The National Reference Centre (NRC) for *S. aureus* of Université Libre de Bruxelles (ULB) provides the following tasks:

- Identification and antimicrobial susceptibility testing of *Staphylococcus sp*. strains using:
  - ❖ Phenotypic methods: protein profiles (MALDI-TOF), biochemical tests, minimal inhibitory concentration (MIC).
  - ❖ Genotypic methods: detection by PCR of *nuc* gene (*S. aureus* identification), *mec*A and *mecC* genes (coding for resistance to oxacillin/cefoxitin), *mup*A gene (coding for mupirocin resistance), *cfr* gene (coding for resistance to linezolid) and genes coding for resistance to macrolides-lincosamides-streptogramines (MLS), tetracyclines and aminoglycosides.
- Detection of genes coding for exfoliatins A, B and D, Panton-Valentine leucocidin (PVL), Toxic Shock Syndrome Toxin (TSST-1), enterotoxins (*se*a to *se*e, *seg* to *se*i and *ser* to *se*t) and enterotoxin-like (*sel*i, *sel*k to *sel*q and *sel*u).
- Molecular typing: pulsed field gel electrophoresis (PFGE) after genomic macrorestriction, multi-locus sequence typing (MLST), *spa* sequence typing, characterisation of the staphylococcal cassette chromosome *mec* (SCC*mec*), determination of *agr* group and detection of the arginine catabolic mobile element (ACME) - *arc*A gene.

These analyses are performed on clinical staphylococcal isolates causing diagnostic problems or collected during epidemiological investigations. Request forms are available on websites of the NRC (http://www.mrsa.be) or ISP-WIV (https://nrchm.wiv-isp.be).

The Microbiology laboratory including the NRC - *S. aureus* is accredited according to standard ISO15189. The list of accredited analyses is available on the BELAC website (http://economie.fgov.be/belac.jsp).

Characterisation of atypical clinical strains

In 2015, the NRC identified and/or determined the antimicrobials susceptibility of 184 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 14 MRSA, 5 MSSA strains. None strain showed a decreased susceptibility to glycopeptides (GISA).

A total of 147 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=142), while the remaining five isolates were coagulase negative *Staphylococcus* including *mecA*-positive *S. epidermidis* (n=1), *S. capitis* (n=1), *S. haemolyticus* (n=1), and *S. hominis* (n=1), as well as one *mecA*-negative *S. hominis*. Of the 142 *S. aureus* isolates, 15 were cryptic (also named heterogeneous) MRSA (10%), containing *mec*A gene but presenting phenotypic susceptibility to both oxacillin (MIC < 2 µg/mL) and cefoxitin (MIC < 4 µg/mL) (n=7), or being only phenotypically susceptible to oxacillin (MIC < 2 µg/mL) (n=8). On the other side, 16 isolates (11%) showing resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=6), to only oxacillin (MIC > 2 µg/mL) (n=6), or to only cefoxitin (MIC > 4 µg/mL) (n=4) lacked the *mec*A gene. From these 16 isolates, two isolates resistant to both β-lactams carried the *mecC* gene (1.4%), and the remaining 14 (9.9%) were classified as BORSA. *Staphylococcus* isolates containing *mec*C gene are difficult to detect by routine laboratory methods, particularly by conventional PCRs or immunochromatographic assays. If immunochromatographic assay is used, we recommend to perform the test after induction with oxacillin and cefoxitin for detecting *mecC*-positive isolates.

Resistance to mupirocin was determined by MIC and *mup*A detection for 37 isolates. Among these, 11 (29.7%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and the presence of *mup*A gene.

Toxin detection and characterisation of community-acquired (CA) *S. aureus* strains

In 2015, 555 isolates of *S. aureus* including 294 MRSA, 257 MSSA and 4 BORSA were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection (Figure 1). All BORSA isolates were exotoxins-negative.

A total of 106 (36%) MRSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). These MRSA isolates were principally recovered from skin lesions, in particular from skin abscess, soft tissues or furunculous (n=62) but also from deep fluids (n=14), screenings (n=15), blood cultures (n=2) or unknown (n=13).

By molecular typing, most of PVL-positive MRSA isolates (n=61, 57.5%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=31), European clone ST80-SCC*mec* IV (n=21), and ST30-SCC*mec* IV (Southwest Pacific clone) (n=9) (Figure 2). Twenty-four of the 31 (77.4%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300. The remaining CA-MRSA were assigned to the following clones: Taiwan ST59-SCC*mec* V (n=19), USA400 ST1 (n=7), West Australia ST88-SCC*mec* IV (n=6), Bengal Bay ST772-SCC*mec* V (n=3), ST152/377-SCC*mec* IV or V (n=2), ST22 (n=4), ST5 (n=2), ST398 (n=1) and undetermined (*spa*-type t15452, n=1).

Figure 1: Number of MRSA and MSSA isolates received for PVL detection, 2005-2015.

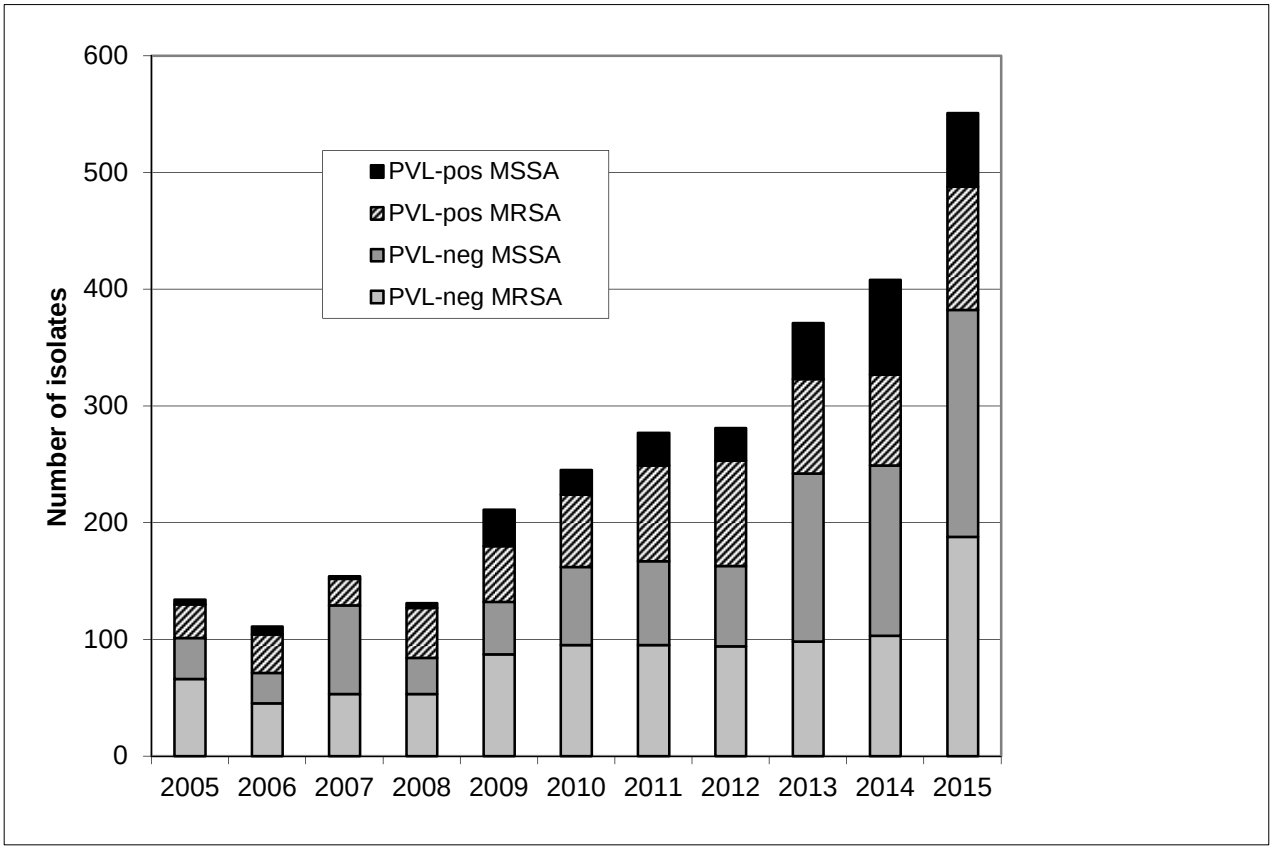
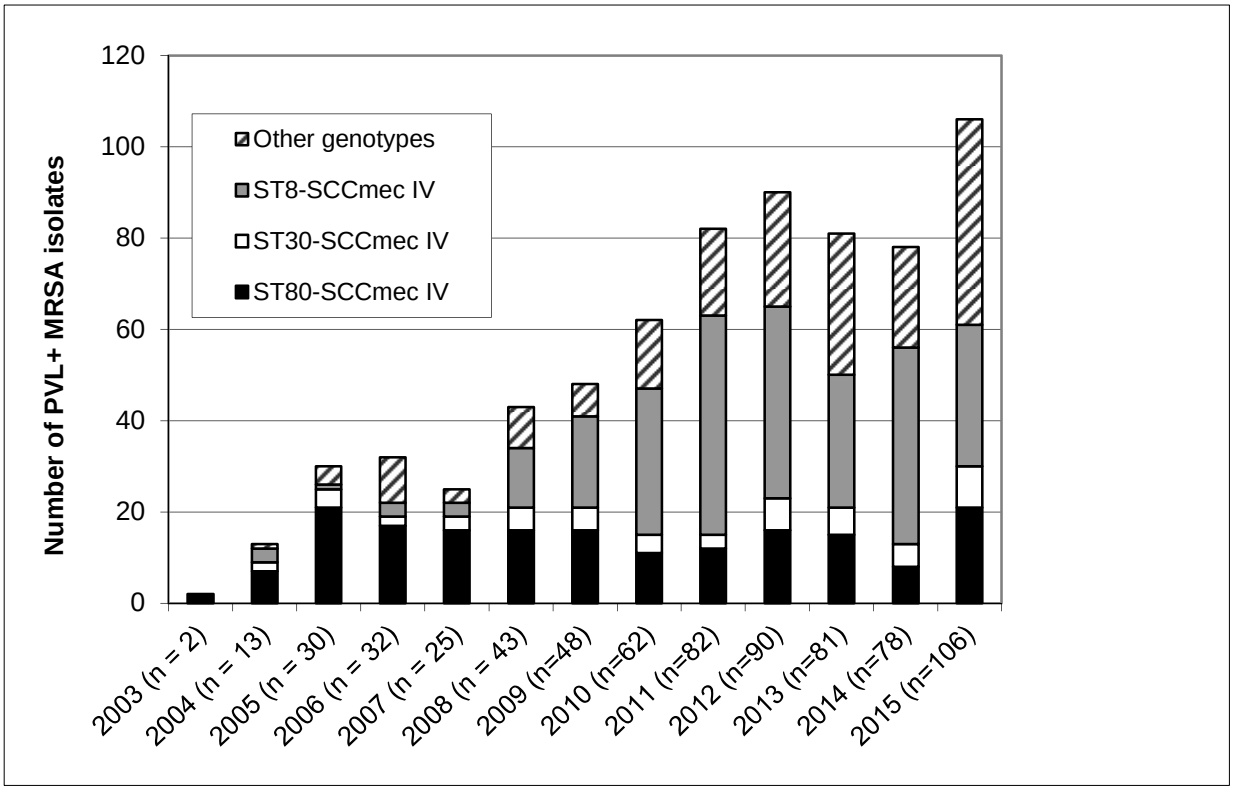


Figure 2: Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2015.



Sixty-three (24.5%) MSSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). Molecular typing of these 63 PVL-positive MSSA isolates revealed more genomic diversity than for MRSA isolates. These isolates were related to the clones: CC121 (n=13), ST152/377 (n=11), CC30 (n=7), CC15 (n=5), CC8 (n=5), CC1 (n=4), CC5 (n=3), ST7 (n=1), ST26 (n=1), CC22 (n=1), and ST88 (n=1) or undetermined clones (n=12)

TSST-1 toxin was detected in 21 MRSA (7%) and 39 MSSA (15.2%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=24), deep fluids (n=4), screenings (n=8), blood cultures (n=6), urines (n=1) or other sites (n=17). Molecular typing showed that majority of TSST-1 positive isolates belonged to ST30 (n=23, 38.3%), ST34 (n=9, 15%) or ST22 (n=8, 13.3%). Six TSST-1 positive MRSA were associated to ST22, currently recognized as the UK-EMRSA- 15 Middle Eastern Variant or Gaza strain.

Genes coding for exfoliatins A (*eta*) and/or B (*etb*) were found in 17 MSSA isolates and one MRSA. The gene coding for exfoliatin A alone was recovered in 8 MSSA isolates belonging to clones ST15 and ST121. Both genes (*eta* and *etb*) were found in 9 MSSA isolates belonging to ST121. Four of these 9 isolates were recovered from skin lesions (n=3) or screening (n=1) samples and belonged to the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC). The MRSA isolate carrying only *eta* gene belonged to the clone ST913-SCC*mec* IV.

Antimicrobial resistance percentages of MRSA and MSSA isolates are summarized in Tables 1 and 2. The 4 BORSA isolates showed additionally resistance to erythromycin-clindamycin (n=1) or tetracycline (n=2).

Table 1: Percentage of antimicrobial resistance of MRSA isolates received for toxin detection.

Antimicrobials	Antimicrobial resistance of MRSA isolates (%)		
	PVL positive (n=106) N (%)	PVL negative (n=188) N (%)	Total (n=294) N (%)
Erythromycin	56 (53)	71 (38)	127 (43)
Clindamycin	36 (34)	69 (37)	105 (36)
Ciprofloxacin	22 (21)	85 (45)	107 (36)
Gentamycin	13 (12)	23 (12)	36 (12)
Tobramycin	13 (12)	69 (37)	82 (28)
Kanamycin	75 (71)	89 (47)	164 (56)
Minocycline	-	22 (12)	22 (7.5)
Tetracycline	32 (30)	74 (39)	96 (33)
Rifampin	-	1 (0.5)	1 (0.3)
Cotrimoxazole	1 (0.9)	2 (1)	3 (1.0)
Linezolid	-	-	-
Fusidic acid	23 (22)	19 (10)	42 (14)
Mupirocin	-	-	-

N, number of resistant isolates; -, absence of resistant isolates.

Table 2: Percentage of antimicrobial resistance (%) of MSSA isolates received for toxin detection.

Antimicrobials	Antimicrobial resistance of MSSA isolates (%)		
	PVL positive (n=63) N (%)	PVL negative (n=194) N (%)	Total (n=257) N (%)
Erythromycin	6 (9.5)	44 (22.7)	50 (19.5)
Clindamycin	4 (6.4)	39 (20.1)	43 (16.7)
Ciprofloxacin	4 (6.4)	7 (3.6)	11 (4.3)
Gentamycin	-	-	-
Tobramycin	-	1 (0.6)	1 (0.4)
Kanamycin	3 (4.8)	4 (2.1)	7 (2.7)
Minocycline	-	4 (2.1)	4 (1.6)
Tetracycline	21 (33.3)	9 (4.6)	30 (11.7)
Rifampin	-	-	-
Cotrimoxazole	1 (1.6)	1 (0.6)	2 (0.8)
Linezolid	-	-	-
Fusidic acid	3 (4.8)	17 (8.8)	20 (7.8)
Mupirocin	1 (1.6)	-	-

N, number of resistant isolates; -, absence of resistant isolates.

Typing for epidemiological investigations

In 2015, molecular typing using *spa* typing and/or PFGE analysis was performed on 585 *S. aureus* isolates including 300 MRSA (299 *mecA*-positive, one *mecC*-positive), 279 MSSA, and 6 BORSA. Among these, 96 MRSA isolates and 55 MSSA isolates were sent for epidemiological investigation of local outbreaks (n=31, 17 MRSA outbreaks, 14 MSSA outbreaks).

The 96 MRSA isolates recovered from 14 hospitals were classified into 10 distinct lineages. The most frequently recovered genotypes were those previously found in our Belgian hospitals: ST5-SCC*mec* II or IV found in 18 (19%) MRSA isolates recovered from 3 hospitals, ST8-SCC*mec* IV found in 18 (19%) MRSA isolates recovered from 7 hospitals, and ST45-SCC*mec* IV found in 20 (21%) MRSA isolates recovered from 5 hospitals. Five isolates belonging to the clone ST8-SCC*mec* IV, containing the ACME pathogenicity island characteristic of MRSA USA300, were associated to an outbreak originated in 2014 in one hospital. Genotype ST1-SCC*mec* IV corresponding to the CA-MRSA (PVL-positive) clone USA400 was recovered from 3 hospitals. The CA-MRSA Taiwan clone ST59-SCC*mec* V (n=14) was recovered in an outbreak from a single hospital. Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 16 of the 17 clusters investigated.

The 55 MSSA isolates recovered from 14 hospitals showed high diversity with 15 distinct lineages. The most frequent genotypes were: CC15 (n=9, 16%), CC22 (n=7, 13%), CC30 (n=5, 9%), CC121 (n=5, 9%), and ST152/377 (n=5, 9%).

An additional *S. capitis* outbreak was investigated by PFGE analysis, all isolates were *mecA*-negative and showed the same pulsotype.

European Antimicrobial Resistance Surveillance Network (EARS-Net) program on *S. aureus* isolated from blood cultures

Since 2005, all MRSA and MSSA bacteraemia are subject to mandatory reporting on a request form including clinical and microbiological data in collaboration with the Public Health Scientific Institut (Institut Scientifique de Santé Publique) (WIV-ISP).

In 2015, 913 patients with a first *S. aureus* bacteraemia episode (per trimester) were recorded. Among these patients, 112 cases of MRSA bacteraemia were confirmed corresponding to a proportion of 12.3% of MRSA (Table 3).

Data from all countries participating to the EARS-Net program are available on the website:

http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial_resistance/EARS-Net/Pages/EARS-Net.aspx

Table 3: EARS-Net Data on methicillin resistance of *S. aureus* isolated from blood cultures in Belgium, 1999-2015.

Year	Number of isolates (%)		Total (N)
	Susceptible	Resistant	
1999	340 (76.4)	105 (23.6)	445
2000	520 (79.1)	137 (20.9)	657
2001	729 (77.4)	213 (22.6)	942
2002	783 (71.7)	309 (28.3)	1092
2003	798 (70.4)	336 (29.6)	1134
2004	819 (66.7)	408 (33.3)	1227
2005	719 (68.6)	329 (31.4)	1048
2006	670 (78.1)	188 (21.9)	858
2007	656 (76.7)	199 (23.3)	855
2008	719 (79.4)	187 (20.6)	906
2009	748 (78.9)	200 (21.1)	948
2010	840 (79.5)	217 (20.5)	1057
2011	1440 (82.6)	304 (17.4)	1744
2012	1308 (83.4)	260 (16.6)	1568
2013	1340 (83.1)	272 (16.9)	1612
2014	855 (86.5)	133 (13.5)	988
2015	801 (87.7)	112 (12.3)	913

Analyse of *S. aureus* from animal origin

Twenty-six (9%, 26/300) MRSA isolates belonging to clone ST398, called livestock-associated MRSA and corresponding to strains from animal origin, were detected in hospitalised or ambulant patients in Flanders (n=24) and Wallonia (n=2). These MRSA were isolated from screenings (n=9), skin lesions (n=10), blood cultures (n=2) or other sites (n=5). Available data allowed to bring out that 9 patients had direct contact with animals (farmers, vets). None toxins were detected in these MRSA ST398 strains.

Two additional MRSA strains related to the lineage CC398, but not associated to livestock were detected in patients from Flanders. One was linked to ST398 and contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). While, the remaining MRSA isolate was related to the clone ST291, a homologue recombinant double locus variant of ST398, which represents a distinct genetic lineage.

Twenty (7.2%, 20/ 279) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from skin lesions (n=9), screenings (n=5), blood cultures (n=4) and deep fluid (n=2). ST398 MSSA strains were not associated with livestock contacts. None toxins were detected in these MSSA ST398 strains.

National surveillance of MRSA in Nursing homes

In 2015, a national study on prevalence of carriage of multi-resistant bacterial pathogens in Nursing homes (NH) was conducted by the Scientific Institute of Public Health (ISP-WIZ), Brussels, the National Reference Centre (NRC) for multi drug resistant Gram-negative bacteria, CHU UCL Namur, Mont-Godinne, the NRC for enterococci, University Hospital Antwerpen, Edegem and the NRC *S. aureus*, Hôpital Erasme– ULB, Brussels. The complete report is available on http://www.nsih.be/download/LTCF/MDRO/Rapport_MDRO_2015_FR.pdf

In this report, only microbiological data concerning MRSA are presented.

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Among the 1441 residents from 29 Belgian NH, 132 (9%) carried MRSA. The positive samples were obtained from rectal swabs (n=130) or wounds (n=2).

Antimicrobial resistance, presence PVL and TSST-1 genes and *spa*-typing were performed on these 132 MRSA isolates

Typing and toxin detection

The 132 MRSA isolates recovered from 29 NH were classified into 6 distinct lineages. The most frequently recovered genotypes were those previously found in our Belgian hospitals: ST5-SCC*mec* II or IV found (n= 49, 37%), ST8-SCC*mec* IV found (n=28, 21%) and ST45-SCC*mec* IV found (n= 40, 30%). The remaining isolates were related to ST22-SCC*mec* IV (n=3), the livestock–associated clones CC97-SCC*mec* V (n=1) and CC398-SCC*mec* V (n=2) or undetermined (n=9).

None of the 132 MRSA isolates carried PVL, and only two isolates belonged to ST45 or undetermined lineage carried TSST-1.

Antimicrobial susceptibility

MICs to 22 antimicrobials were determined by microdilution method using sensititre (TREK Diagnostic Systems) according to EUCAST guidelines (2017) for 130 MRSA isolates (Table 4). All isolates were susceptible to glycopeptides, tigecycline, daptomycin, cotrimoxazole, rifampicine and linezolid. All but two isolates were susceptible to ceftaroline. Four isolates were cryptic MRSA (3.1%), containing the *mecA* gene but presenting phenotypic susceptibility to oxacillin (MIC < 2 µg/mL). More than 90% of MRSA were susceptible to gentamycin (99.2%), minocycline (98.5%) and mupirocin (90%). Majority of MRSA were susceptible to fusidic acid (84.6%). Resistance to MLS was relatively frequent, with 59.2% of resistance to erythromycin and 27.7% to clindamycin. For aminoglycosides, resistance to kanamycin (30.8%) and tobramycin (32.3%) was more frequent than resistance to gentamycin (0.7%). Most isolates were resistant to ciprofloxacin (94.6%) and chloramphenicol (78.4%) and 55.4% were resistant to tetracycline.

Table 4: MICs of MRSA collected in Nursing homes in 2015.

MIC (mg/L)	Susceptible N (%)	Resistant N (%)
Oxacillin (1 - >64)	4 (3.1)	126 (97.0)
Cefoxitin (8 - >64)	-	130 (100)
Ceftaroline (<0.25 - 2)	128 (98.5)	2 (1.5)
Vancomycin (0.5 - 2)	130 (100)	0
Teicoplanin (<0.25 - 1)	130 (100)	0
Telavancine (0.03-0.12)	130 (100)	0
Erythromycin (<0.25 - >64)	77 (59.2)	53 (40.8)
Clindamycin (<0.25 - >64)	94 (72.3)	36 (27.7)
Ciprofloxacin (<0.25 - >64)	3 (2.3)	123 (94.6)
Gentamycin (<0.25 – 2)	129 (99.2)	1 (0.7)
Tobramycin (<0.25 - >64)	92 (70.8)	42 (32.3)
Kanamycin (1- >64)	90 (69.2)	40 (30.8)
Minocycline (<0.25 - 16)	128 (98.5)	2 (1.5)
Tetracycline (<0.25 - >64)	58 (44.6)	72 (55.4)
Tigecycline (<0.25 – 0.5)	130 (100)	0
Daptomycin (<0.25 – 1)	130 (100)	0
Rifampicine (<0.25 – 0.5)	130 (100)	0
Cotrimoxazole (<0.25 - 2)	130 (100)	0
Chloramphenicol (4- >64)	28 (21.5)	102 (78.4)
Linezolid (1 - 4)	130 (100)	0
Fusidic acid (<0.25 - 16)	110 (84.6)	20 (15.4)
Mupirocin (<0.5 - 512)	117 (90.0)	13 (10.0)

Conclusions

In 2015, the number of PVL-positive MRSA cases per year slightly increased compared to 2014, 106 cases in 2015 versus 78 cases in 2014. The antimicrobial resistance profile of these PVL-positive MRSA remained stable, we observed only increase of resistance against clindamycin (34% versus 20%), gentamicin (12% versus 2%) and tobramycin (12% versus 2%). The proportion of CA-MRSA belonging to clone ST8-SCC*mec* IV decreased compared to 2014 (55% to 29%) but the percentage of clone USA-300 within the CA-MRSA ST8 population remained stable (80% in 2014, 77.4% in 2015). An increase of the proportion of CA-MRSA isolates belonging to clone ST80-SCC*mec* IV (19%) was observed returning to the proportion observed in 2013. New emerging CA-MRSA lineages were observed.

The number of MSSA isolates received for toxin detection was similar to 2014. The proportion of PVL-positive MSSA cases decreased (24.5 %) compared to 2014 (36%). High diversity of genotypes was always observed. As in 2014, the most frequent MSSA clones were ST121 (21%) and ST30 (11%). Nevertheless, we observed also 17.5% of isolates belonging to ST152/377.

Genotyping of MRSA from local outbreaks in hospitals showed that MRSA isolates belonged mainly to nosocomial epidemic clones ST8-SSC*mec* IV, ST45-SCC*mec* IV and ST5 SCC*mec* IV ou II.

Study in Belgian Nursing homes showed a low prevalence (9%) of MRSA among residents. These isolates belonged mainly to nosocomial epidemic clones ST8-SSC*mec* IV, ST45-SCC*mec* IV and ST5-SCC*mec* IV or II. These MRSA isolates were resistant to ciprofloxacin, chloramphenicol, tetracycline, erythromycin, aminoglycosides and clindamycin and remained susceptible to other antimicrobials tested.

As in 2014, the livestock-associated MRSA clone ST398 was recovered in persons living principally in Flanders and with direct contact with animals like farmers or vets. These MRSA from animal origin were sporadically (9%) isolated in Belgian hospitals. MSSA strains, presenting the same genotypic characteristics than MRSA ST398, were also rarely (7.2%) recovered from persons without livestock contact.

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