

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), *mecA* and *mecC* genes (coding for resistance to oxacillin/cefoxitin), *mupA* gene (coding for mupirocin resistance), *cfr* gene (coding for resistance to linezolid) and genes coding for resistance to macrolides-lincosamides-streptogramins (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 (*sea* to *see*, *seg* to *sei* and *ser* to *set*) and enterotoxin-like (*seli*, *selk* to *selq* and *selu*).
- 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) - *arcA* 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 (N° 245 – MED). The list of accredited analyses is available on the BELAC website (<http://economie.fgov.be/belac.jsp>).

Characterisation of atypical clinical strains

In 2016, the NRC identified and/or determined the antimicrobials susceptibility of 157 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 8 MRSA, 5 MSSA and 4 coagulase-negative staphylococci strains. None strain showed a decreased susceptibility to glycopeptides (GISA).

A total of 133 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=128), while the remaining five isolates were coagulase negative *Staphylococcus* including *mecA*-positive *S. epidermidis* (n=1) and *S. intermedius* (n=1), as well as *mecA*-negative *S. epidermidis* (n=1), *S. hominis* (n=1) and *S. xylosus* (n=1). Of the 128 *S. aureus* isolates, 8 (6%) were cryptic (also named heterogeneous) MRSA, containing *mecA* gene but presenting phenotypic susceptibility to both oxacillin (MIC < 2 µg/mL) and cefoxitin (MIC < 4 µg/mL) (n=2), or being only phenotypically susceptible to oxacillin (MIC < 2 µg/mL) (n=6). On the other side, 19 isolates (15%) showing resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=8), to only oxacillin (MIC > 2 µg/mL) (n=2), or to only cefoxitin (MIC > 4 µg/mL) (n=9) lacked the *mecA* gene. From these 19 isolates, five isolates resistant to both β-lactams carried the *mecC* gene (4%), and the remaining 14 (11%) were classified as BORSA. *Staphylococcus* isolates containing *mecC* gene are difficult to detect by routine laboratory methods, particularly by conventional PCRs or immunochromatographic assays. If immunochromatographic assay is used, we recommend performing the test after induction with oxacillin and cefoxitin for detecting *mecC*-positive isolates.

Resistance to mupirocin was determined by MIC and *mupA* detection for 18 isolates, among these, 7 (39%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and the presence of *mupA* gene.

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

In 2016, 507 isolates of *S. aureus* including 270 MRSA and 237 MSSA were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection (Figure 1).

A total of 113 (42%) MRSA isolates contained *lukS-lukF* genes coding for Panton-Valentine leucocidin (PVL). These MRSA isolates were principally recovered from skin lesions, in particular from skin abscess, soft tissues or furunculosis (n=58) but also from deep fluids (n=22), screenings (n=16), blood cultures (n=2) or unknown (n=15).

By molecular typing, most of PVL-positive MRSA isolates (n=68, 60%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=38), European clone ST80-SCC*mec* IV (n=14), and ST30-SCC*mec* IV (Southwest Pacific clone) (n=16) (Figure 2). Twenty-four of the 29 (76%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300. The ST8-SCC*mec* IV isolates without ACME are maybe related to the USA300 Latin American variant clone. The remaining CA-MRSA isolates were assigned to the following clones: USA400 ST1 (n=9), ST573/772-SCC*mec* V (n=9), ST5 (n=7), Taiwan ST59-SCC*mec* V (n=6), ST22 (n=5), West Australia ST88-SCC*mec* IV (n=4), and ST398 (n=5).

Figure 1: Number of MRSA and MSSA isolates received for PVL detection, 2005-2016.

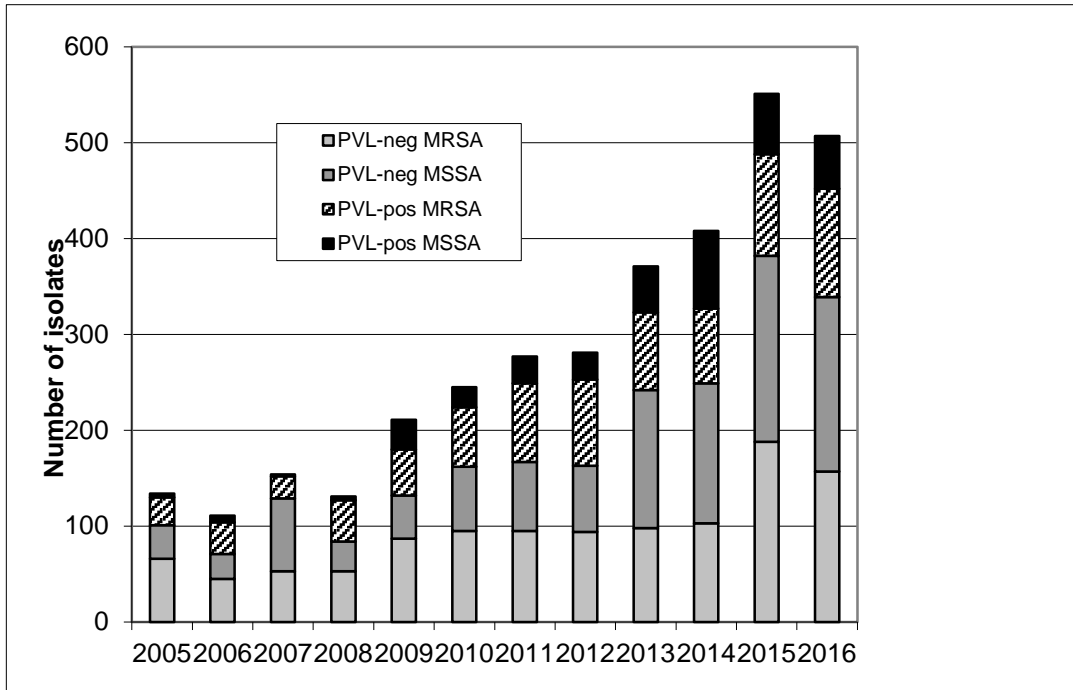
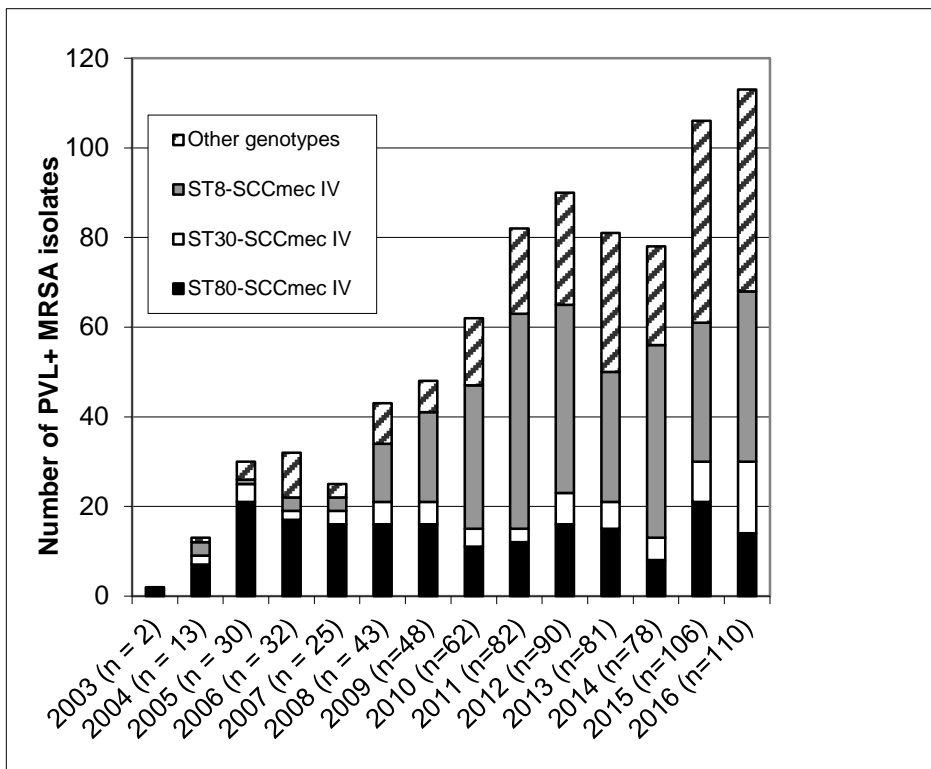


Figure 2: Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2016.



Fifty-five (23%) MSSA isolates contained *lukS-lukF* genes coding for Panton-Valentine leucocidin (PVL). Molecular typing of these 55 PVL-positive MSSA isolates revealed more genomic diversity than for MRSA isolates. These isolates were related to the clones: ST152/377 (n=14), CC1 (n=9), CC8 (n=7), CC30 (n=6), CC15 (n=4), CC121 (n=4), CC22 (n=2), CC80 (n=1), ST88 (n=1), ST188 (n=2), ST291 (n=1), ST398 (n=1), and ST361 (n=3).

TSST-1 toxin was detected in 19 MRSA (7%) and 25 MSSA (10%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=6), deep fluids (n=1), screenings (n=12), blood cultures (n=9), or other sites (n=16). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC30 (n=26, 59%) and or ST22 (n=11, 25%). The eleven ST22 isolates were MRSA and belonged probably to the UK-EMRSA-15 Middle Eastern Variant or Gaza strain.

Interestingly, three strains were positive for both PVL and TSST-1 toxins. One out of the five CA-MRSA isolates associated to CC22 and two out of the six CC30 PVL-positive MSSA isolates.

Genes coding for exfoliatins A (*eta*) and/or B (*etb*) were found in 20 MSSA isolates and 2 MRSA. The gene coding for exfoliatin A alone was recovered in 2 MRSA belonging to the clone ST913-SCC*mec* IV and 8 MSSA isolates belonging to clones ST15, CC25, ST109 or ST121. The gene coding for exfoliatin B alone was recovered in one MSSA isolate belonging to ST121. Both genes (*eta* and *etb*) were found in 11 MSSA isolates belonging to ST121. Five of the 11 *eta-etb* positive isolates, as well as the *etb*-positive isolate, belonged to the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC).

Antimicrobial resistance percentages of MRSA and MSSA isolates are summarized in Tables 1 and 2.

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

Antimicrobials	Antimicrobial resistance of MRSA isolates (%)		
	PVL positive (n=113)	PVL negative (n=157)	Total (n=270)
	N (%)	N (%)	N (%)
Erythromycin	65 (57)	62 (39)	127 (47)
Clindamycin	21 (19)	57 (36)	78 (29)
Ciprofloxacin	34 (30)	78 (50)	112 (41)
Gentamycin	15 (13)	15 (10)	30 (11)
Tobramycin	21 (19)	43 (27)	54 (24)
Kanamycin	62 (55)	54 (34)	116 (43)
Minocycline	-	21 (13)	21 (8)
Tetracycline	30 (26)	51 (32)	81 (30)
Rifampin	1 ()	2 (1)	3 (1)
Cotrimoxazole	1 ()	3 (2)	4 (1.5)
Linezolid	-	-	-
Fusidic acid	16 (14)	11 (7)	27 (10)
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=55)	PVL negative (n=182)	Total (n=237)
	N (%)	N (%)	N (%)
Erythromycin	9 (16)	33 (18)	42 (18)
Clindamycin	9 (16)	29 (16)	38 (16)
Ciprofloxacin	5 (9)	13 (7)	18 (8)
Gentamycin	3 (5)	1 (0.5)	4 (2)
Tobramycin	5 (9)	3 (2)	8 (3)
Kanamycin	5 (9)	4 (2)	9 (4)
Minocycline	1 (2)	1 (0.5)	2 (1)
Tetracycline	14 (25)	7 (4)	21 (9)
Rifampin	2 (4)	-	2 (1)
Cotrimoxazole	2 (4)	-	2 (1)
Linezolid	-	-	-
Fusidic acid	5 (9)	15 (8)	20 (8)
Mupirocin	2 (4)	1 (0.5)	3 (1)

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

Typing for epidemiological investigations

In 2016, molecular typing using *spa* typing and/or PFGE analysis was performed on 545 *S. aureus* isolates including 299 MRSA (*mecA*-positive), 245 MSSA, and one BORSA. Among these, 111 MRSA isolates and 27 MSSA isolates were sent for epidemiological investigation of local outbreaks (n=33, 25 MRSA outbreaks, 8 MSSA outbreaks).

The 111 MRSA isolates recovered from 21 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 34 (31%) MRSA isolates recovered from 10 hospitals, ST8-SCC*mec* IV found in 23 (21%) MRSA isolates recovered from 9 hospitals, and ST45-SCC*mec* IV found in 15 (14%) MRSA isolates recovered from 7 hospitals. Fourteen isolates belonging to the clone ST8-SCC*mec* IV contained the ACME pathogenicity island characteristic of MRSA USA300. Seven out of these 14 isolates were related to the outbreak by USA300 that has been described since 2014 in one hospital. Genotype ST1-SCC*mec* IV corresponding to the CA-MRSA (PVL-positive) clone USA400 (n=10) was recovered from 2 hospitals. The CA-MRSA Taiwan clone ST59-SCC*mec* V (n=3) was recovered from 2 hospitals. One outbreak in one hospital was related with the CA-MRSA ST573-SCC*mec* V (n=3). Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 23 of the 25 clusters investigated.

The 27 MSSA isolates recovered from 8 hospitals showed high diversity with 11 distinct lineages. The most frequent genotypes were: CC5 (n=5, 18%), CC30 (n=4, 15%), and CC121 (n=5, 18%).

Six additional MRSA were sent for SCC*mec* typing due to their false negative results at GeneXpert. All isolates were successfully identified as MRSA ST1-SCC*mec* IV.

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 (Scientifique de Santé Publique) (WIV-ISP).

In 2016, 1364 patients with a first *S. aureus* bacteraemia episode (per trimester) were recorded. Among these patients, a proportion of 12.2% of MRSA were confirmed (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, 1999-2016.

Year	Number total of <i>S. aureus</i> isolates	Number of MRSA isolates (%)
1999	445	105 (23.6)
2000	657	137 (20.9)
2001	942	213 (22.6)
2002	1092	309 (28.3)
2003	1134	336 (29.6)
2004	1227	408 (33.3)
2005	1048	329 (31.4)
2006	858	188 (21.9)
2007	855	199 (23.3)
2008	906	187 (20.6)
2009	948	200 (21.1)
2010	1057	217 (20.5)
2011	1744	304 (17.4)
2012	1568	260 (16.6)
2013	1612	272 (16.9)
2014	988	133 (13.5)
2015	913	112 (12.3)
2016	1364	167 (12.2)

Analyse of *S. aureus* from animal origin

Eighteen (6%, 18/299) 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. These MRSA were isolated from screenings (n=10), skin lesions (n=1), deep fluids (n=2), or other sites (n=5). Available data allowed to bring out that, 3 patients had direct contact with animals (farmers, vets). None toxins were detected in these MRSA ST398 strains. Interestingly, some livestock-associated MRSA clone CC398 (n=6) isolates were related to outbreaks in 2 hospitals.

Five additional MRSA ST398 isolates were related to the human clade population, and carried the exotoxin PVL. Three additional MRSA strains related to the distinct genetic lineage ST291, a homologue recombinant double locus variant of ST398, were detected in patients from Flanders.

Fourteen (6%, 14/245) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from skin lesions (n=6), screenings (n=3), deep fluid (n=4) or other site (n=1). ST398 MSSA strains were not associated with livestock contacts. One MSSA ST398 carried the exotoxin PVL. One additional MSSA carrying PVL belonged to ST291 and was detected in a patient from Wallonia.

Conclusions

In 2016, the number of PVL-positive MRSA cases per year was similar to 2015, 113 cases in 2016 versus 106 cases in 2015. The antimicrobial resistance profile of these PVL-positive MRSA remained similar with a slightly decrease of resistance against clindamycin, kanamycin and fusidic acid. The proportion of CA-MRSA belonging to clone ST8-SCC*mec* IV remained relatively stable compared to 2015 (29% to 33%) and the percentage of clone USA-300 within the CA-MRSA ST8 population was stable (77% in 2015, 76% in 2016). A slightly decrease of the proportion of CA-MRSA isolates belonging to clone ST80-SCC*mec* IV was observed in 2016 (from 19% to 12%), returning to the proportion observed in 2014 (10%). New emerging CA-MRSA lineages detected in 2015 were also detected in 2016.

The number of MSSA isolates received for toxin detection was similar to the number of isolates received in 2015 (257 in 2015, 237 in 2016). The proportion of PVL-positive MSSA cases was also similar (24% in 2015 versus 23% in 2016). High diversity of genotypes was observed in both years. The most frequent MSSA clone in 2016 was ST152/377 (25%), which was also recovered frequently in 2015 (17%).

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.

As in 2015, 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 was sporadically isolated in Belgian hospitals (6%). MSSA strains, presenting the same genotypic characteristics than MRSA ST398, were also rarely recovered from persons without livestock contact (6%).

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