Rapport 2018
NRC *S. aureus* Belgium

National Reference Center *S. aureus*
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Index

The National Reference Centre (NRC) for *S. aureus* of the "Laboratoire Hospitalier Universitaire de Bruxelles - University Laboratory of Brussels (LHUB-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 *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) - *arcA* gene.

These analyses are performed on 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 (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 2018, the NRC identified and/or determined the antimicrobials susceptibility of 138 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 8 MRSA, 3 MSSA, 1 BORSA/MODSA and 6 coagulase-negative staphylococci strains. None of these strains showed a decreased susceptibility to glycopeptides.

A total of 100 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=97), while the remaining 3 isolates were *mecA*-positive *S. epidermidis*. Of the 97 *S. aureus* isolates, 8 were identified as MRSA, containing *mecA* gene and presenting phenotypic resistance to both oxacillin (MIC < 2 µg/mL) and cefoxitin (MIC < 4 µg/mL). A total of 8 isolates (8.2%) 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=3), or being phenotypically susceptible to oxacillin only (MIC < 2 µg/mL) (n=5). On the other side, 15 isolates (15.5%) showing resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=3), to oxacillin only (MIC > 2 µg/mL) (n=3), or to cefoxitin only (MIC > 4 µg/mL) (n=9) lacked the *mecA* gene. From these 15 isolates, one isolate resistant to only cefoxitin (MIC > 4 µg/mL) carried the *mecC* gene (1%), and the remaining 14 (14.4%) were classified as BORSA/MODSA. *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. The presence of *mecB* and *mecD* genes started to be tested by the end of 2018. Currently, we have not detected these novel resistance genes among Belgian strains.

Resistance to mupirocin was determined by MIC and *mupA* detection for 24 *S. aureus* isolates, among these, 7 (29.2%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and the presence of the *mupA* gene.

Resistance to linezolid was determined by MIC for 9 isolates including *S. aureus* (n=4), *S. epidermidis* (n=3) and *S. hominis* (n=2). Only one *S. epidermidis* was resistant (MIC 256 µg/mL) but did not carry the *cfr*, *cfr*(B), *optrA* or *poxtA* genes.

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

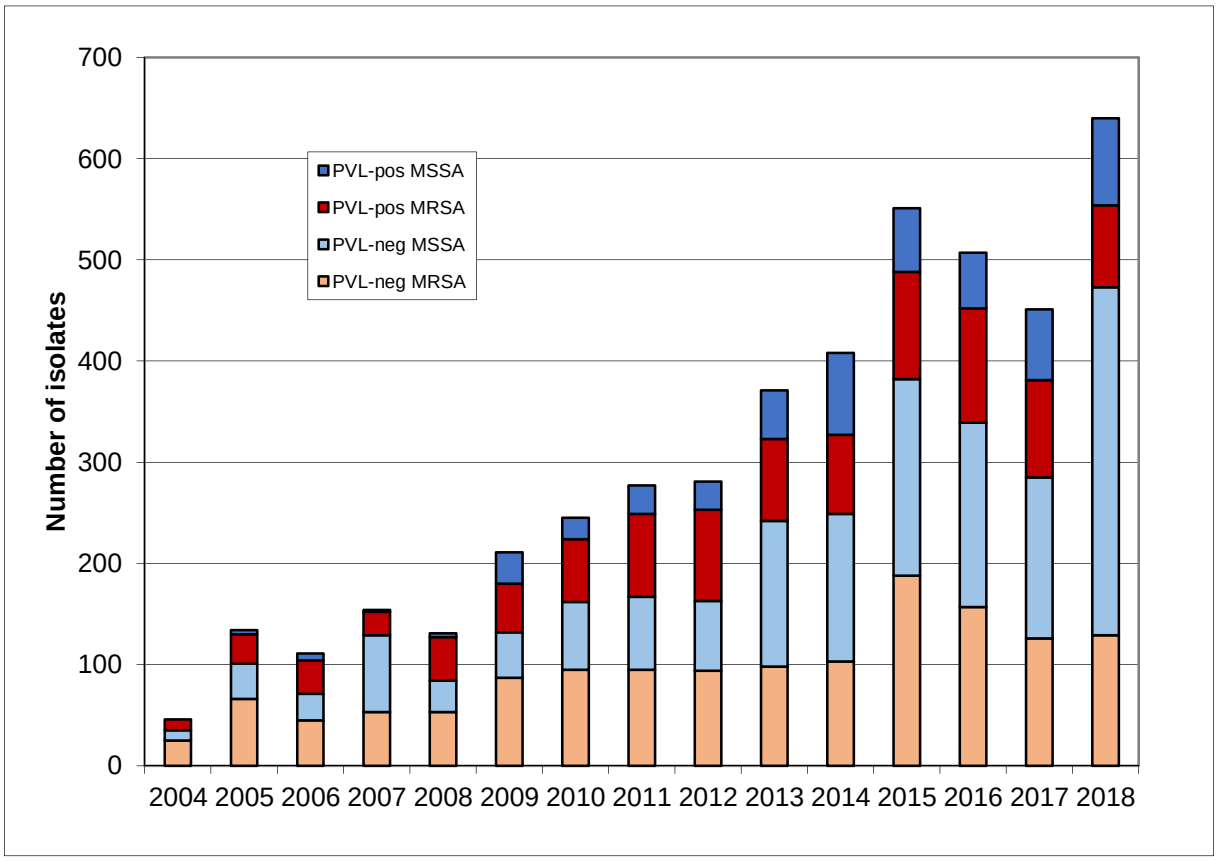
In 2018, 642 isolates of *S. aureus* including 210 MRSA, 430 MSSA and 2 BORSA/MODSA were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection (**Figure 1**).

A total of 81 (38.6%) MRSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). These MRSA isolates were mostly recovered from skin lesions, in particular from skin abscesses, soft tissues or furunculosis (n=52) but also from deep fluids (n=11), screenings (n=2), blood cultures (n=1) or other sites (n=15).

By molecular typing, most of PVL-positive MRSA isolates (n=51, 63%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=21), ST80-SCC*mec* IV (European clone) (n=17), and ST30-SCC*mec* IV (Southwest Pacific clone) (n=13) (**Figure 2**). Eight of the 21 (38.1%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300. It is to note, that this year the proportion of the USA300 has substantially decreased.

The remaining PVL-positive MRSA isolates were assigned to the following clones: ST22 (n=9), ST152/377-SCC*mec* IV or V (n=4), ST398 (n=4), USA400 ST1 (n=3), ST5 (n=3), CC15 (n=1), CC45 (n=1), Taiwan ST59-SCC*mec* V (n=1), CC97 (n=1), CC121 (n=1) and ST573/772-SCC*mec* V (n=2). Since 2015, we observed a great diversification in CA-MRSA clones.

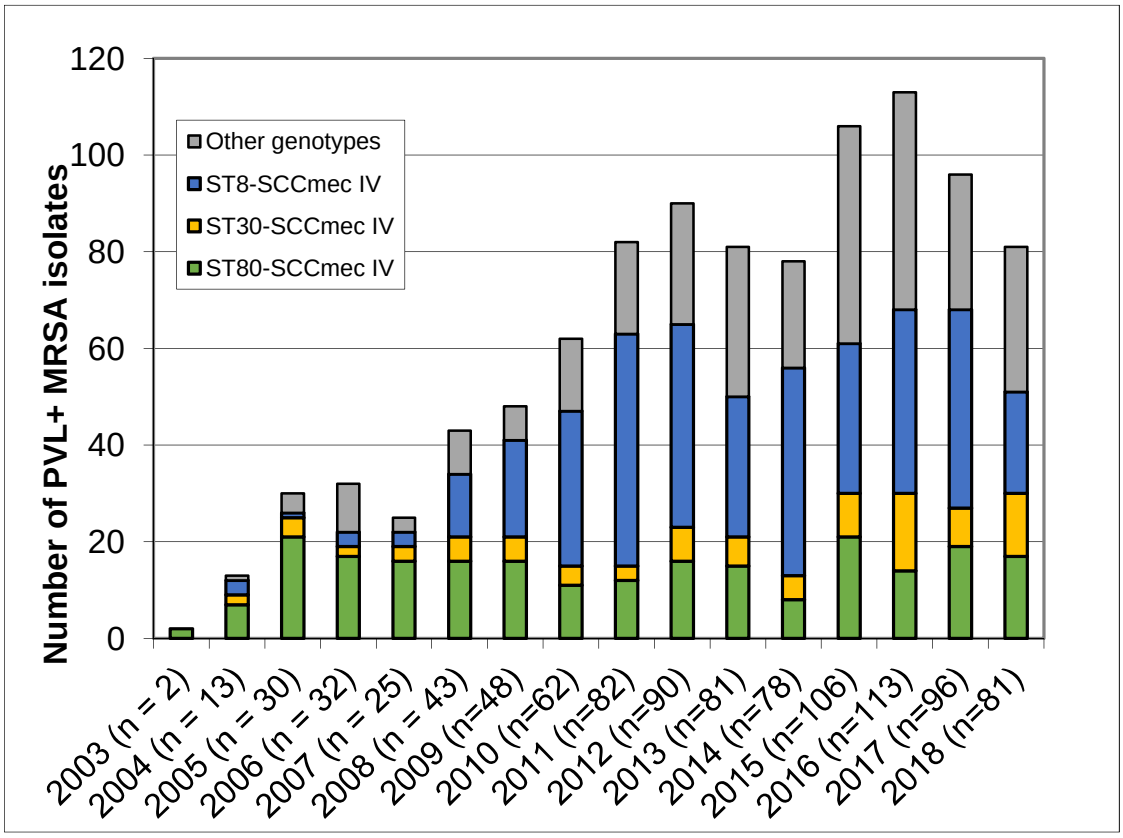
Figure 1: Number of MRSA and MSSA isolates received for PVL detection, 2005-2018.



Eighty-six (20%) MSSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). Molecular typing of these PVL-positive MSSA isolates reveal more genetic diversity than MRSA isolates. These isolates were related to the clones: ST152/377 (n=27), CC30 (n=16), CC121 (n=12), CC15 (n=10), CC22 (n=5), CC1 (n=5), CC8 (n=1), ST88 (n=2), ST188 (n=1), CC398 (n=1), ST1153 (n=1), and others (n=5). This year the number of strains sent for toxin detection has increased, but the number of PVL positive strains remains stable (~ 160 strains per year) since 2014. It is to note that the number of MSSA strains sent for toxin detection has progressively increased for the last five years.

TSST-1 toxin was detected in 31 MRSA (14.8%) and 43 MSSA (10%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=26), deep fluids (n=6), screenings (n=16), blood cultures (n=11), urines (n=1) or other sites (n=14). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC30 (n=35, 47.3%), CC22 (n=17, 23%) and CC5 (n=14, 19%). Seven CC22-MRSA and one ST34/CC30-MSSA carrying TSST-1 were also positive for PVL.

Figure 2: Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2018.



Genes coding for exfoliatins A (*eta*) and/or B (*etb*) were found in 84 MSSA isolates and 3 MRSA. The gene coding for exfoliatin A alone was recovered in one MRSA belonging to CC121 and 21 MSSA isolates belonging to clones CC15 (n=16) and CC121 (n=5). The gene coding for exfoliatin B alone was recovered in three MSSA isolates belonging to CC121. Both genes (*eta* and *etb*) were found in two MRSA isolates belonging to CC121 and 60 MSSA isolates belonging to CC121 (n=59) and ST2657 (n=1).

A high occurrence of exfoliatin(s) positive strains was witnessed this year, because the Agentschap Zorg en Gezondheid sent a letter advising regional general practitioners, dermatologists, pediatricians, Kind en Gezin, the school doctors and some institutions about a cluster of fusidic acid resistant/*eta* positive *S. aureus* causing impetigo in the region of Turnhout. Indeed, most of the MSSA isolates sent to the NRC, including four *eta*-positive, one *etb*-positive, and 54 *eta-etb* positive isolates where fusidic acid resistant and belonged to the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC). The EEFIC strains had a great variety of *spa* types including t159 (n=2), t171 (n=9), t408 (n=19), t876 (n=5), t1636 (n=3), t1994 (n=7), t2524 (n=1), t3274 (n=8), t4956 (n=1), t17015 (n=3), t17175 (n=1) and t18288 (n=1). Two MRSA *eta-etb* positive with *spa*-type t272 showed also resistance against fusidic acid.

It is to note that the current CNR data on *S. aureus* causing CA-infections is based on spontaneous requests for toxin detection. To determine the proportion of community-acquired *S. aureus* strains causing impetigo in Belgium that are resistant to fusidic acid and the prevalence of EEFIC clone in the

Belgian community, a well conducted surveillance of skin community acquired infections will be performed in 2019 in collaboration with Sciensano.

Antimicrobial resistance percentages on the basis of EUCAST clinical breakpoints of MRSA and MSSA isolates received for toxin detection are summarized in **Tables 1** and **2**. One BORSA/MODSA isolate did not show additional resistances, the remaining one showed resistance to kanamycin.

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

Antimicrobials	Antimicrobial resistance of MRSA isolates (%)		
	PVL positive (n=81) N (%)	PVL negative (n=129) N (%)	Total (n=210) N (%)
Erythromycin	40 (49)	44 (34)	84 (40)
Clindamycin [a]	23 (28)	37 (29)	60 (29)
Ciprofloxacin	22 (27)	38 (29)	60 (29)
Gentamycin	16 (20)	20 (16)	36 (17)
Tobramycin	19 (24) [b]	40 (31) [d]	59 (29)
Kanamycin	52 (64)	63 (50) [e]	115 (56)
Minocycline	- [c]	18 (14)	18 (9)
Tetracycline	29 (36)	44 (34)	73 (35)
Rifampicin	-	1 (1)	1 (0.5)
Cotrimoxazole	1 (1)	2 (2)	3 (1)
Linezolid	-	1 (1)	1 (0.5)
Fusidic acid	20 (25)	22 (17) [d]	42 (20)
Mupirocin	-	2 (2)	2 (1)

[a] Included both inducible and constitutive resistance.
[b] Only tested in 79 isolates. [c] Only tested in 80 isolates. [d] Only tested in 128 isolates. [e] Only tested in 126 isolates.
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=86) N (%)	PVL negative (n=343)[d] N (%)	Total (n=429)[d] N (%)
Erythromycin	9 (10)	86 (25)	95 (22)
Clindamycin [a]	6 (8) [b]	59 (17)	65 (15) [h]
Ciprofloxacin	10 (12)	5 (1)	15 (3)
Gentamycin	-	- [e]	- [i]
Tobramycin	2 (2)	14 (4) [e]	16 (4) [i]
Kanamycin	10 (14)[c]	35 (11) [f]	45 (12)[j]
Minocycline	-	3 (1) [g]	3 (1) [k]
Tetracycline	17 (20)	19 (6)	36 (8)
Rifampin	1 (1)	1 (0.3)	2 (0)
Cotrimoxazole	-	1 (0.3)	1 (0)
Linezolid	-	-	-
Fusidic acid	5 (6)	75 (22)	80 (19)
Mupirocin	7 (8)	16 (5)	23 (5)

[a] Included both inducible and constitutive resistance.
[b] Only tested in 80 isolates. [c] Only tested in 71 isolates. [d] A total of 430 was received, but one PVL-negative was not tested for the antibiogram. [e] Only tested in 342 isolates. [f] Only tested in 315 isolates. [g] Only tested in 341 isolates.
[h] Only tested in 423 isolates. [i] Only tested in 428 isolates. [j] Only tested in 386 isolates. [k] Only tested in 427 isolates.
N, number of resistant isolates; -, absence of resistant isolates.

3. Typing for epidemiological investigations

In 2018, molecular typing using *spa* typing and/or PFGE analysis was performed on 610 *S. aureus* isolates including 216 MRSA (*mecA*-positive), 392 MSSA, and two BORSA/MODSA. Among these, 42 MRSA isolates and 79 MSSA isolates were sent for epidemiological investigation of local outbreaks in 2018 (n=21, 13 MRSA outbreaks and 8 MSSA outbreaks).

Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 11 of the 13 clusters investigated. Globally, the most frequently recovered genotype was ST5-SCC*mec* II or IV [found in 14 (34%) MRSA isolates recovered from 6 hospitals]. The remaining isolates belonged to CC1 (found in 7 MRSA isolates recovered from 3 hospitals), ST8-SCC*mec* IV [found in 2 MRSA isolates recovered from 2 hospitals], CC22 (found in 4 MRSA isolates recovered from one hospital), CC45 (found in 6 MRSA isolates recovered from 3 hospitals), LA-MRSA ST398 (found in 8 MRSA isolates recovered from 2 hospitals).

Molecular typing confirmed horizontal transmission of MSSA in 5 of the 8 clusters investigated. MSSA isolates (recovered from 7 hospitals) showed high diversity with 15 distinct lineages. The most frequent genotypes were: CC8 (n=6), CC15 (n=13) and CC121 (n=32). The increase of CC121 isolates is related to the outbreak in the Turnout region.

4. National microbiological surveillance

During 2017, a national microbiological survey to follow the antimicrobial resistance profile and the evolution and geographic distribution of MRSA, MSSA and coagulase negative staphylococci genotypes from patients admitted to Belgian acute-care hospital was performed. Data were produced and analysed during 2018. Details and results of this surveillance are available in a separate report “Microbiologic Survey of Staphylococcus in Belgian Hospitals in 2017”.

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

Among the 210 MRSA strains typed in 2018, 18 (8.6%) ST398 MRSA isolates, called livestock-associated (LA-) MRSA, were observed. These 18 isolates came from hospitalised or ambulant patients in 9 hospitals from Flanders, one from Wallonia and one from Brussels. These MRSA were isolated from screenings (n=12), skin lesions (n=1), blood cultures (n=1), or other sites (n=4). Available data allowed to bring out that 7 patients had direct contact with animals (farmers, vets). No toxin was detected in these MRSA ST398 strains. Interestingly, three outbreaks of livestock-associated MRSA CC398 (n=5) took place in two hospitals in Flanders.

Four non-LA MRSA ST398 isolates (related to the human clade of this lineage and carrying the exotoxin PVL) were also detected. They were obtained from patients attending two hospitals from Flanders and one from Wallonia.

Twenty-five (5.8%, 25/430) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from skin lesions (n=11), screenings (n=6), deep fluid (n=1), blood (n=1) or other sites (n=6). ST398 MSSA strains were not associated with livestock contacts. One MSSA ST398 was positive for PVL.

6. Conclusions

In 2018, a total of 97 *S. aureus* isolates were received for confirmation of oxacillin/cefoxitin resistance. Among these, 8 isolates were cryptic (also named heterogeneous) MRSA and 15 isolates lacked the *mecA* gene. From these 15 isolates, one isolate carried the *mecC* gene and the remaining 14 were classified as BORSA/MODSA.

Resistance against glycopeptides was requested for 8 MRSA, 3 MSSA, and 1 BORSA/MODSA. None of these strains showed a decreased susceptibility to glycopeptides.

The number of PVL-positive strains among MRSA received by the NRC for exotoxin detection was slightly lower than in 2017: 81 versus 96, but this difference was not statistically significant. The total number of PVL positive *S. aureus* strains (MRSA and MSSA) remains stable (~ 160 strains per year) since 2014. The antimicrobial resistance profile of these PVL- positive MRSA remains stable with a slight increase of resistance against tetracycline and fusidic acid.

The proportion of CA-MRSA belonging to clone ST8-SCC*mec* IV decreased compared to 2017 (43% to 26%), and was similar to values found in 2016 (34%) and 2015 (29%). Interestingly, the percentage of clone USA-300 within the CA-MRSA ST8 population has drastically decreased (76% in 2016, 74% in 2017, and 38% in 2018). The proportion of CA-MRSA isolates belonging to clone ST80-SCC*mec* IV remains stable compared to 2017 (20% both years). A great diversification in CA-MRSA clones has been observed since 2015.

This year, the number of MSSA isolates received for toxin detection (n=430) has significantly increased compared to 2017 (n=229) and 2016 (n=237), due to a cluster of fusidic acid resistant/*eta* positive *S. aureus* causing impetigo in the region of Turnhout. Accordingly, the proportion of PVL-positive MSSA cases was lower (20%) than in 2017 (31%), returning to values found in 2016 (23%) and 2015 (24%). The most frequent PVL-positive MSSA clone in 2018 was ST152/377 (31.4%), which was also the most frequent recovered in 2017 (26%) and 2016 (25%). Wide diversity of genotypes was also observed this year.

Genotyping of MRSA from local outbreaks in hospitals showed that these were mainly due to the nosocomial epidemic clone ST5 SCC*mec* IV.

Finally, as in 2017, most livestock-associated ST398 MRSA isolates were recovered from persons living in Flanders and with direct contact with animals.

7. NRC publications of 2018

Argudín MA, Deplano A, Vandendriessche S, Dodémont M, Nonhoff C, Denis O, Roisin S. CC398 *Staphylococcus aureus* subpopulations in Belgian patients. Eur J Clin Microbiol Infect Dis. 2018 May;37(5):911-916.

Argudín MA, Roisin S, Nienhaus L, Dodémont M, de Mendonça R, Nonhoff C, Deplano A, Denis O. Genetic Diversity among *Staphylococcus aureus* Isolates Showing Oxacillin and/or Cefoxitin Resistance Not Linked to the Presence of *mec* Genes. Antimicrob Agents Chemother. 2018 Jun 26;62(7). pii: e00091-18.

Deplano A, Dodémont M, Denis O, Westh H, Gumpert H, Larsen AR, Larsen J, Kearns A, Pichon B, Layer F, Schulte B, Wolz C, Spiliopoulou I, Brennan G, Empel J, Hryniewicz W, de Lencastre H, Faria NA, Codita I, Sabat AJ, Friedrich AW, Deurenberg RH, Tristan A, Laurent F, Vandenesch F. European external quality assessments for identification, molecular typing and characterization of *Staphylococcus aureus*. J Antimicrob Chemother. 2018 Oct 1;73(10):2662-2666.

Lee JYH, Monk IR, Gonçalves da Silva A, Seemann T, Chua KYL, Kearns A, Hill R, Woodford N, Bartels MD, Strommenger B, Laurent F, Dodémont M, Deplano A, Patel R, Larsen AR, Korman TM, Stinear TP, Howden BP. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. Nat Microbiol. 2018 Oct;3(10):1175-1185.