Rapport 2017
NRC *S. aureus* Belgium

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The National Reference Centre (NRC) for *S. aureus* of "Université Libre de Bruxelles (ULB) - Hôpital Erasme" provides the following services:

- 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*, *mecB*, *mecC* and *mecD* genes (coding for resistance to oxacillin/cefoxitin), *mupA* and *mupB* genes (coding for mupirocin resistance), *cfr*, *cfr*(B), *optrA* and *poxtA* genes (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 *se*r 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) - *arcA* gene.

These analyses are performed on clinical staphylococcal isolates causing clinical and/or diagnostic problems or collected during epidemiological investigations. Request forms are available on the website of Sciensano (https://nrchm.wiv-isp.be).

The Microbiology laboratory of the ULB - Hôpital Erasme, now Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB) site Anderlecht, hosting 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).

1. Characterisation of atypical clinical strains

In 2017, the NRC identified and/or determined the antimicrobials susceptibility of 135 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 8 MRSA, 7 MSSA, 1 BORSA and 4 coagulase-negative staphylococci strains. None strain showed a decreased susceptibility to glycopeptides.

A total of 95 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=90), while the remaining five isolates were coagulase negative *Staphylococcus* including *mecA*-positive *S. epidermidis* (n=1) and *S. lugdunensis* (n=1), as well as *mecA*-negative *S. epidermidis* (n=1), *S. condimenti* (n=1) and *S. cohnii* (n=1). Of the 90 *S. aureus* isolates, 3 (3%) 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=1), or being phenotypically susceptible to oxacillin only (MIC < 2 µg/mL) (n=2). On the other side, 14 isolates (16%) showing resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=7), to oxacillin only (MIC > 2 µg/mL) (n=6), or to cefoxitin only (MIC > 4 µg/mL) (n=1) lacked the *mecA* gene. From these 14 isolates, four isolates resistant to both β-lactams carried the *mecC* gene (4%), and the remaining 10 (11%) 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 to this end, we recommend performing the test after induction with oxacillin and cefoxitin. It is to note that BORSA isolates in 2017 were not checked for the presence of *mecB*, which has not been described associated to *S. aureus* until 2018.

Resistance to mupirocin was determined by MIC and *mupA* detection for 43 *S. aureus* isolates, among these, 7 (16%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and the presence of *mupA* gene.

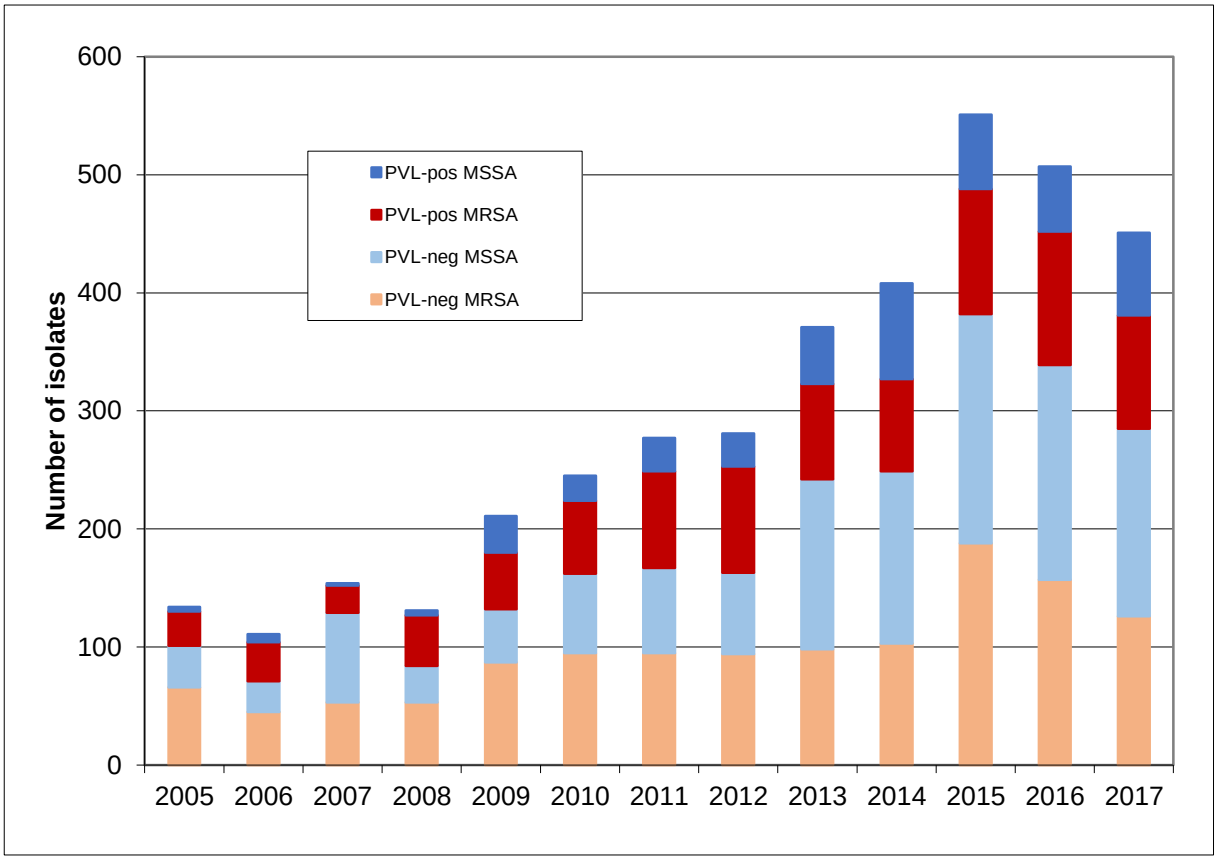
2. Toxin detection and characterisation of community-acquired (CA) *S. aureus* strains

In 2017, 451 isolates of *S. aureus* including 222 MRSA and 229 MSSA were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection (**Figure 1**).

A total of 96 (43%) 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 abscesses, soft tissues or furunculosis (n=55) but also from deep fluids (n=12), screenings (n=8), blood cultures (n=3) or other sites (n=18).

By molecular typing, most of PVL-positive MRSA isolates (n=68, 71%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=41), ST80-SCC*mec* IV (European clone) (n=19), and ST30-SCC*mec* IV (Southwest Pacific clone) (n=8) (**Figure 2**). Thirty of the 41 (73%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300. The remaining CA-MRSA isolates were assigned to the following clones: ST5 (n=5), Taiwan ST59-SCC*mec* V (n=5), ST152/377-SCC*mec* IV or V (n=4), ST22 (n=3), USA400 ST1 (n=2), West Australia ST88-SCC*mec* IV (n=2), ST398 (n=1) and ST573/772-SCC*mec* V (n=1).

Figure 1: Number of MRSA and MSSA isolates received for PVL detection, 2005-2017.

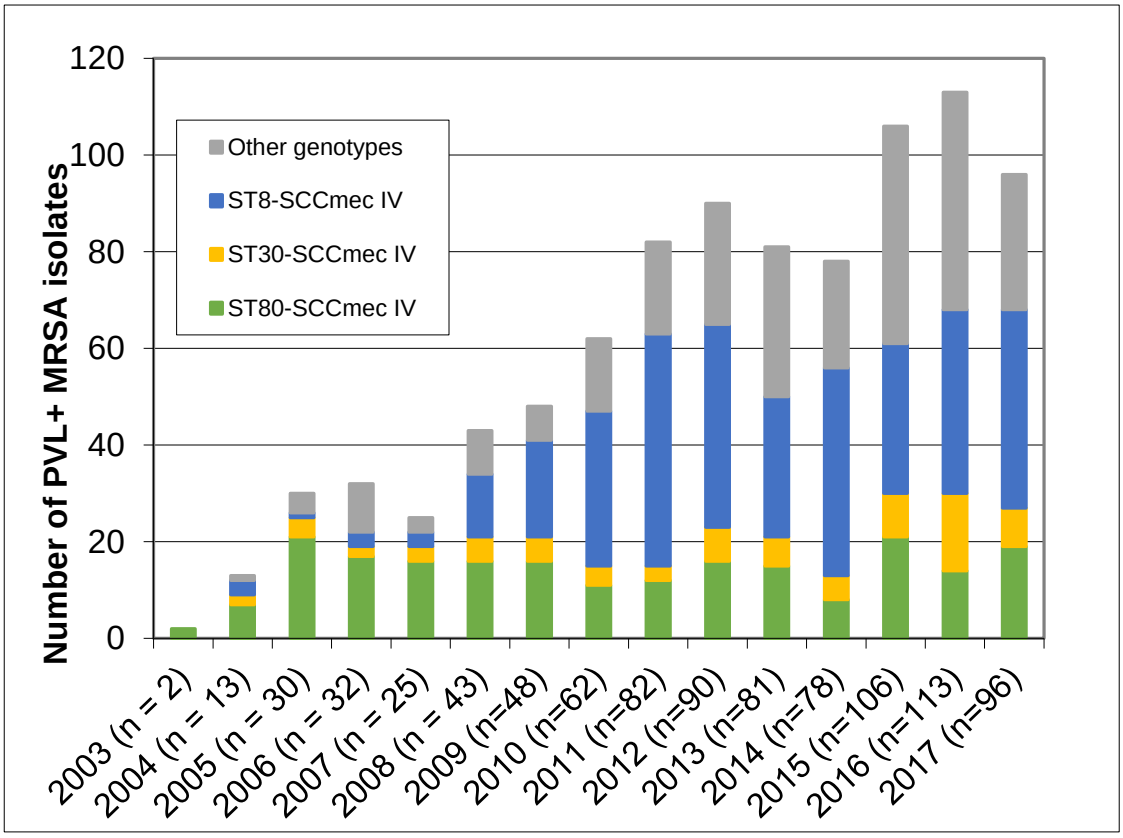


Seventy (31%) MSSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). Molecular typing of these 70 PVL-positive MSSA isolates reveal more genomic diversity than for MRSA isolates. These isolates were related to the clones: ST152/377 (n=18), CC30 (n=12), CC1 (n=11), CC121 (n=11), CC15 (n=5), CC8 (n=3), CC80 (n=3), CC5 (n=1), and others (n=6). The number of strains sent for toxin detection has increased these five last years, but the number of PVL positive strains remains stable (~ 160 strains per year) since 2014.

TSST-1 toxin was detected in 24 MRSA (11%) and 25 MSSA (11%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=20), deep fluids (n=6), screenings (n=10), blood cultures (n=6), or other sites (n=7). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC30 (n=18, 37%) and or CC22 (n=13, 26%). One isolate, from the 12 MRSA-ST22, was also positive for PVL.

Genes coding for exfoliatins A (*eta*) and/or B (*etb*) were found in 26 MSSA isolates and 4 MRSA. The gene coding for exfoliatin A alone was recovered in four MRSA belonging to the clone ST913-SCC*mec* IV and 11 MSSA isolates belonging to clones CC121 (n=5), CC15 (n=2), ST109 (n=3) and CC1 (n=1). The gene coding for exfoliatin B alone was recovered in one MSSA isolate belonging to ST121. Both genes (*eta* and *etb*) were found in 14 MSSA isolates belonging to ST121. Eleven of the 14 *eta-etb* positive isolates, as well as four *eta*-positive isolate and the *etb*-positive isolate, belonged to the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC).

Figure 2: Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2017.



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=96) N (%)	PVL negative (n=126) N (%)	Total (n=222) N (%)
Erythromycin	56 (58)	53 (42)	109 (49)
Clindamycin	18 (19)	44 (35)	62 (28)
Ciprofloxacin	32 (33)	30 (24)	62 (28)
Gentamycin	18 (19)	19 (15)	37 (17)
Tobramycin	26 (27)	36 (29)	62 (28)
Kanamycin	66 (69)	55 (46)	121 (55)
Minocycline	-	12 (9)	12 (5)
Tetracycline	24 (25)	45 (36)	69 (31)
Rifampicin	-	-	-
Cotrimoxazole	2 (2)	8 (6)	10 (4)
Linezolid	-	-	-
Fusidic acid	18 (19)	19 (15)	37 (17)
Mupirocin	1 (1)	2 (2)	3 (1)

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=70) N (%)	PVL negative (n=159) N (%)	Total (n=229) N (%)
Erythromycin	7 (10)	39 (24)	46 (20)
Clindamycin	4 (6)	27 (17)	31 (13)
Ciprofloxacin	5 (7)	2 (1)	7 (3)
Gentamycin	-	-	-
Tobramycin	5 (7)	-	5 (2)
Kanamycin	7 (10)	7 (4)	14 (6)
Minocycline	-	3 (2)	3 (1)
Tetracycline	14 (20)	8 (5)	22 (10)
Rifampin	3 (4)	1 (0.6)	4 (2)
Cotrimoxazole	1 (1)	-	1 (0.4)
Linezolid	-	-	-
Fusidic acid	12 (17)	28 (18)	40 (17)
Mupirocin	4 (6)	1 (0.6)	5 (2)

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

3. Typing for epidemiological investigations

In 2017, molecular typing using *spa* typing and/or PFGE analysis was performed on 501 *S. aureus* isolates including 259 MRSA (*mecA*-positive), 241 MSSA, and one BORSA. Among these, 70 MRSA isolates and 46 MSSA isolates were sent for epidemiological investigation of local outbreaks in 2017 (n=34, 20 MRSA outbreaks, 13 MSSA outbreaks). Additionally 2 MRSA and one MSSA were related to samples recovered previous years or in 2018.

The 70 MRSA isolates recovered from 15 hospitals were classified into 11 distinct lineages. The most frequently recovered genotypes were those previously found in our Belgian hospitals: ST5-SCC*mec* II or IV found in 11 (16%) MRSA isolates recovered from 5 hospitals and ST8-SCC*mec* IV found in 16 (23%) MRSA isolates recovered from 5 hospitals. Two isolates belonging to the clone ST8-SCC*mec* IV contained the ACME pathogenicity island characteristic of MRSA USA300. One outbreak in one hospital was related with the LA-MRSA ST398 (n=5). Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 15 of the 20 clusters investigated.

The 46 MSSA isolates recovered from 13 hospitals showed high diversity with 11 distinct lineages. The most frequent genotypes were: CC121 (n=11), CC1 (n=7) and CC30 (n=8). Molecular typing confirmedMSSA horizontal transmission in 8 of the clusters investigated.

Eight additional MRSA were sent for SCC*mec* typing due to false negative results of molecular tests (GeneXpert). The isolates were successfully identified as MRSA ST1-SCC*mec* IV (n=3), MRSA ST5-SCC*mec* IV (n=1), MRSA ST80 SCC*mec* V (n=2) and MRSA ST9/ST398-SCC*mec* IV (n=1) and V (n=1).

4. 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 Sciensano.

In 2017, 1511 patients with a first *S. aureus* bacteraemia episode (per trimester) were recorded. Among these patients, a proportion of 8.5% of MRSA was 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, 1999-2017.

Year	Number total of *S. aureus* 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)
2017	1511	128 (8.5)

5. Analyse of *S. aureus* from animal origin

Twenty (8%, 20/259) ST398 MRSA isolates, called livestock-associated MRSA, were detected in hospitalised or ambulant patients in six hospitals from Flanders and one from Wallonia. These MRSA were isolated from screenings (n=5), skin lesions (n=3), blood cultures (n=2), or other sites (n=10). Available data allowed to bring out that seven patients had direct contact with animals (farmers, vets). No toxin was detected in these MRSA ST398 strains. Interestingly, an outbreak of livestock-associated MRSA CC398 (n=5) took place in one hospital in Wallonia.
Two additional MRSA ST398 isolates were related to the human clade of this lineage and carried the exotoxin PVL or the exotoxin TSST-1. They were obtained from patients attending one hospital from Brussels and one from Wallonia.

Twelve (5%, 12/241) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from skin lesions (n=7), screenings (n=1), deep fluid (n=2) or other site (n=2). ST398 MSSA strains were not associated with livestock contacts. No toxin was detected in these MSSA ST398 strains.

6. Conclusions

In 2017, the number of PVL-positive MRSA cases per year was slightly lower than 2016, 96 cases in 2017 versus 113 cases in 2016 but this difference is not statistically significant. The antimicrobial resistance profile of these PVL- positive MRSA remained similar with a slightly increase of resistance against kanamycin and tobramycin. The proportion of CA-MRSA belonging to clone ST8-SCC*mec* IV increased compared to 2016 (34% to 43%), but the percentage of clone USA-300 within the CA-MRSA ST8 population remained stable (76% in 2016, 74% in 2017). The proportion of CA-MRSA isolates belonging to clone ST80-SCC*mec* IV increased compared to 2016 (12% versus 20%), returning to the proportion observed in 2015 (19%). New emerging CA-MRSA lineages detected in 2015 and 2016 were also detected in 2017.

The number of MSSA isolates received for toxin detection was similar to the number of isolates received in 2016 (237 in 2016, 229 in 2017). The proportion of PVL-positive MSSA cases was higher (23% in 2016 versus 31% in 2017). High diversity of genotypes was observed in both years. The most frequent MSSA clone in 2017 was ST152/377 (26%), which was also the most frequent recovered in 2016 (25%).

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

As in 2016, most livestock-associated MRSA clone ST398 isolates were recovered in persons living principally in Flanders and with direct contact with animals like farmers or vets. MSSA strains, presenting the same genotypic characteristics than MRSA ST398, were also rarely recovered, but from persons without livestock contact (5%).

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