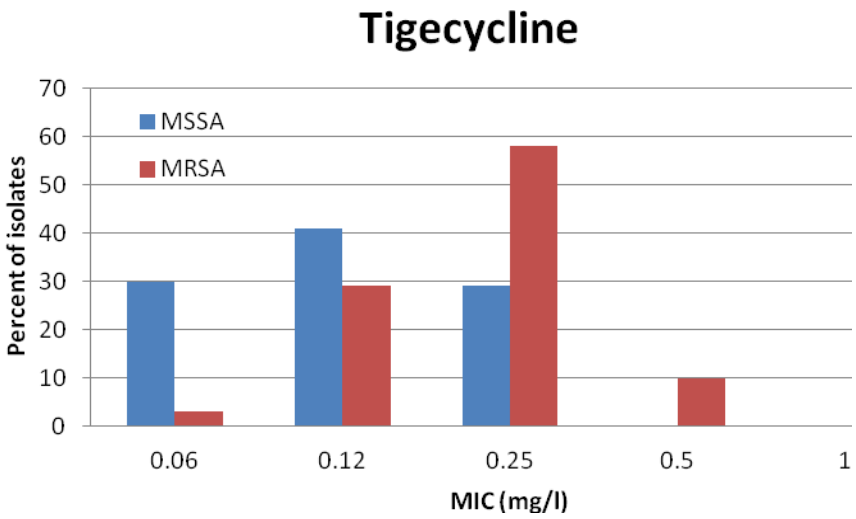
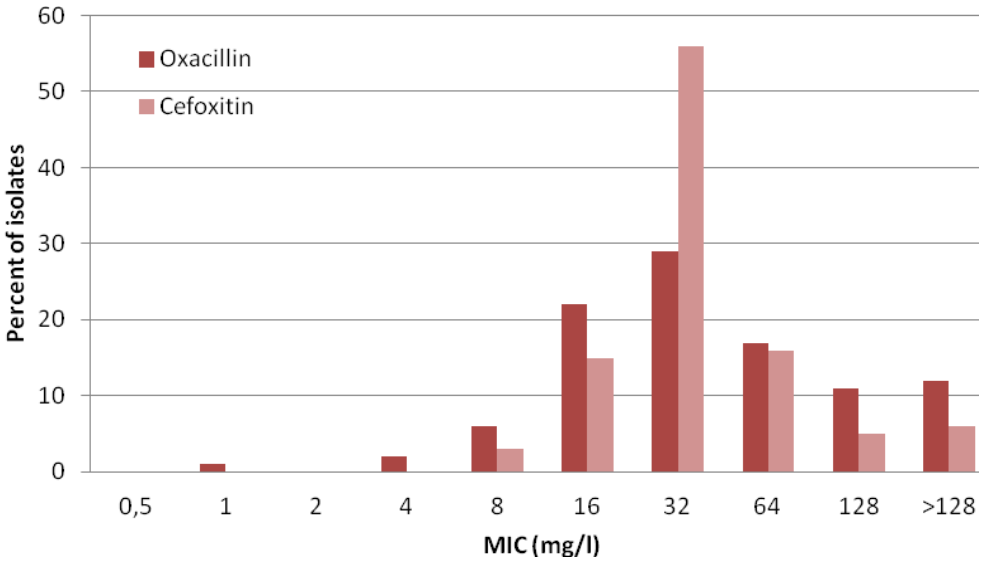
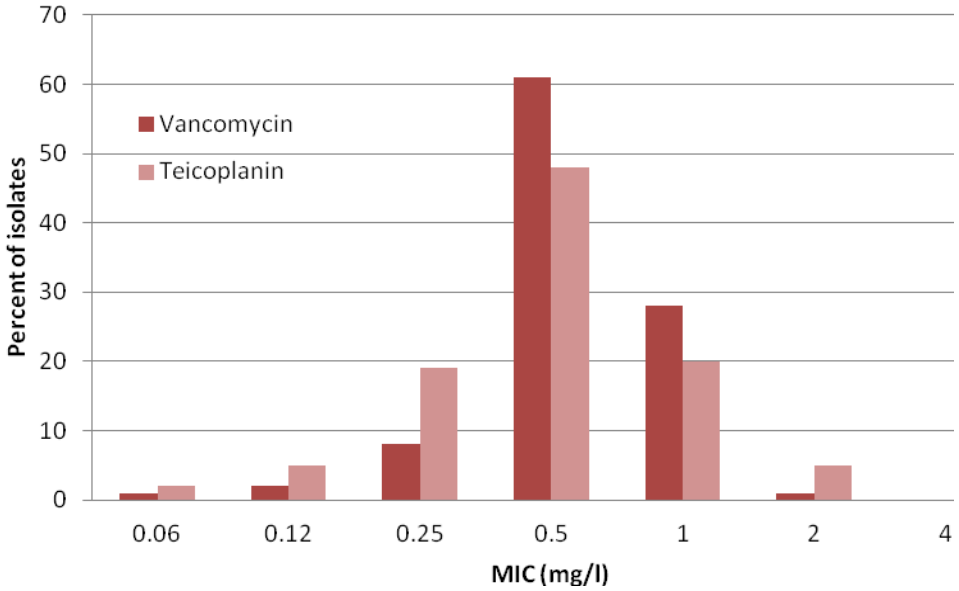
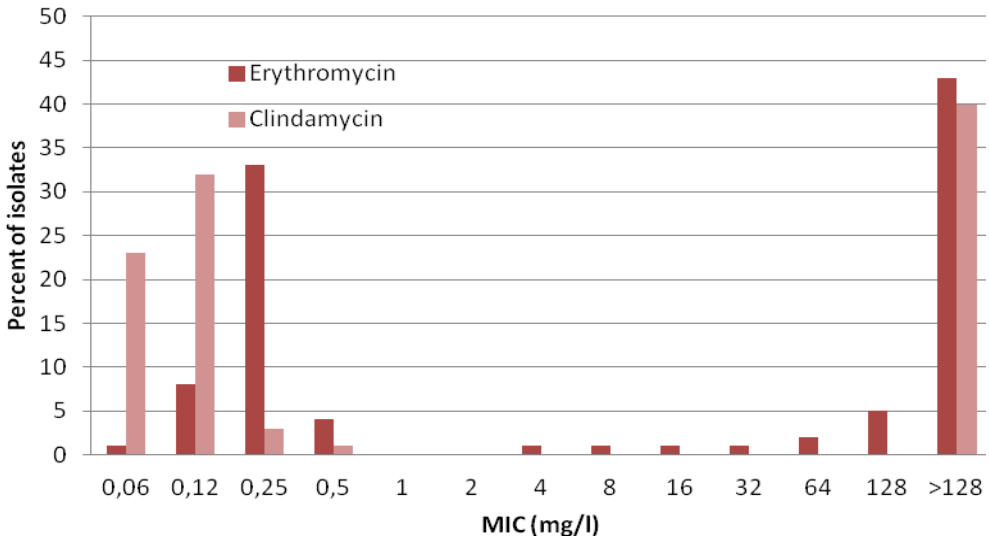
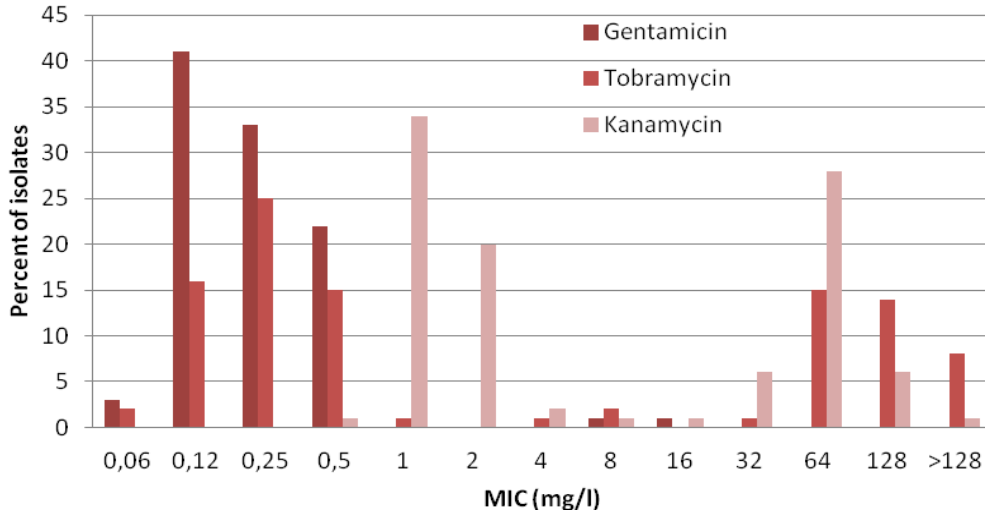


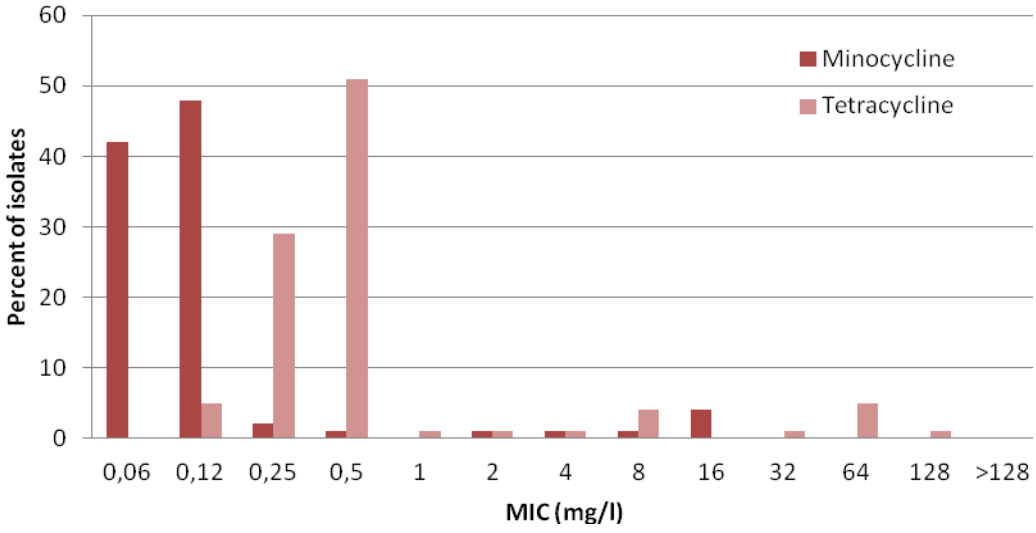
Figure 2: MIC distribution by antimicrobial class for 314 MRSA isolates, Belgian hospitals, 2011






Erythromycin
Clindamycin
Percent of isolates
0
5
10
15
20
25
30
35
40
45
50
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


Gentamicin
Tobramycin
Kanamycin
Percent of isolates
0
5
10
15
20
25
30
35
40
45
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


Minocycline
Tetracycline
Percent of isolates
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10
20
30
40
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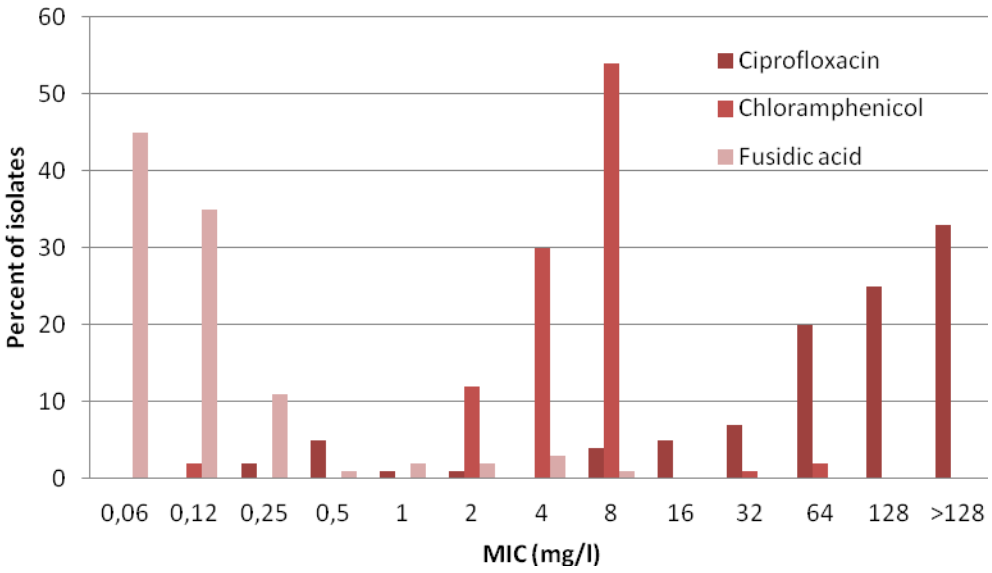
60
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)


60
50
40
30
20
10
0
Percent of isolates
Ciprofloxacin
Chloramphenicol
Fusidic acid
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0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)

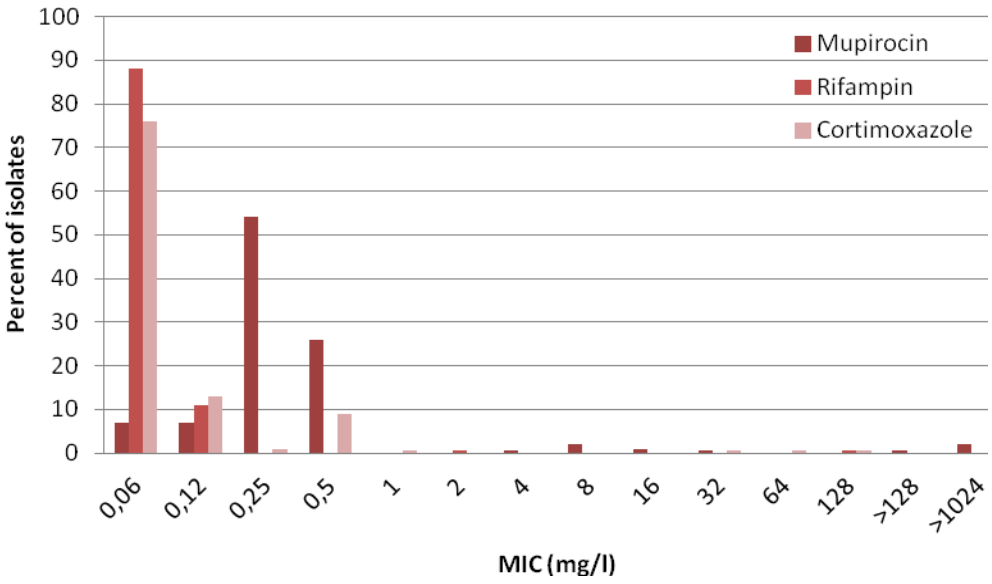
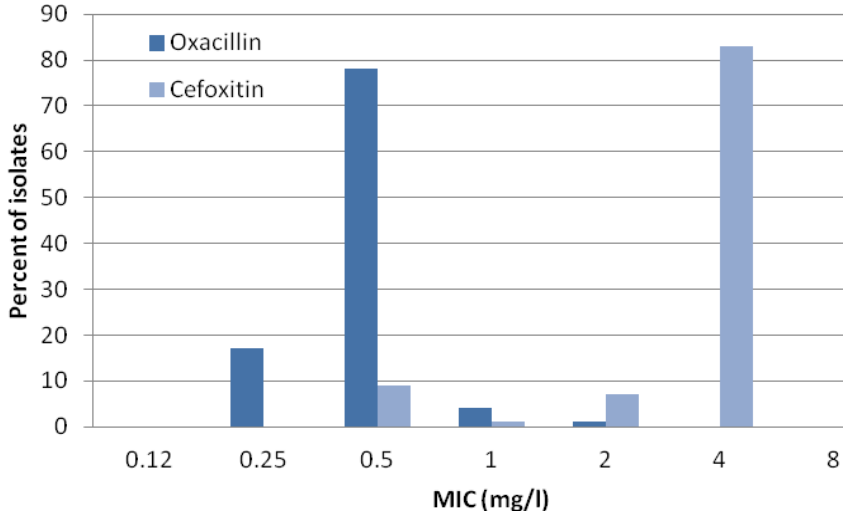
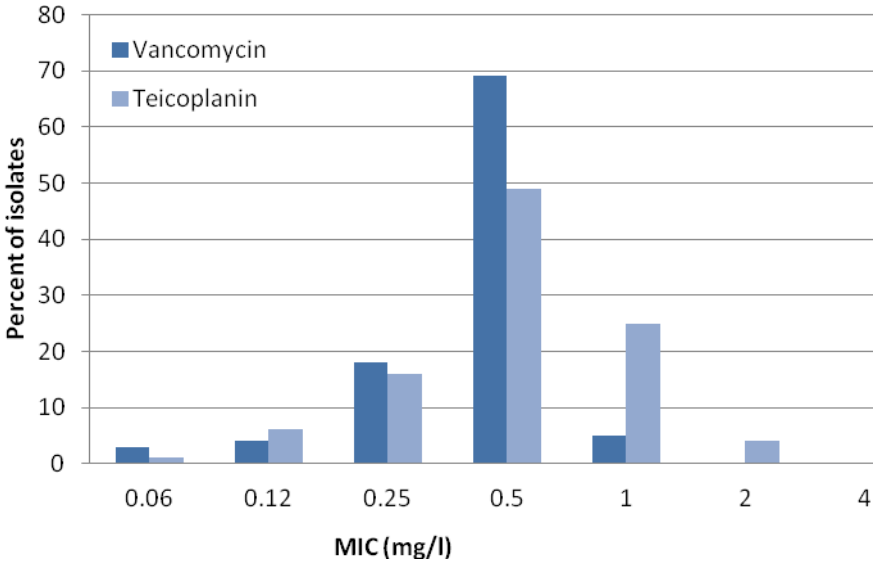
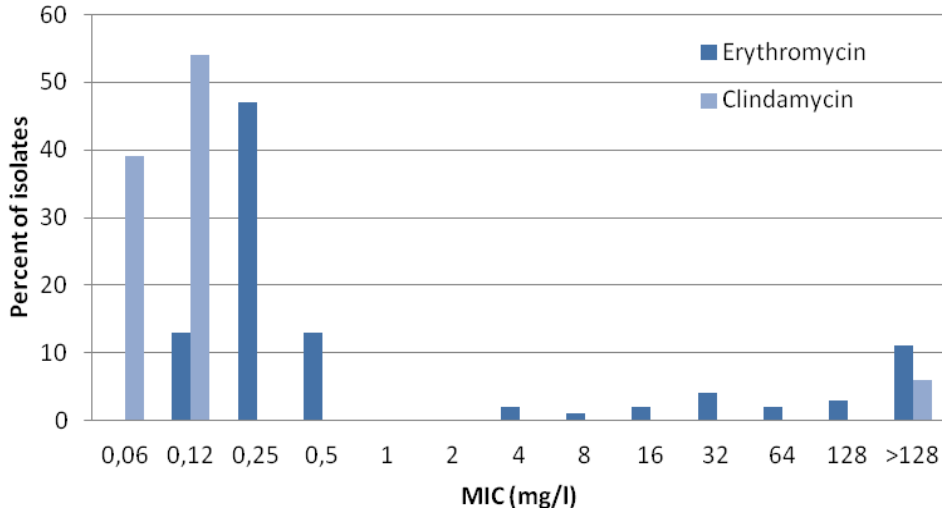
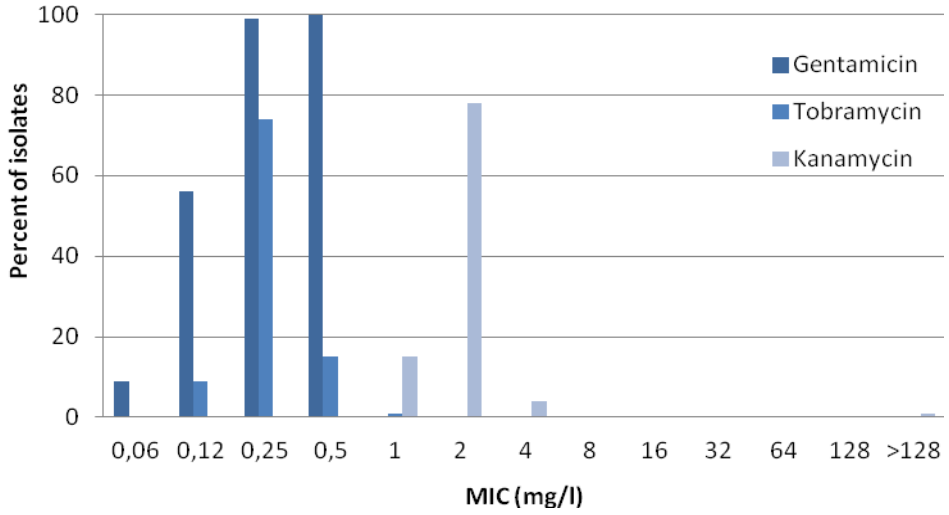

100
90
80
70
60
50
40
30
20
10
0
Percent of isolates
Mupirocin
Rifampin
Cortimoxazole
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
>1024
MIC (mg/l)

Figure 3 : MIC distribution by antimicrobial class for 210 MSSA isolates, Belgian hospitals, 2011






Erythromycin
Clindamycin
Percent of isolates
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
Kanamycin
Percent of isolates
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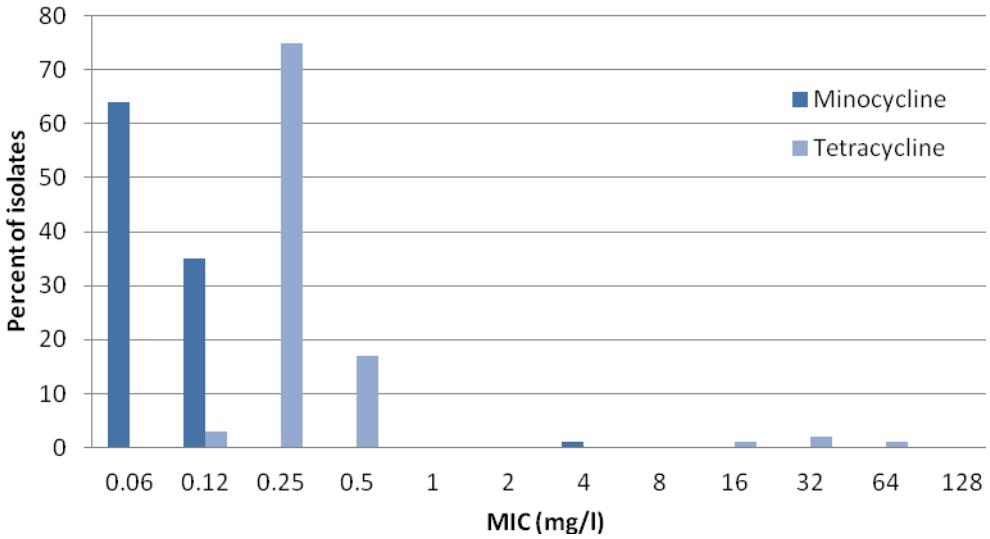
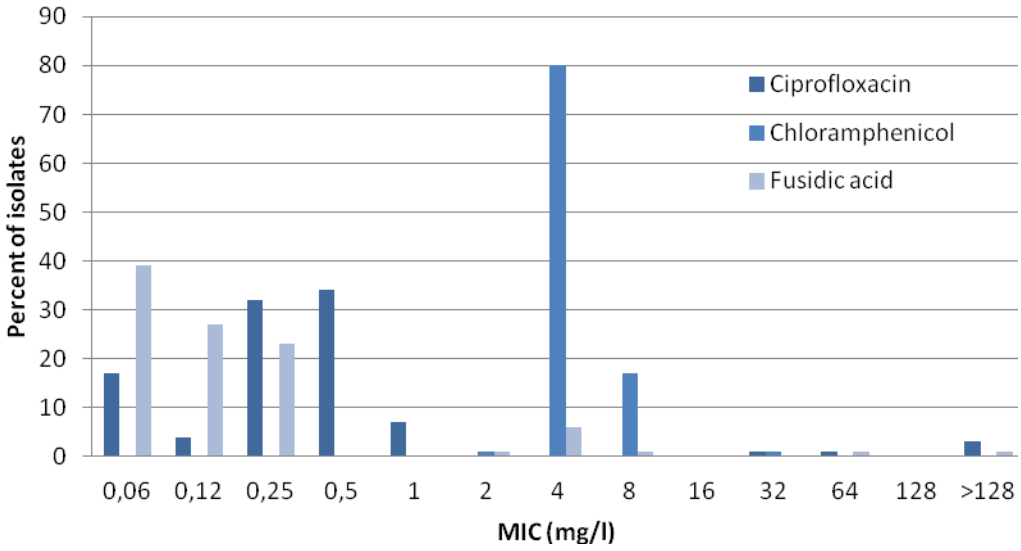
80
60
40
20
0
0,06 0,12 0,25 0,5 1 2 4 8 16 32 64 128 >128
MIC (mg/l)


Minocycline
Tetracycline
Percent of isolates
80
70
60
50
40
30
20
10
0
0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128
MIC (mg/l)


90
80
70
60
50
40
30
20
10
0
Percent of isolates
Ciprofloxacin
Chloramphenicol
Fusidic acid
0,06
0,12
0,25
0,5
1
2
4
8
16
32
64
128
>128
MIC (mg/l)

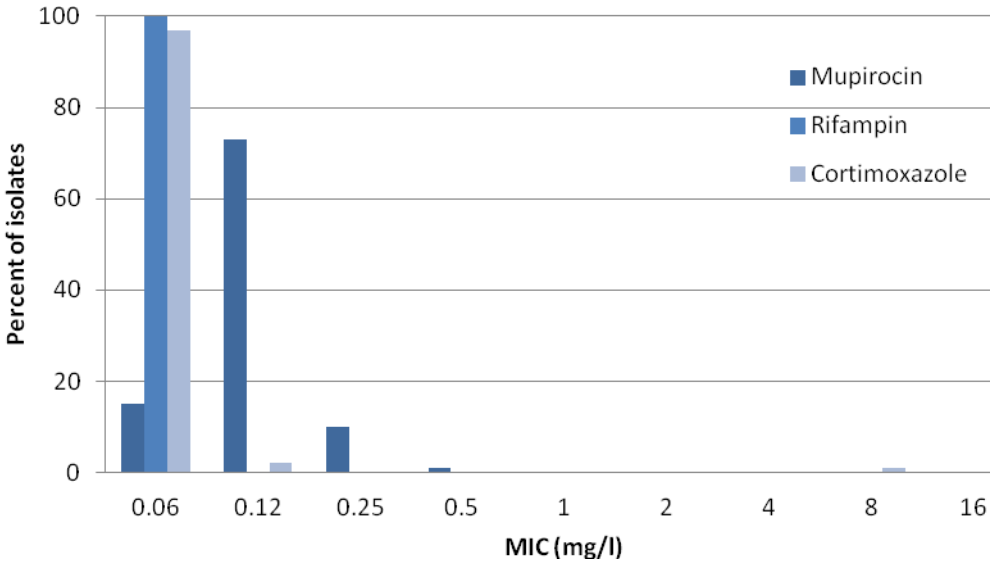
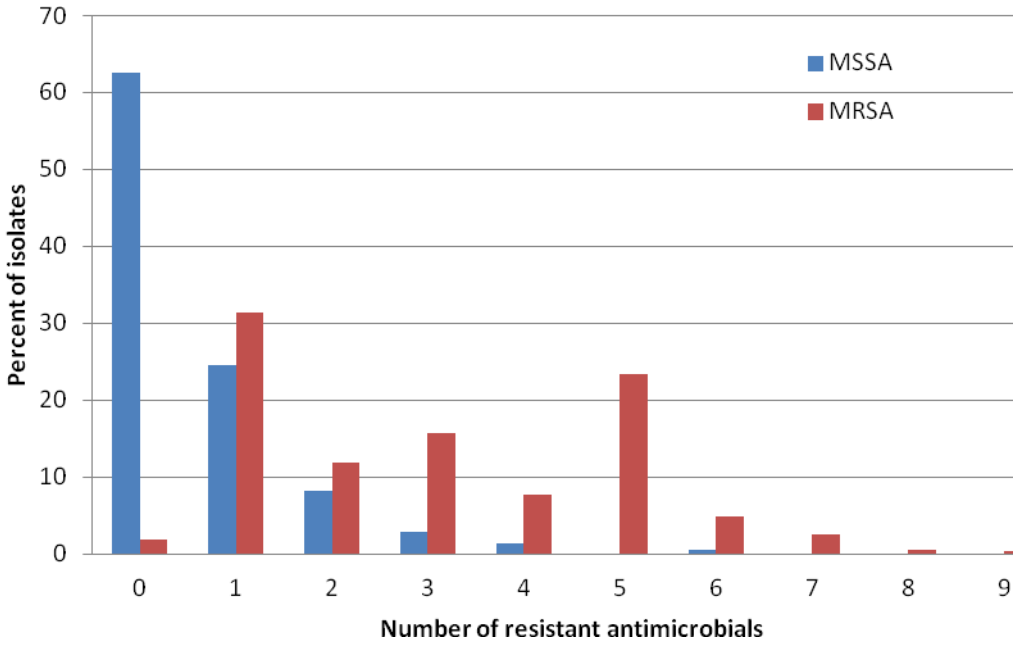

100
80
60
40
20
0
Percent of isolates
Mupirocin
Rifampin
Cortimoxazole
0.06
0.12
0.25
0.5
1
2
4
8
16
MIC (mg/l)

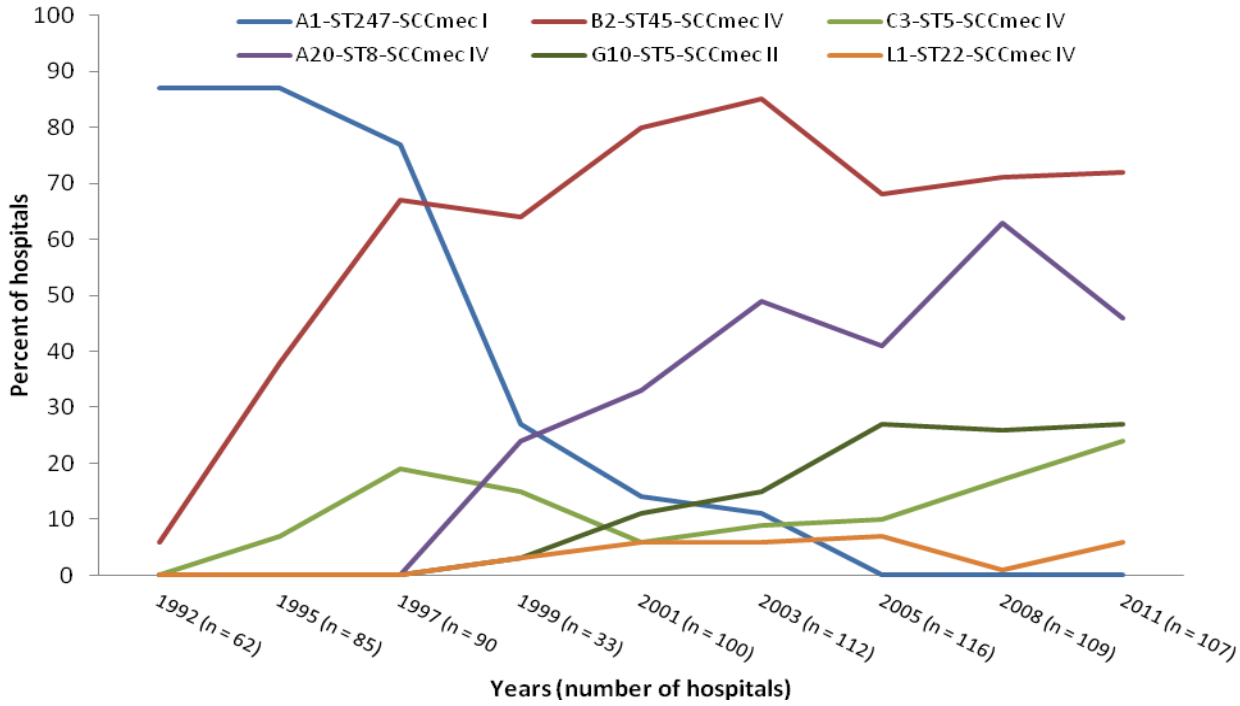
Figure 4: Comparison of co-resistance to non beta-lactam drugs of MSSA versus MRSA isolates, Belgian hospitals, 2011



Genotype distribution

SCC*mec* were classified into type IV (72.9%), type II (15.9%), type V (2.5%), type VI (0.6%) and type I (1.0%). Twenty-two MRSA (7%) had a non-typeable SCC*mec* element. By the combination of *spa* typing and MLST, 90% of MRSA strains belonged to four epidemic clones: *spa* CC38 (formerly PFGE type B2) ST45-SCC*mec* IV (42.4%); *spa* CC8 (formerly PFGE type A20) ST8-SCC*mec* IV (19.1%), *spa* CC2 (formerly PFGE type G10) ST5-SCC*mec* II (15.6%), *spa* CC2 (formerly PFGE type C3) ST5-SCC*mec* IV (6.7%), which were found in 77 (72.0%), 49 (45.8%), 29 (27.1%), 26 (24.3%) hospitals, respectively (Figure 5). Among three PVL positive isolates, two belonged to CA-MRSA clone USA ST8-SCC*mec* IV. Seven MRSA isolates belonged to the LA-MRSA ST398 clone.

Figure 5: Distribution of Epidemic MRSA PFGE Types National Surveillance, Belgium, 1992-2011



Conclusions

1. **The antimicrobial drugs linezolid and tigecycline showed excellent activities against MRSA and MSSA strains recovered from hospitalised patients in Belgian hospitals.**

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

3. **A high proportion of MRSA isolates were resistant to fluoroquinolones (>90%) and to MLS (>40%).**

4. **Resistance to erythromycin (>25%) and fusidic acid (>10%) was frequent in MSSA isolates.**

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

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

7. **Few MRSA isolates belonged to PVL-positive CA-MRSA USA300 clone ST8-SCC*mec* IV and to LA-MRSA clone ST398.**

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