# Rapport 2019
# NRC *S. aureus* Belgium


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The National Reference Centre (NRC) for *Staphylococcus aureus* held by the "Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB)" provides the following services:

- Identification and antimicrobial susceptibility testing of *Staphylococcus sp*. strains using:
  - Phenotypic methods: protein profiles (MALDI-TOF), biochemical tests, disk diffusion method, 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), *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 (*seA* to *seE*, *seG* to *seI* and *seR* to *seT*) and enterotoxin-like (*selI*, *selK* to *selG* 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 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). Sending isolates to the NRC is voluntary-based.

The Microbiology laboratory of LHUB-ULB - site Anderlecht, hosting the NRC *S. aureus* is accredited according to standard ISO15189 (N° 650 – 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 2019, the NRC identified and/or determined the antimicrobials susceptibility of 159 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 8 MRSA, 11 MSSA, and 3 coagulase-negative staphylococci strains. Only 2 coagulase-negative staphylococci showed a decreased susceptibility to teicoplanin.

A total of 101 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=92), while the remaining isolates were *mecA*-negative *Staphylococcus argenteus* (n=1), *mecA*-positive *Staphylococcus epidermidis* (n=5), *mecA*-positive *Staphylococcus haemolyticus* (n=1), *mecA*-positive (n=1) and *mecA*-negative (n=1) *Staphylococcus lugdunensis*. Of the 92 *S. aureus* isolates, 19 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 5 (5.4%) isolates were cryptic (also named heterogeneous) MRSA, containing *mecA* gene but being phenotypically susceptible to oxacillin (MIC < 2 µg/mL). Three isolates (3.3%) showing resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) carried the *mecC* gene. *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 or cefoxitin disks. A total of 18 isolates (19.6%) were classified as BORSA/MODSA presenting phenotypic resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=5), to only oxacillin (MIC > 2 µg/mL) (n=2) or to only cefoxitin (MIC > 4 µg/mL) (n=11). These BORSA/MODSA isolates proved to be negative for the presence of *mecA*, *mecB*, *mecC* and *mecD* genes. The remaining *S. aureus* isolates investigated were MSSA (n=47).

Resistance to mupirocin was determined by MIC and *mupA* detection for 37 *S. aureus* isolates. Among these, 15 (40%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and carried the *mupA* gene. Two additional *S. epidermidis* were also tested and showed a high level resistance to mupirocin.

Resistance to linezolid was determined by MIC for 8 isolates including *S. aureus* (n=5) and *S. epidermidis* (n=3). All *S. aureus* were susceptible and the three *S. epidermidis* were 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

The CNR data on *S. aureus* causing CA-infections is based on spontaneous requests for toxin detection. In 2019, 662 isolates of *S. aureus* including 222 MRSA (220 *mecA*-positive, 2 *mecC*-positive), 438 MSSA and 2 BORSA/MODSA, as well as two *S. argenteus* were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection.

A total of 103 (46.4%) MRSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL) (**Figure 1**). These MRSA isolates were mostly recovered from skin lesions, in particular from skin abscesses, soft tissues or furunculosis (n=55) but also from deep fluids (n=20), screenings (n=7), blood cultures (n=2) or other sites (n=19).

By molecular typing, 50 PVL-positive MRSA isolates (48.5%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=21, 20.4%), ST30-SCC*mec* IV (Southwest Pacific clone) (n=17, 16.5%) and ST80-SCC*mec* IV (European clone) (n=12, 11.7%) (**Figure2**). Eleven of the 21 (52.4%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300.

The remaining PVL-positive MRSA isolates were assigned to the following clones: ST152/377-SCC*mec* IV or V (n=8), CC5 (n=9), CC398 (n=8), CC22 (n=7), Taiwan ST59-SCC*mec* V (n=4), ST88 (n=4), CC1 (n=4), CC1/ST573/772-SCC*mec* V (n=3) and CC121 (n=3). Since 2015, we observe a great diversification of CA-MRSA circulating clones.

Ninety-five (21.7%) MSSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL) (**Figure 1**). Molecular typing of these PVL-positive MSSA isolates revealed even more genetic diversity than MRSA isolates. These isolates were related to the clones: ST152/377 (n=32), CC121 (n=20), CC30 (n=12), CC15 (n=6), CC1 (n=5), CC8 (n=3), CC22 (n=3), CC5 (n=2), ST88 (n=2), CC1/ST573/772-SCC*mec* V (n=1) and others (n=9). These two last years (2018, 2019) the number of strains sent for toxin detection has increased, but the number of PVL positive strains remains stable since 2014.

**Figure 1:** Number of MRSA and MSSA isolates received for PVL detection, 2004-2019.

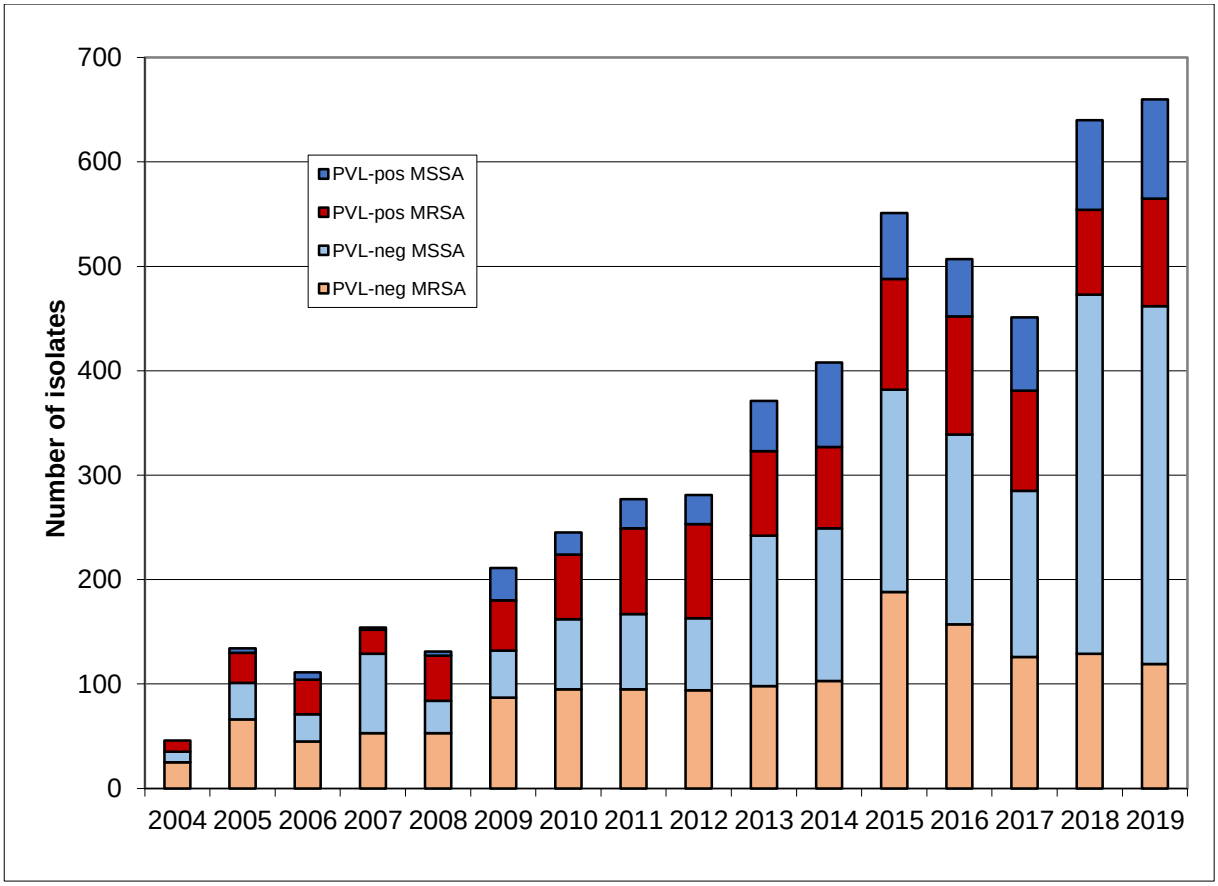
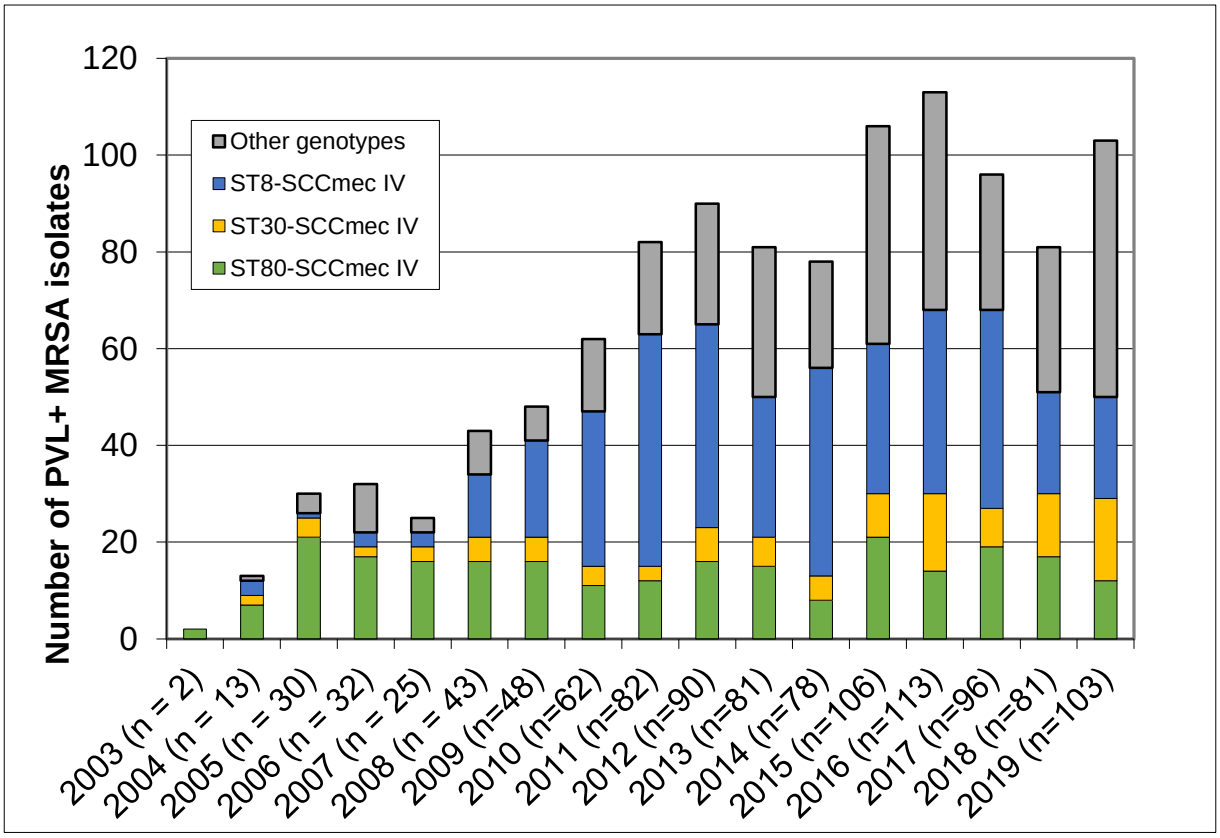


**Figure 2:** Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2019.




TSST-1 toxin was detected in 31 MRSA (14%) and 44 MSSA (10%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=20), screenings (n=18), deep fluids (n=9), blood cultures (n=2), or other sites (n=26). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC30 (n=30, 40%), CC22 (n=25, 33.3%) and CC5 (n=8, 10.7%). CC22 MRSA (n=6) and MSSA (n=1), as well as one MRSA belonging to ST152/377 carried both TSST-1 and PVL.

During recent years, strains causing toxic shock syndrome have had special attention due the decease of young people suffering this shock after using tampons. In January 2020 a young girl (17 years old) died from a toxic shock syndrome in Belgium. From 2015 to 2019, the NRC has received 32 request related to strains causing toxic shock (**Table 1**), but only for 6 TSST-1 positive isolates the request mentioned the use of tampons. Interestingly, in two toxic shock cases from 2015 and 2018 the *S. aureus* strain carried the enterotoxin *sec*. The proportion of isolates received by the NRC in the context of a toxic shock diagnosis is 2-3% (between 10 to 20 requests per year), one out of five effectively reveals the presence of a toxin (mainly TSST-1). The number of TSST-1 positive strains has been stable this last 5 years (from 2015 to 2019, ~11% of the ~500-600 strains analysed per year).

**Table 1:** Number of requests with toxic shock diagnosis and confirmed TSST-1 isolates per year.

| Year | N° of requests with toxic shock diagnosis | Confirmed TSST-1 positive isolates |
|---|---|---|
| 2019 | 14 | 2 |
| 2018 | 14 | 7 |
| 2017 | 9 | 6 |
| 2016 | 17 | 8 |
| 2015 | 18 | 9 |

Genes coding for exfoliatin A (*eta*) and/or B (*etb*) were found in 65 MSSA (14.8%) and 2 MRSA (0.9%) isolates. Both genes (*eta* and *etb*) were found in 51 MSSA isolates, most of them (n=47) belonging to CC121. A total of 39 MSSA isolates have the characteristics of the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC): carrying *eta* and/or *etb* genes, belonging to CC121 and showing fusidic acid resistance. Additionally, the gene coding for exfoliatin A was recovered 'alone' in two MRSA (belonging to CC1 and CC121 each), and 12 MSSA isolates most belonging to clones CC15 (n=4) and CC121 (n=7). The gene coding for exfoliatin B was recovered 'alone' in two MSSA isolates belonging to CC121.

Antimicrobial resistance percentages on the basis of EUCAST clinical breakpoints of MRSA and MSSA isolates received for toxin detection are summarized in **Tables 2** and **3**.

**Table 2:** Percentage of antimicrobial resistance of MRSA isolates received for toxin detection.

| Antimicrobials | Antimicrobial resistance of MRSA isolates (%) | | |
|---|---|---|---|
| | PVL positive (n=103) N (%) | PVL negative (n=115) N (%) | Total (n=218) N (%) |
| Erythromycin | 54 (52.4) | 35 (30.4) | 89 (40.8) |
| Clindamycin[a] | 35 (34.0) | 37 (32.5)[d] | 72 (33.2)[f] |
| Ciprofloxacin | 30 (29.4)[b] | 29 (25.2) | 59 (27.2)[f] |
| Kanamycin | 61 (62.2)[c] | 44 (41.1)[e] | 105 (51.2)[g] |
| Tobramycin | 29 (28.2) | 30 (26.1) | 59 (271) |
| Gentamycin | 24 (23.3) | 13 (11.3) | 37 (17.0) |
| Minocycline | - | 24 (20.9) | 24 (11..0) |
| Tetracycline | 48 (46.6) | 45 (39.1) | 93 (42.7) |
| Cotrimoxazole | 2 (1.9) | 2 (1.7) | 4 (1.8) |
| Fusidic acid | 22 (21.4) | 17 (14.8) | 39 (17.9) |
| Mupirocin | 2 (1.9) | 1 (0.9) | 3 (1.4) |

[a] Included both inducible and constitutive resistance.
[b] Only tested in 102 isolates. [c] Only tested in 98 isolates. [d] Only tested in 114 isolates. [e] Only tested in 107 isolates. [f] Only tested in 217 isolates. [g] Only tested in 205 isolates.
N, number of resistant isolates; -, absence of resistant isolates.

**Table 3:** Percentage of antimicrobial resistance (%) of MSSA isolates received for toxin detection.

| Antimicrobials | Antimicrobial resistance of MSSA isolates (%) | | |
|---|---|---|---|
| | PVL positive (n=95) N (%) | PVL negative (n=336) N (%) | Total (n=431) N (%) |
| Erythromycin | 14 (14.7) | 78 (23.2) | 92 (21.3) |
| Clindamycin[a] | 10 (10.6)[b] | 62 (18.5) | 72 (16.7)[f] |
| Ciprofloxacin | 9 (9.5) | 5 (1.5) | 14 (3.2) |
| Gentamycin | 1 (1.1) | 1 (0.3) | 2 (0.5) |
| Tobramycin | 2 (2.1) | 15 (4.5) | 17 (3.9) |
| Kanamycin | 4 (4.4)[c] | 21 (6.9)[d] | 25 (6.3)[g] |
| Minocycline | 1 (1.1)[b] | 4 (1.2) | 5 (1.2)[f] |
| Tetracycline | 27 (28.4) | 18 (5.4) | 45 (10.4) |
| Rifampicin | 2 (2.1) | - | 2 (0.5) |
| Cotrimoxazole | 8 (8.4) | 2 (0.6) | 10 (2.3) |
| Fusidic acid | 5 (5.3) | 77 (22.9) | 82 (19.0) |
| Mupirocin | 1 (1.1) | 10 (3.0)[e] | 11 (2.6) |

[a] Included both inducible and constitutive resistance.
[b] Only tested in 94 isolates. [c] Only tested in 90 isolates. [d] Only tested in 306 isolates. [e] Only tested in 334 isolates. [f] Only tested in 430 isolates. [g] Only tested in 396 isolates.
N, number of resistant isolates; -, absence of resistant isolates.

## 3. Typing for epidemiological investigations

In 2019, molecular typing using *spa*-typing was performed on 601 *S. aureus* isolates including 287 MRSA (285 *mecA*-positive, 2 *mecC*-positive) and 314 MSSA. Among these, 136 MRSA isolates and 48 MSSA isolates were sent for epidemiological investigation of local outbreaks in 2019 (17 MRSA outbreaks and 8 MSSA outbreaks). Additionally, 3 MRSA and 32 MSSA were sent in the context of a screening of hospital staff.

Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 13 of the 17 clusters investigated (involving 11 hospitals). Nevertheless, in four outbreaks more than one strain was involved. Globally, the most frequently recovered genotype was ST5-SCC*mec* II or IV [30 (22%) MRSA isolates recovered from 7 hospitals]. The remaining isolates belonged to CC1 (10 MRSA recovered from 2 hospitals), ST8-SCC*mec* IV (21 MRSA recovered from 5 hospitals), CC22 (6 MRSA recovered from 3 hospitals), CC30 (7 MRSA recovered in 3 hospitals), CC45 (12 MRSA recovered from 5 hospitals), ST80 (2 MRSA recovered from one hospital), ST88 (27 MRSA recovered from one hospital) and LA-MRSA ST398 (19 MRSA recovered from 2 hospitals).

Molecular typing confirmed horizontal transmission of MSSA in 6 of the 8 clusters investigated. MSSA isolates (recovered from 7 hospitals) showed high diversity with 13 distinct lineages. The most frequent genotypes were: CC5 (n=9), CC398 (n=8), CC121 (n=7) and CC30 (n=6).

Regarding the isolates sent in the context of a screening of hospital personnel, most isolates recovered were MSSA. These isolates belonged to more than 9 different clones CC398 (n=5) and CC30 (n=7) being the most frequent lineages.

Additionally, this year, two MRSA isolates were investigated for their SCC*mec* type, and molecular typing by PFGE analysis was performed to confirm an outbreak of *Staphylococcus capitis* (n=10).

## 4. Analyse of *S. aureus* from animal origin

Among the 287 MRSA strains typed in 2019, 37 (13%) ST398 MRSA isolates, called livestock-associated (LA-) MRSA, were observed. These 37 isolates came from hospitalised or ambulant patients in 10 hospitals from Flanders, two from Wallonia and one from Brussels. These MRSA were isolated from screenings (n=26), skin lesions (n=5), or other sites (n=6). Among these, available data showed that eight patients had direct contact with animals (farmers, vets). Eight MRSA ST398 strains carried PVL but they have other markers suggesting they were LA-MRSA (tetracycline and erythromycin/clindamycin resistance). Interestingly, most LA-MRSA CC398 (n=19) isolates were related to three outbreaks that took place in ~~two hospitals in~~ Flanders.

Thirteen (4.1%, 13/314) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from screenings (n=7), deep fluid (n=2), or other sites (n=4). These ST398 MSSA strains were not associated with livestock contacts and were all associated to outbreaks.

## 5. Conclusions

In 2019, a total of 92 *S. aureus* isolates were received for confirmation of oxacillin resistance. Among these, 5 isolates were cryptic (also named heterogeneous) MRSA and 21 oxacillin and/or cefoxitin resistant isolates lacked the *mecA* gene. From these 21 isolates, three isolates carried the *mecC* gene and the remaining 18 were classified as BORSA/MODSA.

Investigation of resistance against glycopeptides was requested for 8 MRSA and 11 MSSA. 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 remains stable (103 in 2019 versus 81in 2018). The antimicrobial resistance profile of these PVL- positive MRSA remains stable. Nevertheless, since 2016, the PVL-positive MRSA isolates that are resistant to tetracycline is increasing (2016-2017: 25%, 2018: 36%, 2019: 47%).

The proportion of CA-MRSA belonging to the ST8-SCC*mec* IV clone has drastically decreased since 2017 (2017: 43%, 2018: 26%, 2019: 20.4%). The percentage of the USA-300 clone within the CA-MRSA ST8 population has also decreased these recent years (74% in 2017, and 38% in 2018), although in 2019, half of the CA-MRSA ST8 belonged to USA300. The proportion of CA-MRSA isolates belonging to the ST80-SCC*mec* IV clone (12%) has also decreased compared to 2017-2018 (20%). In fact, we have observe a great diversification of circulating CA-MRSA clones since 2015.

The number of MSSA isolates received for toxin detection per year has increased (more than 400 isolates) compared to 2016 and 2017 (n≈230). Nevertheless, the proportion of PVL-positive MSSA cases remains stable (~20%). Wide diversity of genotypes was observed as usual. Interestingly, the most frequent PVL-positive MSSA clone was again ST152/377 (33.7%).

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

Finally, as in 2018, most livestock-associated ST398 MRSA isolates were recovered from persons living in Flanders and with direct contact with animals. The proportion of CC398 has decreased in 2019,

perhaps because since this year the NRC only performs *spa*-typing in case of epidemiological investigation or for toxin-positive isolates.

## 6. NRC publications - 2019

Nguyen TK, Argudín MA, Deplano A, Nhung PH, Nguyen HA, Tulkens PM, Dodemont M, Van Bambeke F. Antibiotic Resistance, Biofilm Formation, and Intracellular Survival As Possible Determinants of Persistent or Recurrent Infections by *Staphylococcus aureus* in a Vietnamese Tertiary Hospital: Focus on Bacterial Response to Moxifloxacin. Microb Drug Resist. 2019; doi: 10.1089/mdr.2019.0282.

Tajdar M, Reynders M, Van Praet J, Argudín MÁ, Vandecasteele SJ, Nulens E. A case of a surgical-site infection with *Staphylococcus condimenti*. Infection. 2019; 47(5):853-856.

Argudín MA, Hoefer A, Butaye P. Heavy metal resistance in bacteria from animals. Res Vet Sci. 2019; 122:132-147.

Dodémont M, Argudín MA, Willekens J, Vanderhelst E, Pierard D, Miendje Deyi VY, Hanssens L, Franckx H, Schelstraete P, Leroux-Roels I, Nonhoff C, Deplano A, Knoop C, Malfroot A, Denis O. Emergence of livestock-associated MRSA isolated from cystic fibrosis patients: Result of a Belgian national survey. J Cyst Fibros. 2019;18(1):86-93.