



**8th National epidemiological and antimicrobial
susceptibility survey of *Staphylococcus aureus* and
coagulase negative staphylococci in Belgian hospitals
2013-2014**

Final report

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1. Introduction

Staphylococcus aureus is a leading cause of skin and soft tissue infection, surgical site and catheter infection, pneumonia, bacteraemia and osteo-articular infections (Lowy, 1998). 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) (Chambers et al., 2001). CA-MRSA strains belong to genetically distinct clones and frequently produce the Panton-Valentine leukocidin (PVL) (Tristan et al., 2007). More recently a third MRSA reservoir was reported in livestock animals (livestock-associated MRSA, LA-MRSA) and in persons in contact with them. LA-MRSA strains belong to ST398 which are distinct from HA- and CA-MRSA found in the general human population (Denis et al., 2009a; Garcia-Graells et al., 2012).

Sequence based typing methods have been increasingly used to follow accurately the epidemiology and evolution of *S. aureus*. Multilocus sequence typing (MLST) defines each isolate by its allelic profile as determined by sequencing a ~ 500 bp region of seven housekeeping genes (sequence type, ST). By comparing the DNA sequences with those in an international database (<http://www.mlst.net/>), an allelic profile is assigned, each of which corresponds to a specific ST. Statistical analysis of MLST data allows to identify groups of related *S. aureus* genotypes (clonal complexes, CCs) and to trace the genetic lineages of *S. aureus* (Enright et al., 2000). In *spa* typing, the polymorphic X-region of the gene encoding surface protein A is sequenced. This region consists of a varying number of 24 bp repeats. Different repeats are assigned an alpha-numerical code and the order of repeats defines the *spa* type, which is assigned by the international database Ridom Staph Type (available at <http://www.ridom.de/staphtype/>) (Hallin et al., 2007). The staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element that carries the *mec* gene conferring beta-lactam resistance, can be divided into different types. To date, eleven different SCC*mec* types have been described, which differ from each other by their physical structure and the number of genes coding for additional antibiotic resistance determinants (IWG-SCC, 2009; Garcia-Alvarez et al., 2011, Li et al., 2011). As previous studies have shown that MRSA clones emerged repeatedly from successful methicillin susceptible *S. aureus* (MSSA) clones by acquisition of the SCC*mec* element (Hiramatsu et al., 2001), studying the evolution of MRSA clones cannot be dissociated from the study of the overall *S. aureus* population structure (Strommenger et al., 2006; Vandendriessche et al. 2012).

Since 1992, the ULB – National Reference Centre for *S. aureus* (NRC), in collaboration with the Scientific Institute for Public Health (ISP-WIV) and the Belgian Infection Control Society (BICS), contributes to the epidemiological surveillance of MRSA infections by conducting bi-annual microbiological surveys (Deplano et al., 2000; Denis et al., 2006; Hallin et al., 2008; Denis et al., 2009b). The objectives of this national MRSA survey are to follow the evolution and geographic distribution of MRSA genotypes and the antimicrobial resistance profiles of MRSA isolated from patients admitted to Belgian acute-care hospitals. The last survey conducted in 2011 showed that the majority of MRSA can be classified into two major epidemic clones, *spa* CC38 (formerly PFGE type B2) ST45-SCC*mec* IV (43%) and *spa* CC8 (formerly PFGE type A20) ST8-SCC*mec* IV (24%) and two minor epidemic clones, *spa* CC2 (formerly PFGE type G10) ST5-SCC*mec* II (13%) and *spa* CC2 (formerly PFGE type C3) ST5-SCC*mec* IV (11%). Among three PVL positive isolates, two belonged to CA-MRSA clone USA300 ST8-SCC*mec* IV. Seven MRSA isolates belonged to the LA-MRSA ST398 clone (www.mrsa.be).

In this report, we describe the results of molecular typing and antimicrobial susceptibility of MRSA and MSSA strains from the national survey conducted in 2013-2014 in 113 Belgian hospitals. Clinically relevant coagulase negative staphylococci (CoNS) were also collected to determine their antimicrobial susceptibility profiles and molecular epidemiology.

2. Materials and methods

2.1. Survey methods and collection of bacterial strains

From October 2013 until March 2014, laboratories of all Belgian acute-care hospital (n = 155) 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. One clinically relevant CoNS isolate was collected per laboratory. These strains were sent to the NRC with a case report form describing the following demographic data: hospital code, city, isolation date, patient age, sex, type of specimen, category of hospital unit, infection or colonization, MRSA acquisition (nosocomial or imported). Nosocomial acquisition was defined as a MRSA/MSSA strain firstly isolated from a patient who had been hospitalised for more than 48 hours. CA-MRSA strains were defined as MRSA isolated within 48h upon hospital admission. A clinical relevant CoNS was defined as a CoNS isolated from a single patient from at least two pairs of blood cultures obtained during a single bacteraemic episode, belonging to the same species and with identical antimicrobial profiles. Strains were stored at -80°C until further analysis.

2.2. Identification of staphylococci

S. aureus isolates were confirmed genotypically by PCR for detection of *16S*, *mecA* and *nuc* genes as previously described (Maes et al., 2002). Detection of the *mecA* homologue *mecC* (*mecA*_{LGA251}) was performed by PCR (Stegger et al., 2012). CoNS identification at species level was further performed using Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF).

2.3. Antimicrobial susceptibility

Minimal inhibitory concentrations (MIC) (with a test dilution ranged from 0.03 to 128 mg/l) were determined by the broth dilution method according to clinical laboratory standards institute guidelines (CLSI, 2014) for oxacillin, cefoxitin, ceftaroline, vancomycin, teicoplanin, telavancin, erythromycin, clindamycin, ciprofloxacin, gentamicin, tobramycin, kanamycin, tetracycline, minocycline, tigecycline, daptomycin, rifampin, cotrimoxazole, fusidic acid, linezolid, chloramphenicol and mupirocin. MICs for mupirocin resistant strains (MIC >128 mg/l) 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, telavancin, tigecycline, daptomycin, and mupirocin. Fusidic acid, telavancin, tigecycline and, daptomycin breakpoints were interpreted according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). Mupirocin resistant strains were classified into two categories according to the British Society for Antimicrobial Chemotherapy (BSAC, 2015): 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.

2.4. Toxin gene detection

The exotoxin production profile was characterized by PCR for genes encoding for toxic shock syndrome toxin (*tss*) and Panton-Valentine leukocidin toxin (PVL) (*lukS-lukF*) (Jarraud et al., 2002; Lina et al., 1999a).

2.5. Molecular epidemiology of *S. aureus* isolates

a) SCC*mec* typing

For MRSA isolates the SCC*mec* type was determined by two multiplex PCR for determination of *ccr* and *mec* complex by PCR (Kondo et al., 2007).

b) Surface protein A (*spa*) typing

The *spa* typing was performed as previously described by Harmsen et al. (2003). *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 CC if the cost is less than or equal to six (Mellmann et al., 2007).

c) MLST

MLST was performed on selected MRSA and MSSA strains belonging to the major epidemic types as previously described (Enright et al., 2000). In brief, seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) 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>). Similar STs were grouped into CCs using the Based Upon Related Sequence Types (BURST) algorithm.

2.6. Analysis of *S. epidermidis* isolates

2.6.1. Detection of resistance and virulence genes

a) Macrolide-lincosamide-streptogramin (MLS) resistance genes encoding for modified target site proteins (*ermA*, *ermB*, *ermC*) and efflux system pump (*msrA*) were detected by multiplex PCR (Lina et al., 1999b).

b) Aminoglycoside resistance genes *aacA/aphD*, *aphA3* and *aadC* encoding for the aminoglycoside-modifying enzymes aac(6['])-aph(2[']), aph(3[']) and ant(4['],4[']), respectively, were determined by PCR (Vanhoof et al., 1994).

c) Tetracycline resistance genes (*tetK*, *tetL* encoding for an efflux system and *tetM* for a ribosomal protective protein) were tested by PCR (Ng et al., 2001).

d) The *mupA* gene which codes for high-level resistance to mupirocin was detected by PCR for strains with an increased MIC (> 256 µg/ml) to mupirocin (Ramsey et al., 1996).

e) The *icaA* gene which belonged to the *ica* operon involved in biofilm formation was detected by PCR (Cherifi et al., 2014).

2.6.2. Molecular epidemiology of *S. epidermidis* isolates

a) SCC*mec* typing

For methicillin resistant *S. epidermidis* (MRSE), the SCC*mec* types were determined by multiplex PCRs for *ccr* and *mec* gene complexes (Kondo et al., 2007).

b) Pulsed Field Gel Electrophoresis (PFGE)

S. epidermidis strains were genotyped by SmaI PFGE analysis (Cookson et al., 1996; Cookson et al., 2007; Murchan et al., 2003). The similarity PFGE patterns were analysed in BioNumerics using Dice coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

c) MLST

A subset of representative PFGE types of *S. epidermidis* was further genotyped by MLST (<http://sepidermidis.mlst.net/>) (Thomas et al., 2007). Sequencing of 7 housekeeping genes (*arcC*, *aroE*, *gtr*, *mutS*, *pyr*, *tpi* and *yqiL*) yielded the allelic profile, which was assigned a ST. Similar sequence types were grouped into CCs.

3. Results

3.1. Hospital participation and bacterial strains

One hundred thirteen hospitals (72.9% of all sites) participated. They were located in Brussels (n = 15), Flanders (n = 63) and Wallonia (n = 35). Among 298 isolates sent as MRSA to the NRC, 287 MRSA strains (96.3%) were confirmed as such by multiplex PCR whereas one isolate was identified as methicillin resistant CoNS and three strains were identified as MSSA. Seven isolates did not grow after subculture. Among 200 isolates sent as MSSA to the Reference Laboratory, the identification of 194 isolates (97%) was confirmed genotypically. One isolate was identified as MRSA and five did not grow after subculture. MRSA strains were mainly recovered from screening swabs at muco-cutaneous sites and skin and soft tissue infections (SSTIs) whereas MSSA strains were mainly isolated from SSTI and respiratory tract (Table 1). Among 83 isolates sent as clinically relevant CoNS to the NRC, 80 CoNS strains (96.4%) were confirmed as such by multiplex PCR, whereas three samples were contaminated or did not grow after subculture. The CoNS were identified by MALDI-TOF as *S. epidermidis* (76%, n=61), *S. hominis* (12%, n=10), *S. capitis* (7%, n= 5), *S. haemolyticus* (4%, n=3) and *S. warneri* (1%, n=1).

Table 1 Distribution of *S. aureus* strains by sample category, Belgium 2013-2014.

| Type of sample | MRSA Number (%) | MSSA Number (%) |
|-------------------|--------------------|--------------------|
| Blood | 6 (2.1) | 22 (11.2) |
| SSTIs | 41 (14.2) | 70 (35.5) |
| Respiratory tract | 38 (13.2) | 51 (25.9) |
| Screening swabs | 164 (56.9) | 6 (3.0) |
| Pus | 20 (6.9) | 32 (16.2) |
| Urine | 11 (3.8) | 2 (1.0) |
| Other | 7 (2.4) | 11 (5.6) |
| Not determined | 1 (0.3) | 3 (1.5) |
| Total | 288 (100) | 197 (100) |

SSTIs, skin and soft tissue infections; NA, not applicable

3.2. Demographic data

The majority of case patients with MRSA infection or colonisation were elderly with a median age of 79 years old (range: 1-96 years) (Table 2). Patients were mainly hospitalised in geriatric wards (26.0%), medical wards (22.9%), surgical wards (15.3%) or intensive care units (ICU) (9.0%). MRSA strains were recovered from MRSA screening at muco-cutaneous sites (56.9%), SSTIs (14.2%), respiratory tract (13.2%), pus (6.9%), urine (3.8%) and blood (2.1%) and other specimens (2.4%) (Table 1). The proportion of total MRSA detected within 48h of admission was 31% in 2013-14 versus 39% in 2011.

The median age of patients with MSSA infection or colonisation was 72 years old (range: <1-92) (Table 2). Patients were mainly hospitalised in medical wards (22.8%), surgery wards (16.8%), ICU (13.7%) and geriatric wards (12.7%). MSSA were recovered from SSTIs (35.5%), respiratory tract (25.9%), pus (16.2%), blood (11.2%), urine (1.0%) and other specimen (5.6%) (Table 1).

The median age of patients with CoNS infection (the samples were obtained from blood cultures) was 70 years old (range: 4-104 years) (Table 2). Patients were mainly hospitalised in medical wards (31.3%), surgery wards (17.5%), geriatric wards (13.8%) and ICU (12.5%).

Table 2: Age and sex distribution of patients with *S. aureus* or CoNS, Belgium 2013-2014.

| Age group (years) | No patients with MRSA (% total by category) ^a | | No patients with MSSA (% of total by category) ^a | | No patients with CoNS (% of total by category) | |
|----------------------|--|-----------|--|-----------|---|-----------|
| | Female | Male | Female | Male | Female | Male |
| <1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (1.0) | 0 (0.0) | 0 (0.0) |
| 1-19 | 2 (1.6) | 5 (3.5) | 3 (3.5) | 4 (3.9) | 0 (0.0) | 1 (2.3) |
| 20-49 | 4 (3.1) | 14 (9.7) | 9 (10.6) | 13 (12.6) | 3 (8.1) | 5 (11.6) |
| 50-69 | 18 (14.1) | 32 (22.2) | 25 (29.4) | 32 (31.1) | 9 (24.3) | 20 (46.5) |
| 70-79 | 31 (24.2) | 35 (24.3) | 22 (25.9) | 26 (25.2) | 13 (35.1) | 9 (20.9) |
| ≥80 | 73 (57.0) | 58 (40.3) | 26 (30.6) | 27 (26.2) | 12 (32.4) | 8 (18.6) |
| Total | 128 (100) | 144 (100) | 85 (100) | 103 (100) | 37 (100) | 43 (100) |

^aThe age of 16 patients with MRSA and 9 patients with MSSA was not stated.

3.3. Antimicrobial susceptibility

MICs for 19 antimicrobials were determined for all (n=288) MRSA isolates (Table 3 and Table 4, Figure 1, Figure 2). All MRSA isolates were susceptible to linezolid and tigecycline (Figure 1) with MIC₅₀ and MIC₉₀ of 2 and 4 mg/l for linezolid and 0.25 and 0.25 mg/l for tigecycline, respectively. Few isolates (n=4) showed intermediate resistance (MIC = 2 mg/l) to ceftaroline. Presence of limited number of ceftaroline-intermediate *S. aureus* has been already described (Fernandez et al., 2014; Kelley et al., 2015). All MRSA isolates were susceptible to glycopeptides and daptomycin. More than 90% of isolates were susceptible to rifampin (99.3%), cotrimoxazole (98.3%), gentamicin (95.1%), and mupirocin (93.1%). More than 85% of isolates was susceptible to fusidic acid (87.5%) and ciprofloxacin (85.1%). Resistance to tetracycline (21.2%) was higher than for minocycline (8.0%). Resistance to MLS was frequent, ranging from 44.1% for erythromycin to 29.5% for clindamycin. For aminoglycosides, resistance was more frequent to kanamycin (39.2%) and tobramycin (37.8%) than to gentamicin (4.5%). Finally, 2.4% of the isolates were resistant to chloramphenicol.

One hundred thirty-one MSSA isolates were tested for their susceptibility to 19 antimicrobials (Table 5 and 6, Figure 3). All MSSA isolates were susceptible to linezolid, ceftaroline and tigecycline (Figure 1) with MIC₅₀ and MIC₉₀ of 2 and 4 mg/l for linezolid, 0.25 and 0.5 mg/l for ceftaroline, and 0.25 and 0.25 mg/l for tigecycline, respectively. All MSSA isolates were susceptible to glycopeptides, daptomycin and gentamicin. Most of the MSSA isolates were susceptible to cotrimoxazole (99.2%), mupirocin (97.7%), rifampin (92.2%), kanamycin (99.2%), tobramycin (98.5%), tetracyclines (98.5-100%), fusidic acid (92.4%), and ciprofloxacin (90.8%). Resistance to macrolides-lincosamides-streptogramins (MLS) was more frequent ranging from 23.7% for erythromycin to 6.1% for clindamycin. Finally, 5.8% of the isolates were resistant to chloramphenicol.

Table 4: Range of MIC, MIC₅₀, MIC₉₀ and proportion of 288 MRSA isolates by susceptibility category to 19 antimicrobial agents from Belgian Hospitals, 2013-2014.

| 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 | 1.4 | 0.0 | 98.6 |
| Cefoxitin | 8->128 | 32 | 64 | 0.0 | 0.0 | 100.0 |
| Ceftaroline | 0.06->128 | 0.5 | 1 | 98.6 | 1.4 | 0.0 |
| Vancomycin | 0.06-2 | 1 | 2 | 100.0 | 0.0 | 0.0 |
| Teicoplanin | 0.06-2 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Telavancin | 0.03-2 | 0.06 | 0.06 | 100.0 | 0.0 | 0.0 |
| Daptomycin | 0.06->128 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Erythromycin | 0.06->128 | 0.5 | 128 | 55.9 | 0.0 | 44.1 |
| Clindamycin | 0.06->128 | 0.25 | 128 | 70.1 | 0.3 | 29.5 |
| Chloramphenicol | 0.12-64 | 8 | 16 | 54.5 | 43.1 | 2.4 |
| Ciprofloxacin | 0.25->128 | 128 | 128 | 13.9 | 1.0 | 85.1 |
| Linezolid | 0.25-2 | 2 | 4 | 100.0 | 0.0 | 0.0 |
| Gentamicin | 0.06->128 | 0.5 | 1 | 95.1 | 0.3 | 4.5 |
| Tobramycin | 0.06->128 | 0.5 | 128 | 60.8 | 1.4 | 37.8 |
| Kanamycin | 0.5->128 | 4 | 128 | 59.4 | 1.4 | 39.2 |
| Tetracycline | 0.12-128 | 0.5 | 64 | 78.1 | 0.7 | 21.2 |
| Minocycline | 0.06-32 | 0.25 | 0.5 | 91.7 | 0.3 | 8.0 |
| Tigecycline | 0.06-0.5 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Rifampin | 0.06->128 | 0.25 | 0.25 | 99.3 | 0.7 | 0.0 |
| Cotrimoxazole | 0.06-128 | 0.25 | 0.25 | 98.3 | 0.0 | 1.7 |
| Fusidic acid | 0.06-64 | 0.25 | 4 | 87.5 | 0.0 | 12.5 |
| Mupirocin | 0.06->1024 | 0.5 | 1 | 93.1 | 0.0 | 6.9 |

Table 6: Range of MIC, MIC₅₀, MIC₉₀ and proportion of 131 MSSA isolates by susceptibility category to 19 antimicrobial agents from Belgian Hospitals, 2013-2014.

| Antimicrobial agents | Range (mg/l) | MIC ₅₀ (mg/l) | MIC ₉₀ (mg/l) | % of strains per susceptibility category | | |
|------------------------|-----------------|-----------------------------|-----------------------------|--|------|------|
| | | | | S | I | R |
| Oxacillin | 1->128 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Cefoxitin | 8->128 | 4 | 4 | 100.0 | 0.0 | 0.0 |
| Ceftaroline | 0.06->128 | 0.25 | 0.5 | 100.0 | 0.0 | 0.0 |
| Vancomycin | 0.06-2 | 1 | 2 | 100.0 | 0.0 | 0.0 |
| Teicoplanin | 0.06-2 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Telavancin | 0.03-2 | 0.06 | 0.06 | 100.0 | 0.0 | 0.0 |
| Daptomycin | 0.06->128 | 0.5 | 0.5 | 100.0 | 0.0 | 0.0 |
| Erythromycin | 0.06->128 | 0.5 | 128 | 76.3 | 0.0 | 23.7 |
| Clindamycin | 0.06->128 | 0.25 | 0.25 | 93.1 | 0.8 | 6.1 |
| Chloramphenicol | 0.12-64 | 8 | 16 | 65.0 | 29.2 | 5.8 |
| Ciprofloxacin | 0.25->128 | 0.5 | 1 | 90.8 | 0.8 | 8.4 |
| Linezolid | 0.25-2 | 2 | 4 | 100.0 | 0.0 | 0.0 |
| Gentamicin | 0.06->128 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Tobramycin | 0.06->128 | 0.5 | 1 | 98.5 | 0.0 | 1.5 |
| Kanamycin | 0.5->128 | 2 | 4 | 99.2 | 0.0 | 0.8 |
| Tetracycline | 0.12-128 | 1 | 1 | 98.5 | 0.0 | 1.5 |
| Minocycline | 0.06-32 | 0.5 | 0.5 | 100.0 | 0.0 | 0.0 |
| Tigecycline | 0.06-0.5 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Rifampin | 0.06->128 | 0.25 | 0.25 | 99.2 | 0.0 | 0.8 |
| Cotrimoxazole | 0.06-128 | 0.25 | 0.25 | 99.2 | 0.0 | 0.8 |
| Fusidic acid | 0.06-64 | 0.25 | 1 | 92.4 | 0.0 | 7.6 |
| Mupirocin | 0.06->1024 | 0.5 | 1 | 97.7 | 0.0 | 2.3 |

Figure 1: MIC distribution of ceftaroline, linezolid and tigecycline resistance for 288 MRSA and 131 MSSA isolates, Belgian hospitals, 2013-2014.

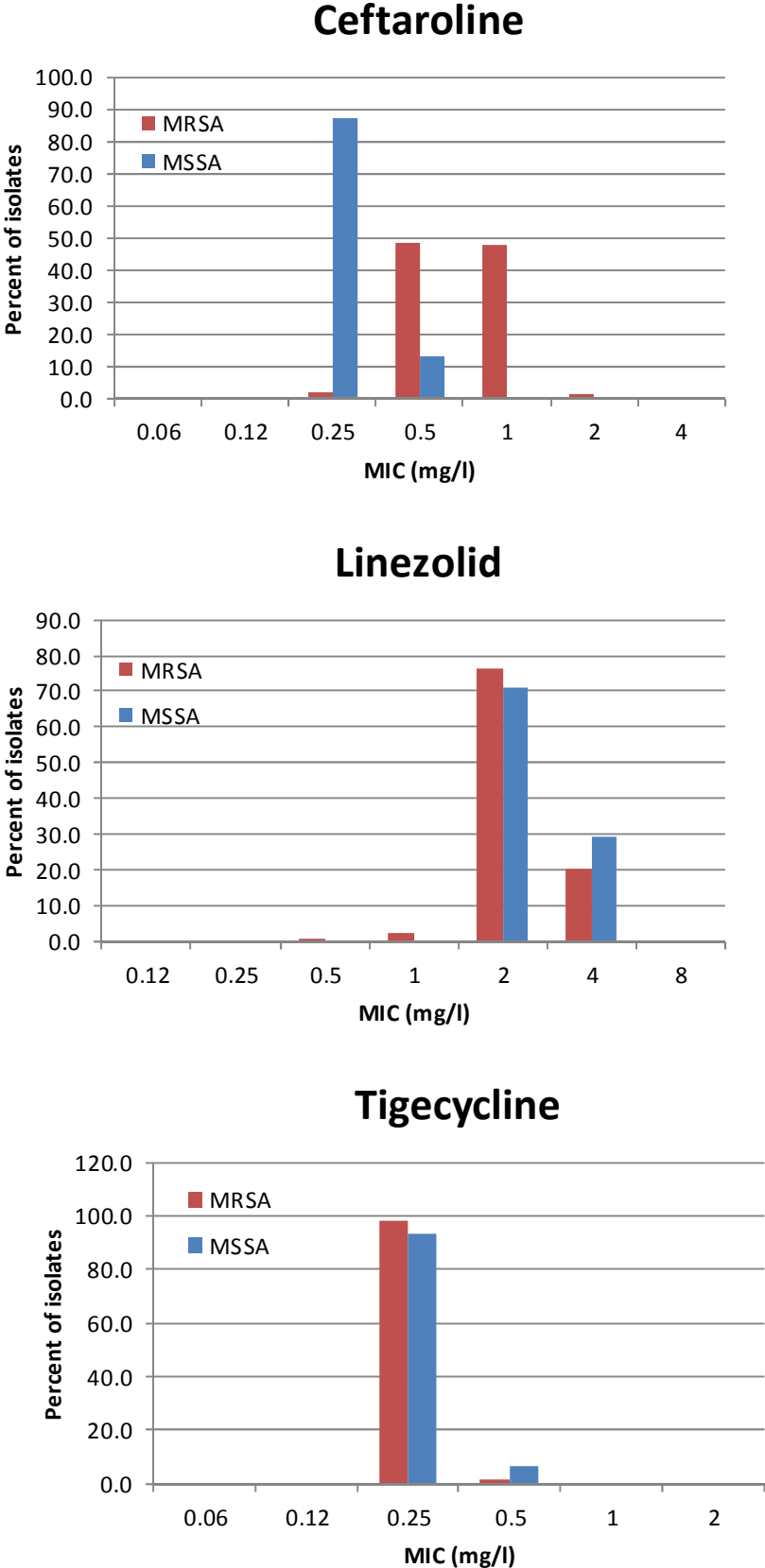
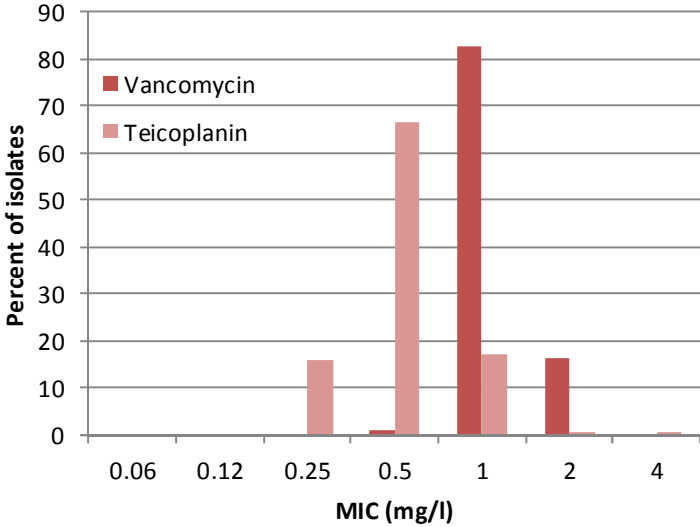
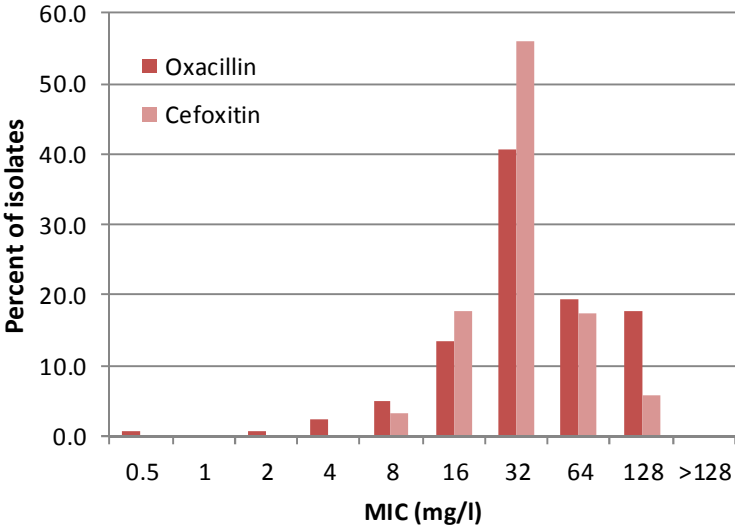
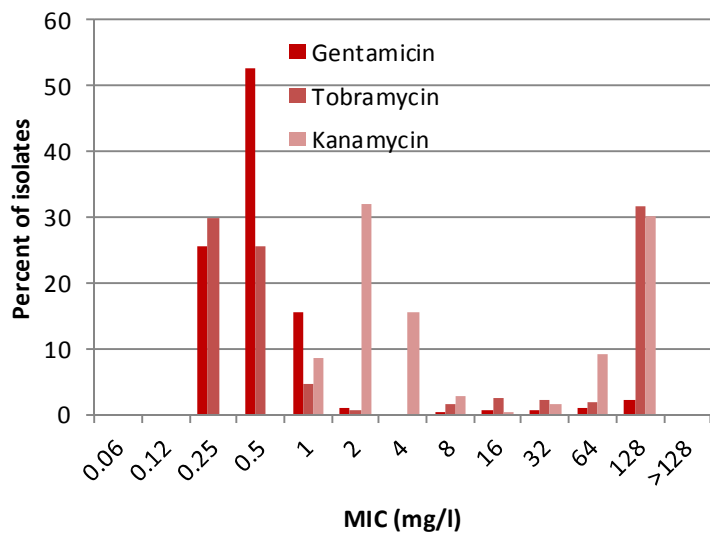
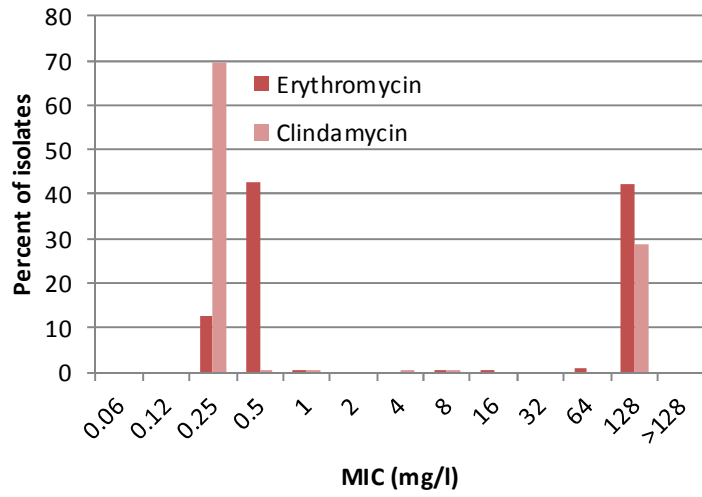
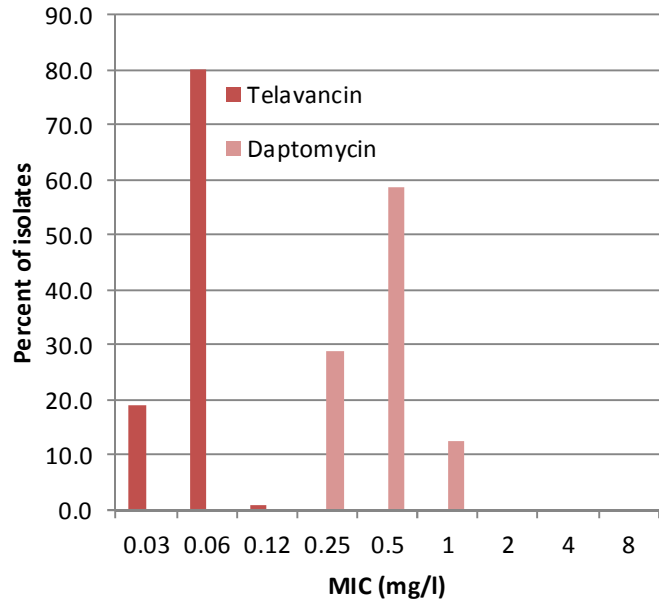


Figure 2: MIC distribution by antimicrobial for 288 MRSA isolates, Belgian hospitals, 2013-2014.





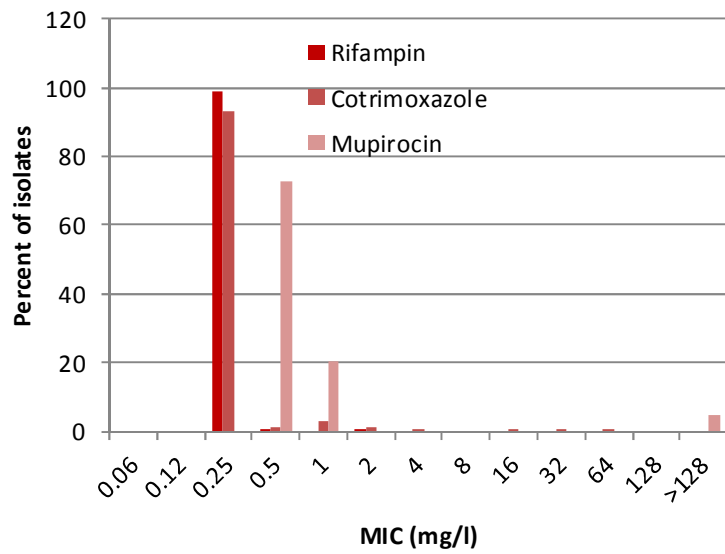
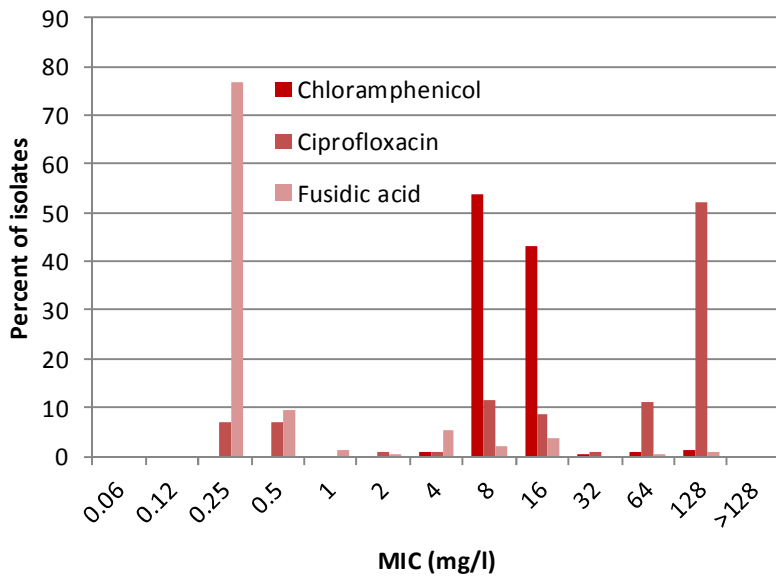
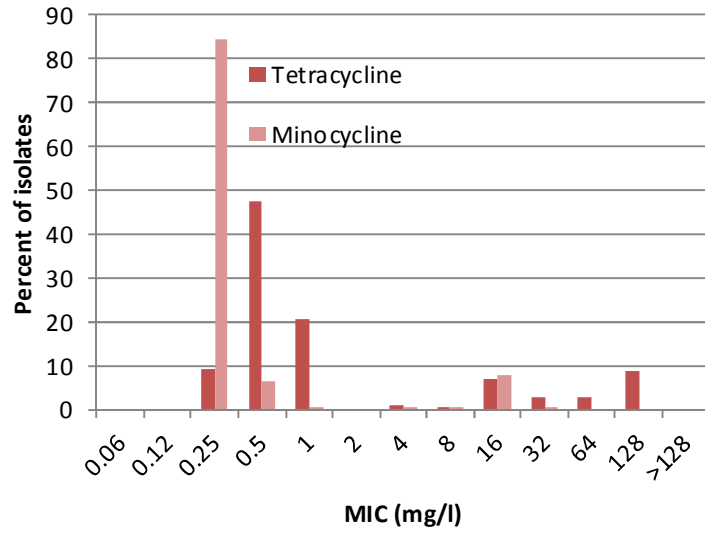
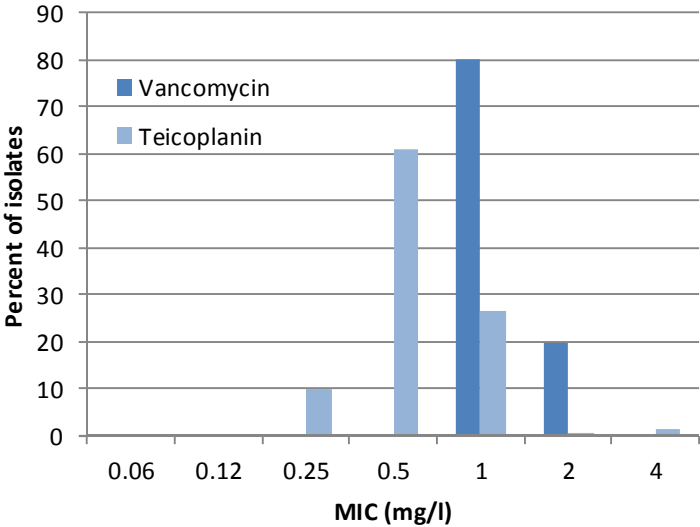
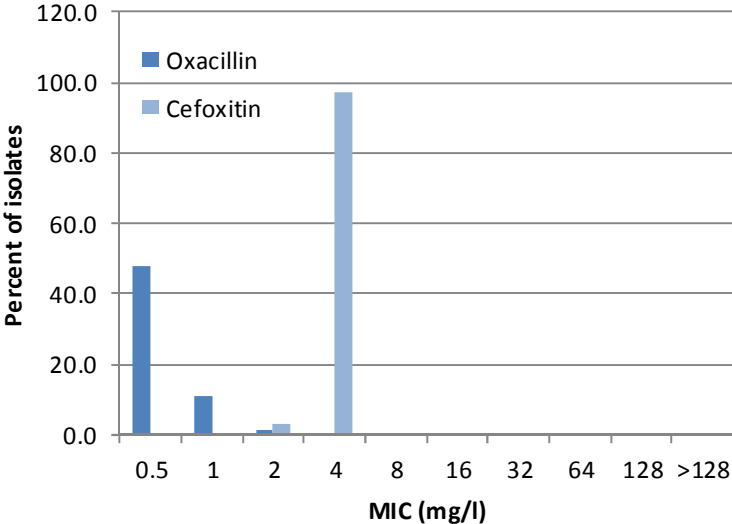
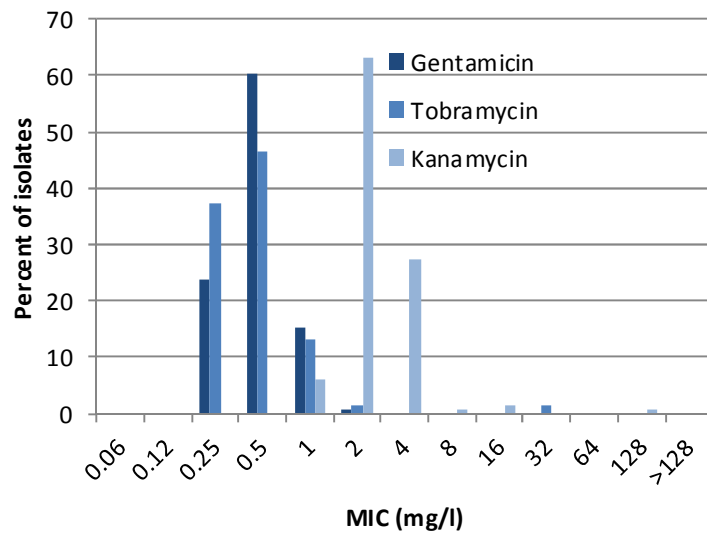
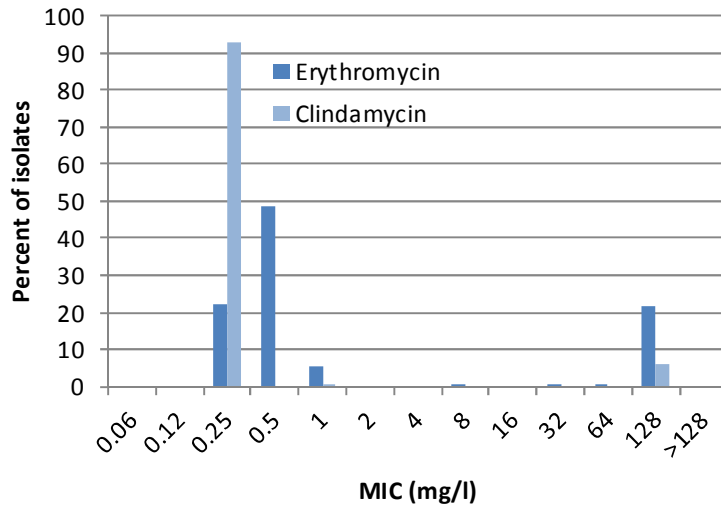
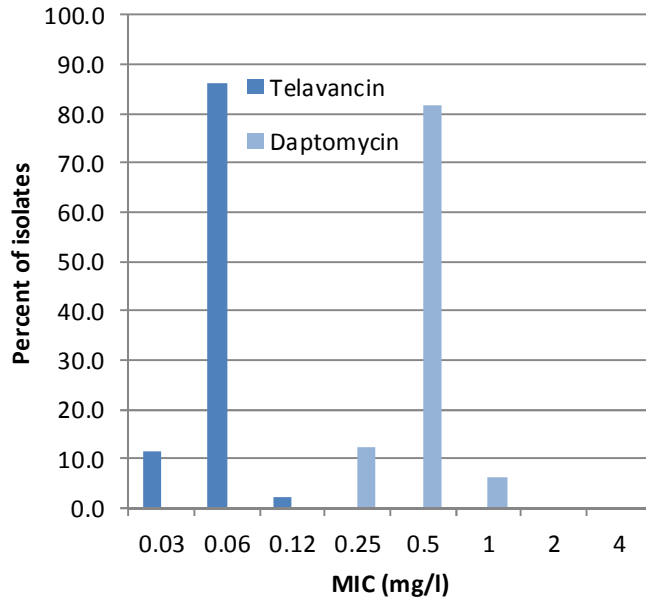


Figure 3: MICs distribution by antimicrobials for 131 MSSA isolates, Belgian hospitals, 2013-2014.





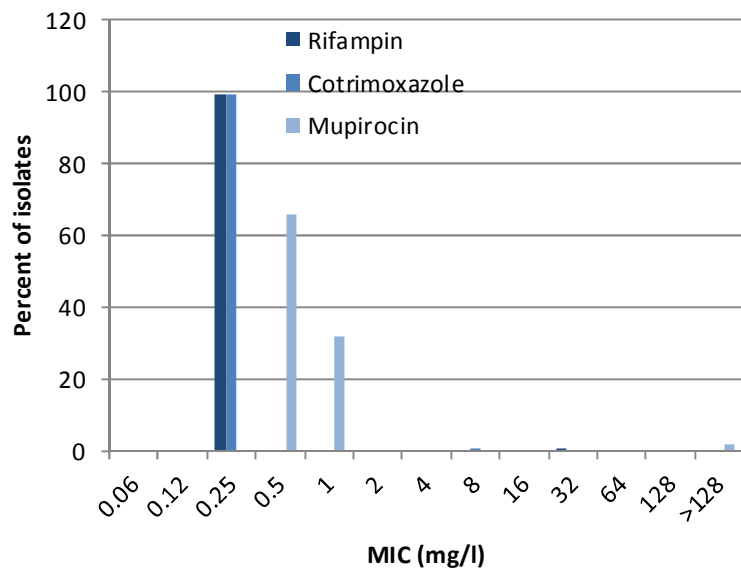
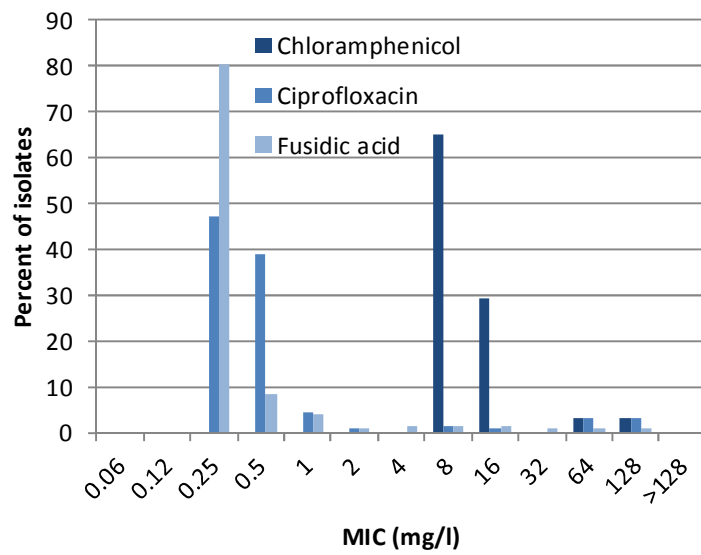
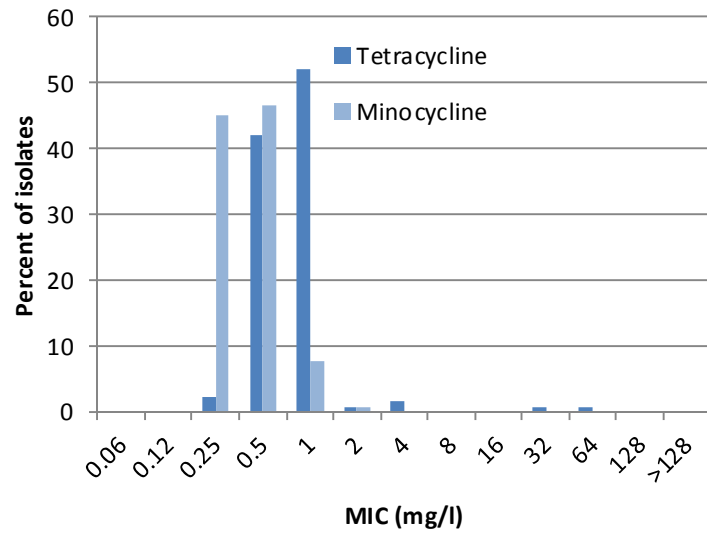
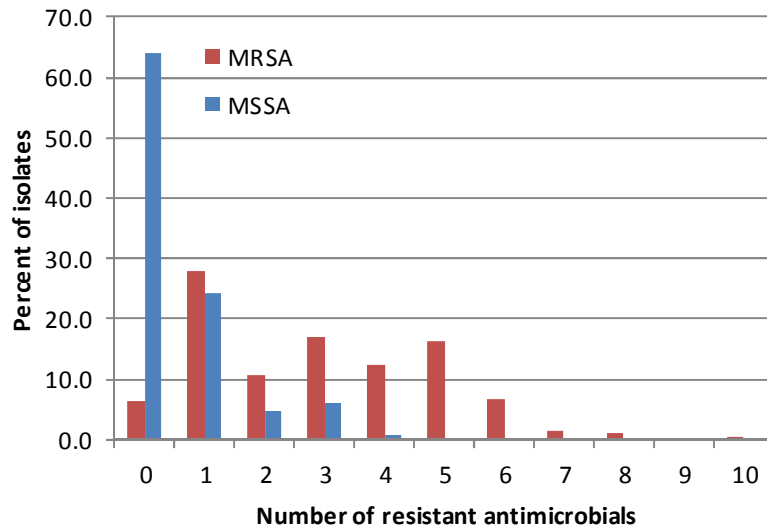


Figure 4: Comparison of co-resistance to non-beta-lactam drugs MSSA versus MRSA isolates from hospitals, Belgium, 2013-2014.



MICs for 19 antimicrobials were determined for all (n=60) methicillin resistant CoNS (MRCoNS, *mecA* positive) isolates (Table 7 and Table 8, Figure 5). All MRCoNS isolates were susceptible to linezolid, ceftaroline, vancomycin, telavancin, daptomycin, minocycline and tigecycline. Most MRCoNS isolates were resistant against fusidic acid (82%) and ciprofloxacin (78.3%). They also showed high resistances against cotrimoxazole (52%), aminoglycosides (35-38%) and tetracycline (20%). Resistance to MLS was frequent, ranging from 66.7% for erythromycin to 31.7% for clindamycin. Few MRCoNS isolates were resistant to chloramphenicol (15.3%) and rifampin (8.3%).

MICs for 19 antimicrobials were determined for all (n=20) methicillin susceptible CoNS (MSCoNS, *mecA* negative) isolates (Table 9 and Table 10, Figure 6). All MSCoNS isolates were susceptible to cefoxitin, ceftaroline, glycopeptides, daptomycin, gentamicin, kanamycin, minocycline, tigecycline, linezolid and rifampin. Most were also susceptible (90%-95%) to clindamycin, chloramphenicol, cotrimoxazole, mupirocin, ciprofloxacin and tobramycin. Resistance was frequent for erythromycin (40%), fusidic acid (40%) and tetracycline (15%).

Among the *S. epidermidis* (n=61) isolates, most were methicillin-resistant *S. epidermidis* (MRSE, n=46, 75%) and less were classified as methicillin-susceptible *S. epidermidis* (MSSE, n=15, 25%). The MRSE isolates showed more additional resistances than the MSSE strains (Figure 7). Most MRSE isolates were MLS resistant (76%) and carried *ermC*, *msrA* and/or *ermA* genes. More than the half MRSE (59%) showed aminoglycoside resistance and carried the genes *aacA-aphD* and/or *aadC*. 41% of MRSE isolates were mupirocin resistant and carried the gene *mupA*. Finally 20% of MRSE isolates were tetracycline resistant and carried the gene *tetK*.

Table 7: Cumulative proportions of MRCoNS isolates (n = 60) inhibited by increasing concentrations of 19 antimicrobial agents, Belgian hospitals, 2013-2014.

| Antimicrobial agents | % of strains resistant at MIC (mg/l) of : | | | | | | | | | | | | | | |
|----------------------|---|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|
| | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | >1024 |
| Oxacillin | 0 | 0 | 0 | 2 | 2 | 5 | 10 | 22 | 32 | 40 | 52 | 72 | 100 | 100 | - |
| Cefoxitin | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 12 | 38 | 88 | 97 | 100 | 100 | - |
| Ceftaroline | 0 | 0 | 0 | 37 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Vancomycin | 0 | 0 | 0 | 0 | 10 | 22 | 93 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Teicoplanin | 0 | 0 | 0 | 18 | 18 | 25 | 30 | 55 | 87 | 100 | 100 | 100 | 100 | 100 | - |
| Telavancin | 0 | 0 | 0 | 28 | 97 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Daptomycin | 0 | 0 | 0 | 23 | 58 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Erythromycin | 0 | 0 | 0 | 25 | 33 | 33 | 33 | 33 | 33 | 33 | 35 | 38 | 100 | 100 | - |
| Clindamycin | 0 | 0 | 0 | 60 | 65 | 68 | 68 | 70 | 70 | 70 | 70 | 70 | 100 | 100 | - |
| Chloramphenicol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 73 | 85 | 85 | 93 | 100 | 100 | - |
| Ciprofloxacin | 0 | 0 | 0 | 13 | 17 | 20 | 22 | 35 | 55 | 58 | 62 | 77 | 100 | 100 | - |
| Linezolid | 0 | 0 | 0 | 0 | 0 | 35 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Gentamicin | 0 | 0 | 0 | 60 | 62 | 62 | 62 | 62 | 63 | 72 | 82 | 93 | 100 | 100 | - |
| Tobramycin | 0 | 0 | 0 | 33 | 33 | 35 | 37 | 40 | 65 | 85 | 88 | 92 | 100 | 100 | - |
| Kanamycin | 0 | 0 | 0 | 0 | 22 | 33 | 38 | 50 | 55 | 62 | 62 | 63 | 100 | 100 | - |
| Tetracycline | 0 | 0 | 0 | 15 | 37 | 38 | 57 | 80 | 80 | 85 | 85 | 92 | 100 | 100 | - |
| Minocycline | 0 | 0 | 0 | 52 | 87 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Tigecycline | 0 | 0 | 0 | 83 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Rifampin | 0 | 0 | 0 | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 100 | - |
| Cotrimoxazole | 0 | 0 | 0 | 32 | 35 | 38 | 48 | 65 | 78 | 100 | 100 | 100 | 100 | 100 | - |
| Fusidic acid | 0 | 0 | 0 | 15 | 17 | 18 | 18 | 25 | 40 | 72 | 95 | 100 | 100 | 100 | - |
| Mupirocin | 0 | 0 | 0 | 0 | 60 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 100 | 100 |

Table 8: Range of MIC, MIC₅₀, MIC₉₀ and proportion of 60 MRCoNS isolates by susceptibility category to 19 antimicrobial agents from Belgian Hospitals, 2013-2014.

| 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 | 1.7 | 0.0 | 98.3 |
| Cefoxitin | 8->128 | 32 | 64 | 3.3 | 0.0 | 96.7 |
| Ceftaroline | 0.06->128 | 0.5 | 0.5 | 100.0 | 0.0 | 0.0 |
| Vancomycin | 0.06-2 | 2 | 2 | 100.0 | 0.0 | 0.0 |
| Teicoplanin | 0.06-2 | 4 | 16 | 86.7 | 8.0 | 0.0 |
| Telavancin | 0.03-2 | 0.5 | 0.5 | 100.0 | 0.0 | 0.0 |
| Daptomycin | 0.06->128 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Erythromycin | 0.06->128 | 128 | 128 | 33.3 | 0.0 | 66.7 |
| Clindamycin | 0.06->128 | 0.25 | 128 | 65.0 | 2.0 | 31.7 |
| Chloramphenicol | 0.12-64 | 8 | 16 | 72.9 | 7.0 | 15.3 |
| Ciprofloxacin | 0.25->128 | 8 | 128 | 20.0 | 1.0 | 78.3 |
| Linezolid | 0.25-2 | 2 | 2 | 100.0 | 0.0 | 0.0 |
| Gentamicin | 0.06->128 | 0.25 | 64 | 61.7 | 1.0 | 36.7 |
| Tobramycin | 0.06->128 | 8 | 64 | 40.0 | 15.0 | 35.0 |
| Kanamycin | 0.5->128 | 4 | 128 | 61.7 | 0.0 | 38.3 |
| Tetracycline | 0.12-128 | 2 | 64 | 80.0 | 0.0 | 20.0 |
| Minocycline | 0.06-32 | 0.25 | 1 | 100.0 | 0.0 | 0.0 |
| Tigecycline | 0.06-0.5 | 0.25 | 0.5 | 100.0 | 0.0 | 0.0 |
| Rifampin | 0.06->128 | 0.25 | 0.25 | 91.7 | 0.0 | 8.3 |
| Cotrimoxazole | 0.06-128 | 4 | 16 | 48.3 | 0.0 | 51.7 |
| Fusidic acid | 0.06-64 | 16 | 32 | 18.3 | 0.0 | 81.7 |
| Mupirocin | 0.06->1024 | 0.5 | >128 | 61.7 | 0.0 | 38.3 |

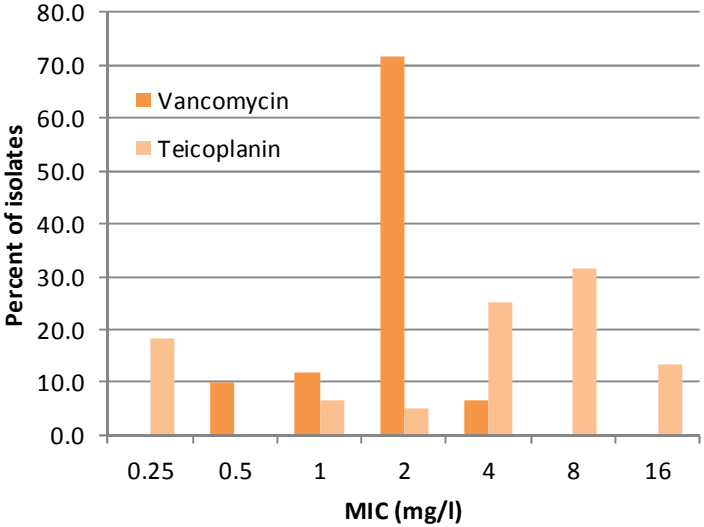
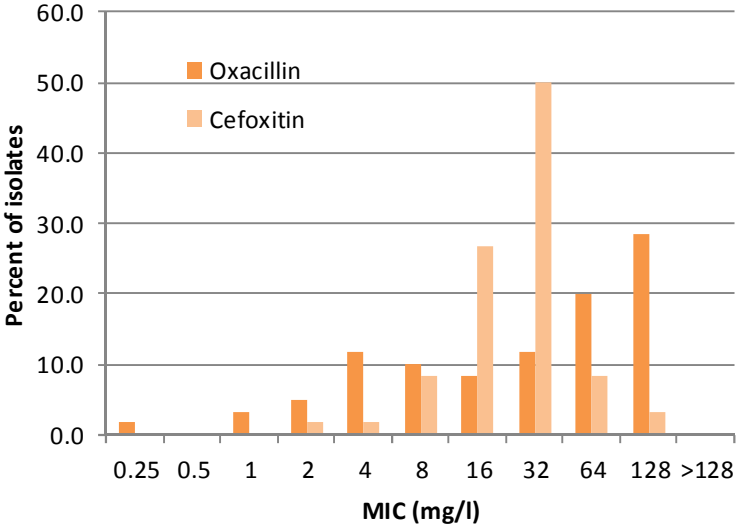
Table 9: Cumulative proportions of MSCoNS isolates (n = 20) inhibited by increasing concentrations of 19 antimicrobial agents, Belgian hospitals, 2013-2014.

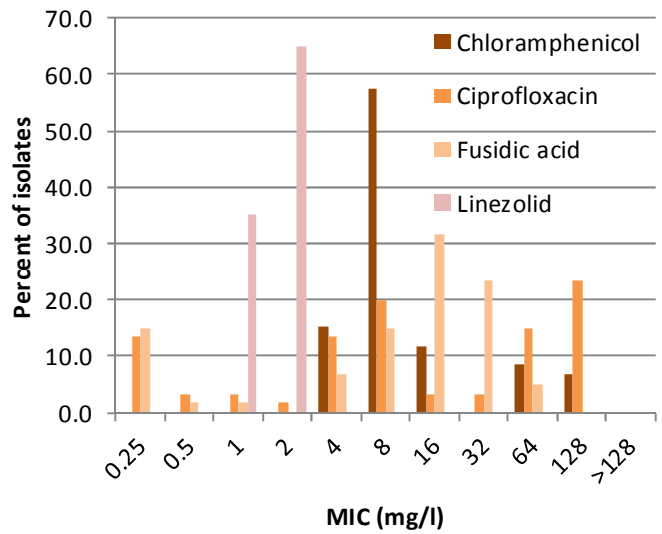
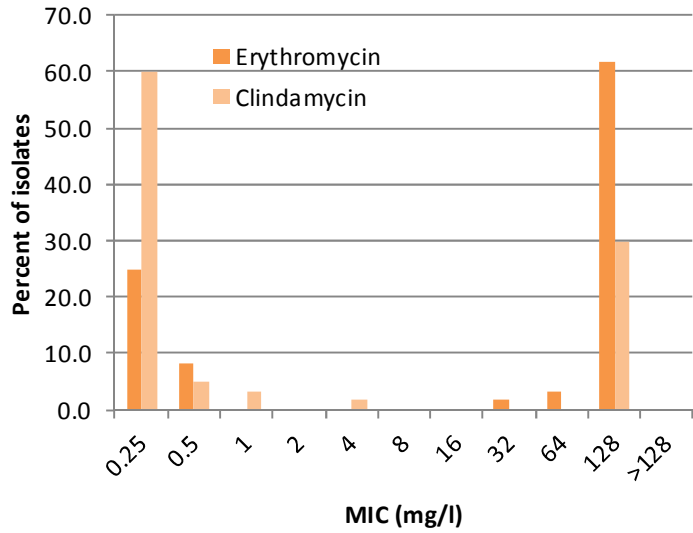
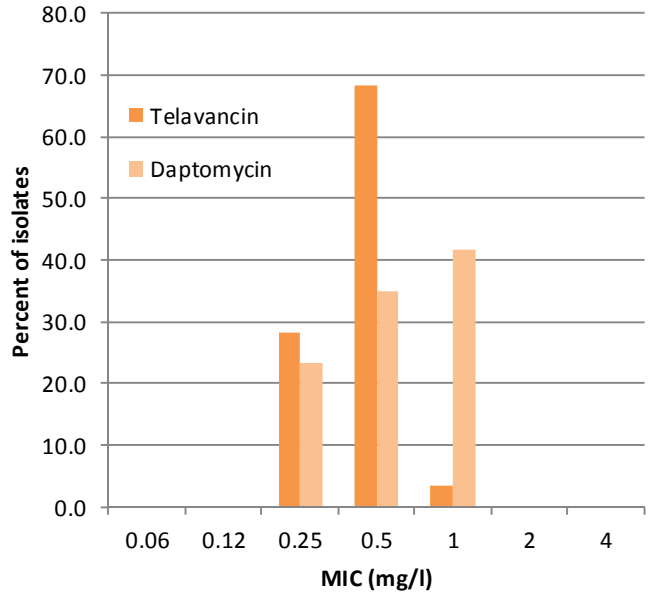
| Antimicrobial agents | % of strains resistant at MIC (mg/l) of : | | | | | | | | | | | | | | |
|----------------------|---|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|
| | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | >1024 |
| Oxacillin | 0 | 0 | 0 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Cefoxitin | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Ceftaroline | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Vancomycin | 0 | 0 | 0 | 0 | 5 | 35 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Teicoplanin | 0 | 0 | 0 | 25 | 25 | 40 | 75 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Telavancin | 0 | 0 | 0 | 55 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Daptomycin | 0 | 0 | 0 | 30 | 70 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Erythromycin | 0 | 0 | 0 | 55 | 60 | 60 | 60 | 60 | 60 | 60 | 65 | 75 | 100 | 100 | - |
| Clindamycin | 0 | 0 | 0 | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 100 | - |
| Chloramphenicol | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 35 | 90 | 100 | 100 | 100 | 100 | 100 | - |
| Ciprofloxacin | 0 | 0 | 0 | 65 | 85 | 90 | 95 | 95 | 95 | 95 | 95 | 95 | 100 | 100 | - |
| Linezolid | 0 | 0 | 0 | 0 | 0 | 30 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Gentamicin | 0 | 0 | 0 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Tobramycin | 0 | 0 | 0 | 85 | 85 | 95 | 95 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Kanamycin | 0 | 0 | 0 | 55 | 55 | 75 | 95 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Tetracycline | 0 | 0 | 0 | 20 | 55 | 55 | 70 | 85 | 85 | 85 | 85 | 90 | 100 | 100 | - |
| Minocycline | 0 | 0 | 0 | 65 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Tigecycline | 0 | 0 | 0 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Rifampin | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Cotrimoxazole | 0 | 0 | 0 | 80 | 90 | 90 | 90 | 95 | 95 | 100 | 100 | 100 | 100 | 100 | - |
| Fusidic acid | 0 | 0 | 0 | 55 | 60 | 60 | 60 | 65 | 65 | 90 | 100 | 100 | 100 | 100 | - |
| Mupirocin | 0 | 0 | 0 | 0 | 90 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 100 | 100 |

Table 10: Range of MIC, MIC₅₀, MIC₉₀ and proportion of 20 MSCoNS isolates by susceptibility category to 19 antimicrobial agents from Belgian Hospitals, 2013-2014.

| Antimicrobial agents | Range (mg/l) | MIC ₅₀ (mg/l) | MIC ₉₀ (mg/l) | % of strains per susceptibility category | | |
|----------------------|-----------------|-----------------------------|-----------------------------|--|-----|------|
| | | | | S | I | R |
| Oxacillin | 1->128 | 0.25 | 0.25 | 95.0 | 0.0 | 5.0 |
| Cefoxitin | 8->128 | 2 | 4 | 100.0 | 0.0 | 0.0 |
| Ceftaroline | 0.06->128 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Vancomycin | 0.06-2 | 2 | 2 | 100.0 | 0.0 | 0.0 |
| Teicoplanin | 0.06-2 | 2 | 4 | 100.0 | 0.0 | 0.0 |
| Telavancin | 0.03-2 | 0.25 | 0.5 | 100.0 | 0.0 | 0.0 |
| Daptomycin | 0.06->128 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| Erythromycin | 0.06->128 | 0.25 | 128 | 60.0 | 0.0 | 40.0 |
| Clindamycin | 0.06->128 | 0.25 | 0.25 | 90.0 | 0.0 | 10.0 |
| Chloramphenicol | 0.12-64 | 8 | 8 | 90.0 | 2.0 | 0.0 |
| Ciprofloxacin | 0.25->128 | 0.25 | 1 | 90.0 | 1.0 | 5.0 |
| Linezolid | 0.25-2 | 2 | 2 | 100.0 | 0.0 | 0.0 |
| Gentamicin | 0.06->128 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Tobramycin | 0.06->128 | 0.25 | 1 | 95.0 | 1.0 | 0.0 |
| Kanamycin | 0.5->128 | 0.5 | 2 | 100.0 | 0.0 | 0.0 |
| Tetracycline | 0.12-128 | 0.05 | 64 | 85.0 | 0.0 | 15.0 |
| Minocycline | 0.06-32 | 0.25 | 0.5 | 100.0 | 0.0 | 0.0 |
| Tigecycline | 0.06-0.5 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Rifampin | 0.06->128 | 0.25 | 0.25 | 100.0 | 0.0 | 0.0 |
| Cotrimoxazole | 0.06-128 | 0.25 | 0.5 | 90.0 | 0.0 | 10.0 |
| Fusidic acid | 0.06-64 | 0.25 | 16 | 60.0 | 0.0 | 40.0 |
| Mupirocin | 0.06->1024 | 0.5 | 0.5 | 95.0 | 0.0 | 5.0 |

Figure 5: MIC distribution by antimicrobial for 60 MRCoNS isolates, Belgian hospitals, 2013-2014.





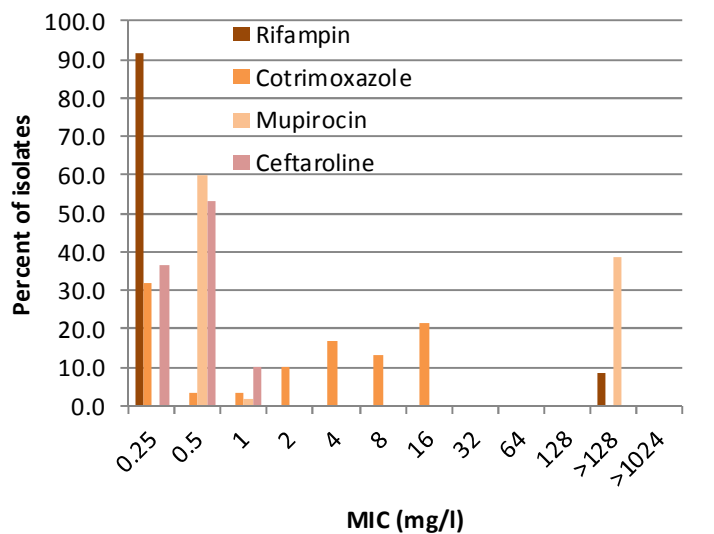
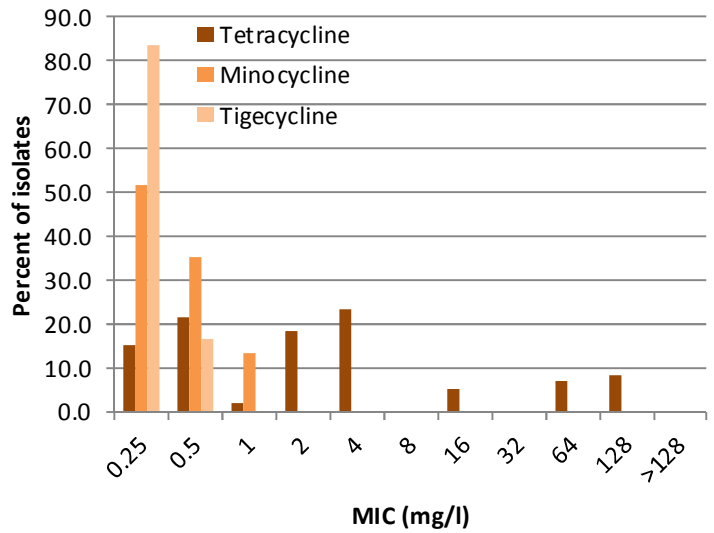
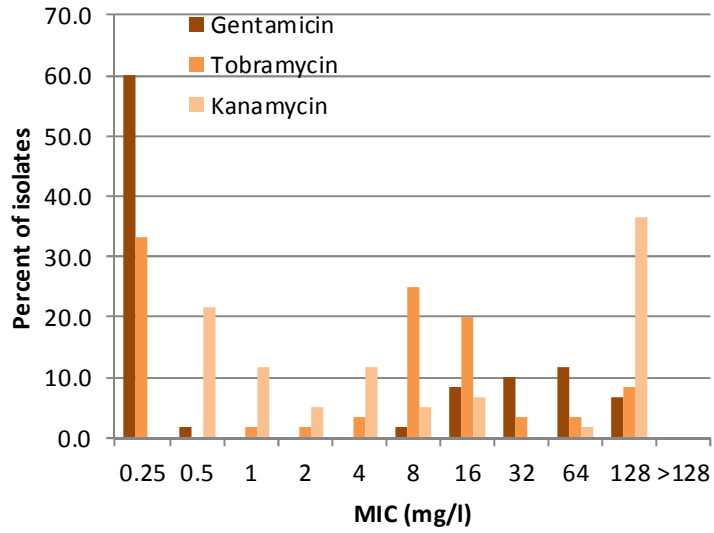
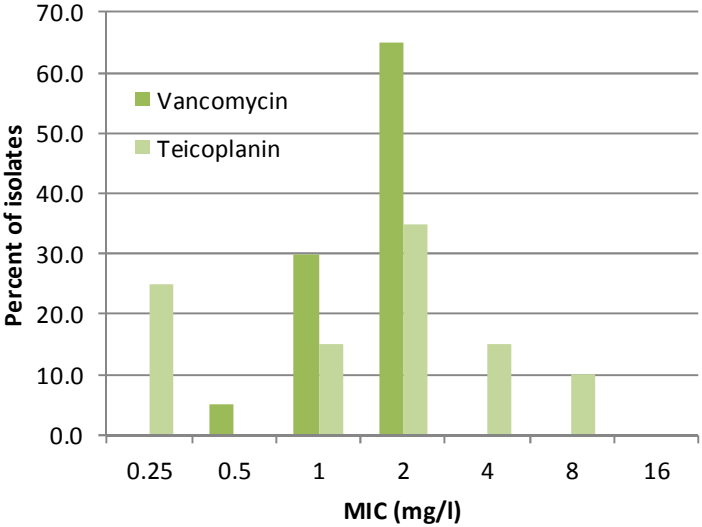
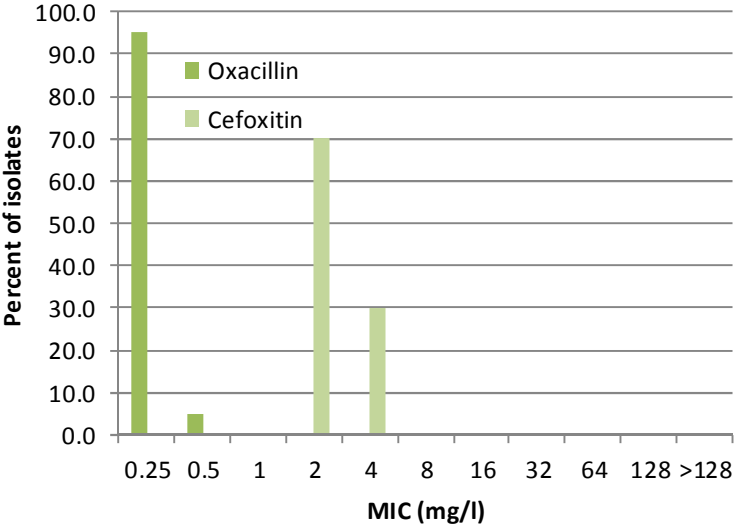
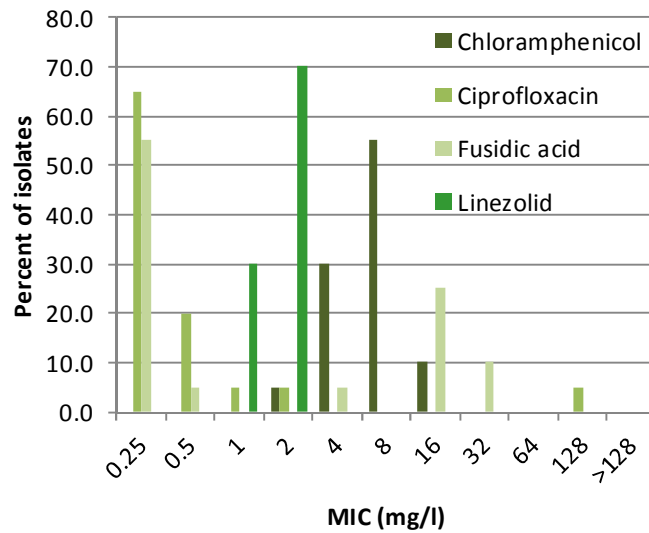
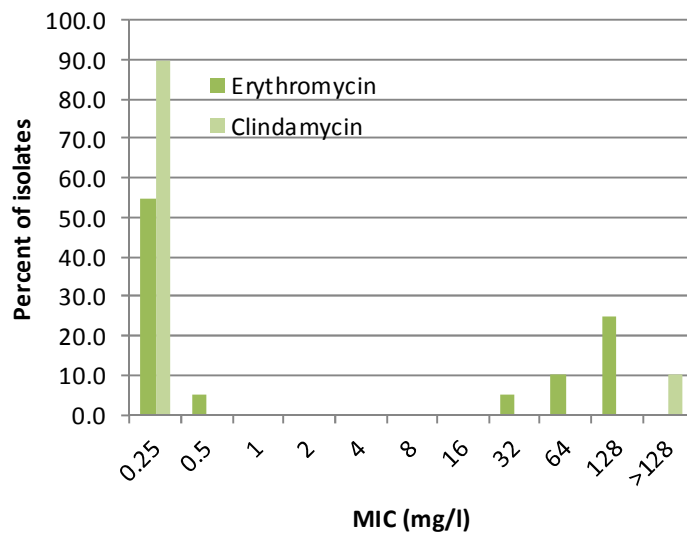
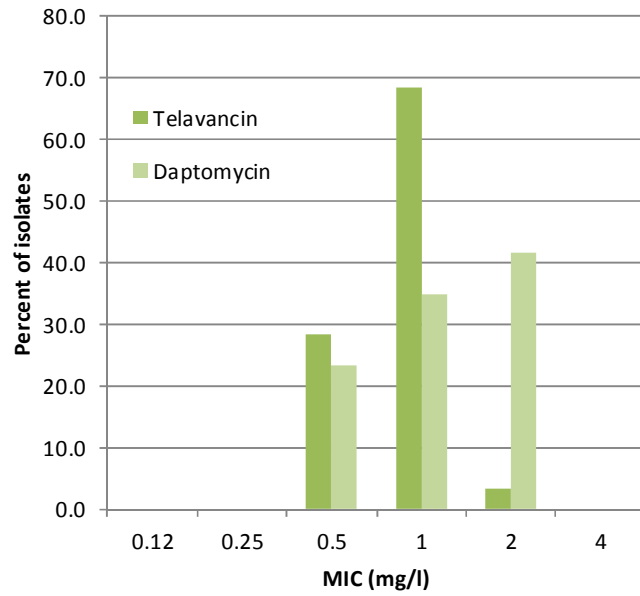


Figure 6: MIC distribution by antimicrobial for 20 MSCoNS isolates, Belgian hospitals, 2013-2014.





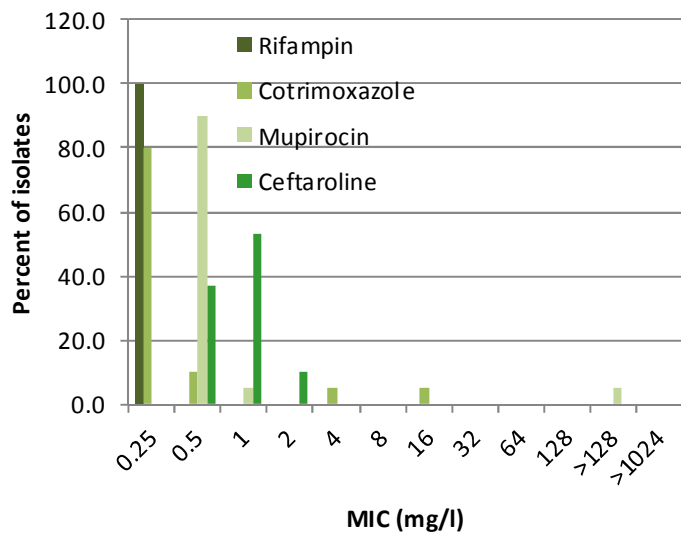
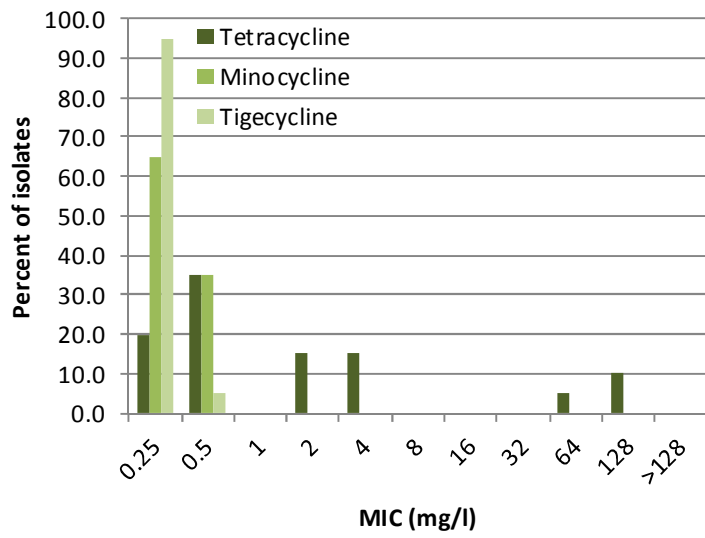
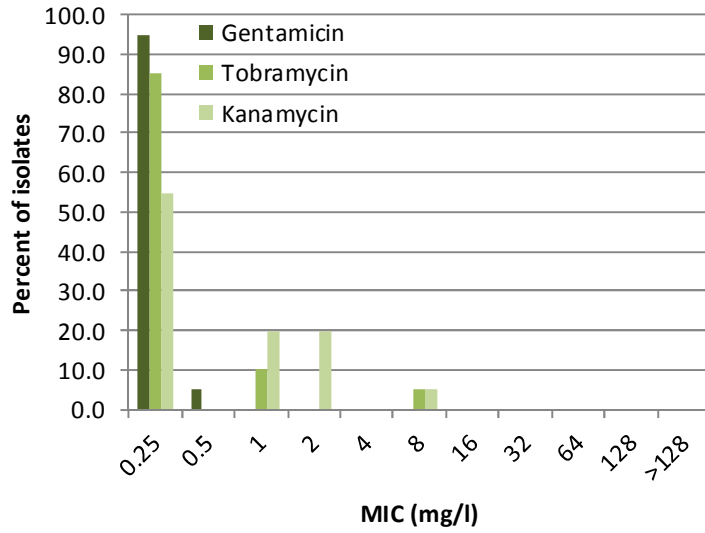
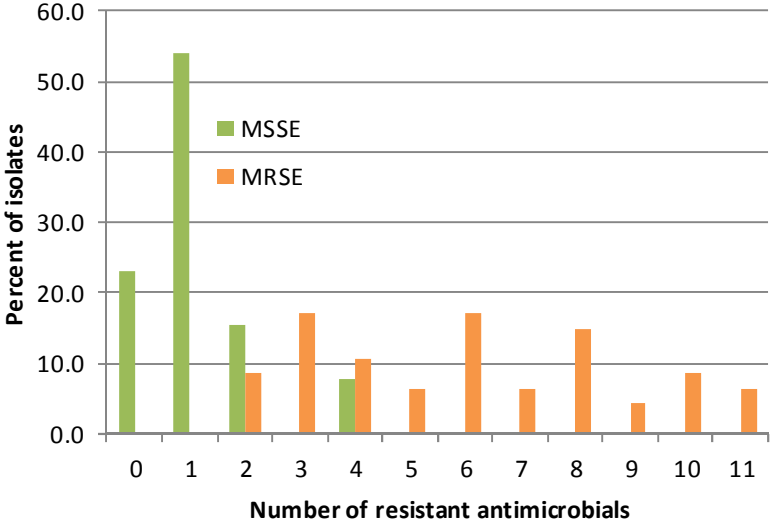


Figure 7: Comparison of co-resistance to non-beta-lactam drugs MSSE versus MRSE isolates from hospitals, Belgium, 2013-2014.



3.4. Genotype distribution

MRSA isolates (n=288) carried SCC mec type IV (2B) [n=217, 75.4%], II (2A) [n=34, 11.8%], V (5C2) [n=12, 4.2 %], IV (2B&5) [n=10, 3.5 %], VI(4B) [n=4, 1.4 %], I (1B) [(n=3, 1.0%), III (3A) + SCCHg [n=1, 0.4 %] or non-typeable [n=7, 2.4 %]. By *spa* typing, nearly 75% of MRSA strains belonged to four epidemic clones: *spa* CC38 (formerly PFGE type B2) ST45-SCC mec IV (n=87, 30.3%); *spa* CC8 (formerly PFGE type A20) ST8-SCC mec IV (n=55, 19.2%), *spa* CC2 (formerly PFGE type G10) ST5-SCC mec II (n=34, 11.8%), *spa* CC2 (formerly PFGE type C3) ST5-SCC mec IV (n=39, 13.6%), which were found in 57 (50.4%), 41 (36.3%), 24 (21.2%), 32 (28.3%) hospitals, respectively (Figure 8). Thirteen isolates belonged to the LA-MRSA ST398 clone. Sixteen MRSA were TSST positive, while 16% of MSSA isolates carried TSST. Most MSSA TSST positive isolates (22 out of 31) belonged to *spa* CC12 CC30. Fifteen *S. aureus* isolates (3 MSSA and 12 MRSA) were PVL positive. Among them, two isolates belonged to CA-MRSA clone USA300 ST8-SCC mec IV, one isolate belonged to ST8-SCC mec non-typeable, three isolates belonged to CA-MRSA European clone ST80-SCC mec IV, and one isolate belonged to CA-MRSA clone USA400 ST1-SCC mec V.

No CoNS isolate carried PVL and TSST genes. Among the *S. capitis* isolates, three were methicillin resistant and carried SCC mec V. Two *S. haemolyticus* were methicillin resistant and carried SCC mec V or non-typeable, respectively. The eight methicillin resistant *S. hominis* carried SCC mec non-typeable. Regarding to the *S. epidermidis* isolates (n=61), 76% of the MRSE belonged to 7 epidemic pulsotypes (found in ≥ 3 hospitals) which corresponded to the clonal complexes CC2 and CC5 (Figure 9). Most MRSE isolates carried SCC mec IV (56%). The 15 MSSE strains showed a great diversity (12 pulsotypes). The *icaA* gene involved in biofilm formation was found in 36% of the isolates (39% of MRSE and 27% of MSSE).

The gene *mecC* was not detected in *S. aureus* and CoNS isolates.

Figure 8: Distribution of Epidemic MRSA PFGE Types National Surveillance, Belgium, 1992-2014

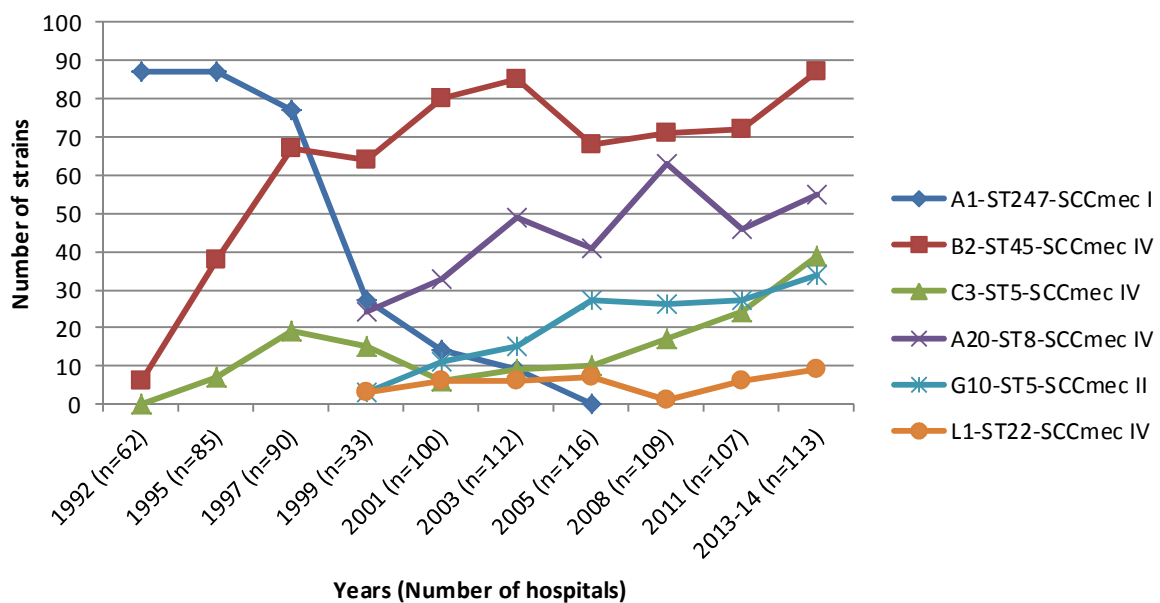
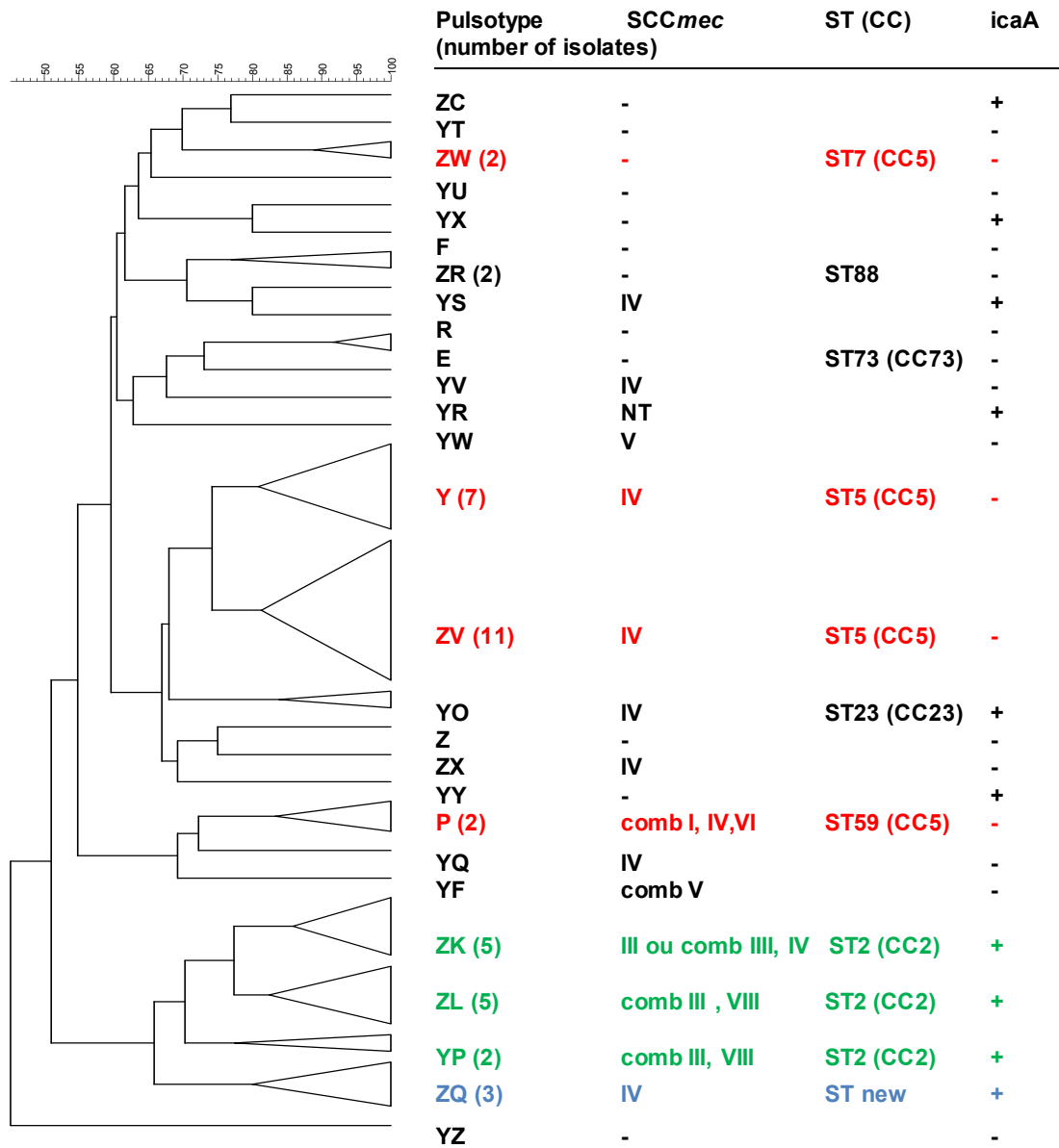


Figure 9: Dendrogram showing the relation between the PFGE types of MRSE and MSSE isolates. ST, sequence type



4. Conclusions

1. Ceftarolin showed excellent activity against methicillin-susceptible and methicillin-resistant *S. aureus*. However, few MRSA isolates (n = 4) showed MIC = 2 mg/l (intermediate)
2. The antimicrobial drugs linezolid and tigecycline showed excellent activities against MRSA and MSSA strains recovered from hospitalised patients in Belgian hospitals.
3. No *S. aureus* isolate resistant to glycopeptides and/or daptomycin was found.
4. A high proportion of MRSA isolates were resistant to fluoroquinolones (85%) and to MLS (>40%).
5. Resistance to erythromycin (>20%), ciprofloxacin (8%) and fusidic acid (8%) was frequent in MSSA isolates.
6. MSSA isolates were more susceptible to antimicrobials than MRSA isolates.
7. MRSA strains belonged to four international MRSA clones with a predominance of ST45-SCC_{mec} IV and ST8-SCC_{mec} IV.
8. Few MRSA isolates belonged to PVL-positive CA-MRSA USA300 clone ST8-SCC_{mec} IV, CA-MRSA European clone ST80-SCC_{mec} IV, CA-MRSA clone USA400 ST1-SCC_{mec} V and to LA-MRSA clone ST398.
9. Most CoNS isolates (76%) involved in bacteraemia were *S. epidermidis*.
10. Most *S. epidermidis* isolates were MRSE-SCC_{mec} IV strains which belonged to the hospital clones CC2 and CC7.

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