# Belgian *S. aureus* and other *Staphylococci* NRC Annual report 2020


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

- Identification and antimicrobial susceptibility testing of *Staphylococcus spp* 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, whole genome sequencing.

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* and other *Staphylococci* 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 2020, the NRC identified and/or determined the antimicrobials susceptibility of 118 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 6 MRSA, 11 MSSA, and one *S. epidermidis* strains. Only the *S. epidermidis* strain showed a decreased susceptibility to teicoplanin.

A total of 50 isolates were received for confirmation of oxacillin/cefoxitin resistance. Most isolates were identified as *S. aureus* (n=40), while the remaining isolates were *mecA*-positive *Staphylococcus argenteus* (n=1), *mecA*-positive (n=4) and *mecA*-negative (n=2) *Staphylococcus epidermidis*, *mecA*-positive (n=1) *Staphylococcus lugdunensis*, *mecA*-positive (n=1) *Staphylococcus saprophyticus* and *mecA*-negative *(n=1) Staphylococcus capitis*. Of the 40 *S. aureus* isolates, 17 were identified as MRSA, carrying the *mecA* gene and presenting phenotypic resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL). One (2%) isolate was cryptic (also named heterogeneous) MRSA, carrying the *mecA* gene but being phenotypically susceptible to oxacillin (MIC < 2 µg/mL). Two isolates (4%) 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 4 isolates (8%) were classified as BORSA/MODSA presenting phenotypic resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=2) or to only oxacillin (MIC > 2 µg/mL) (n=2). 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=17).

Resistance to mupirocin was determined by MIC and *mupA* detection for 13 *S. aureus* isolates. Among these, 5 (38.5%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and carried the *mupA* gene.

Resistance to linezolid was determined by MIC for 6 isolates including *S. aureus* (n=1) and *S. epidermidis* (n=5). The *S. aureus* was susceptible and four *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 NRC data on *S. aureus* causing CA-infections is based on spontaneous requests for toxin detection. In 2020, 454 isolates of *S. aureus* including 201 MRSA (200 *mecA*-positive, 1 *mecC*-positive), 250 MSSA, one *S. epidermidis* and one *S. argenteus* were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection.

A total of 94 (46.8%) MRSA isolates contained *lukS-lukF* 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=52) but also from deep fluids (n=12), screenings (n=11), blood cultures (n=2) or other sites (n=17).

By molecular typing, 30/94 (32%) PVL-positive MRSA isolates belonged to one of the three following clones: ST8-SCC*mec* IV (n=16), ST30-SCC*mec* IV (Southwest Pacific clone) (n=10) and ST80-SCC*mec* IV (European clone) (n=4) (**Figure2**). Six of the 16 (37.5%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of USA300 MRSA clone.

The remaining PVL-positive MRSA isolates were assigned to the following clonal complexes: CC5 (n=2), CC398 (n=8), CC22 (n=9), CC45 (n=4), CC1 (n=10), 7 other clonal complexes or non typeable (n=31). Since 2015, we observe a great diversification of CA-MRSA circulating clones.

Fifty-seven (21.6%) 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 harboured *spa* types related to the clonal complexes: CC15 (n=6), CC1 (n=1), CC8 (n=3), CC121 (n=1), CC30 (n=5), CC5 (n=1), and 21 "other" *spa* types (n=37). This last year, the number of strains sent for toxin detection has substantially decreased, but the number of PVL-positive strains remains stable since 2014.

**Figure 1:** Number of MRSA and MSSA isolates received for PVL detection, 2004-2020

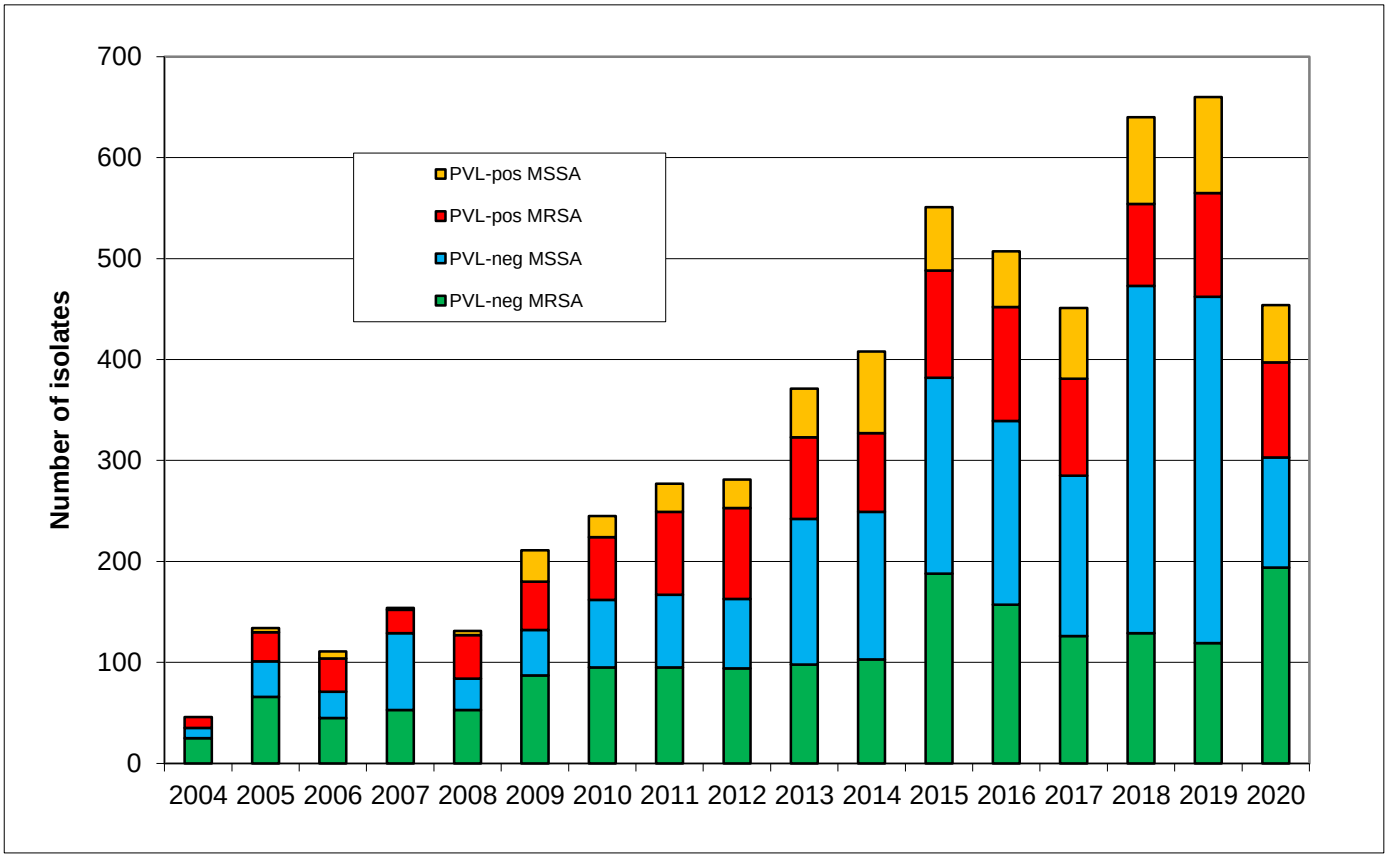

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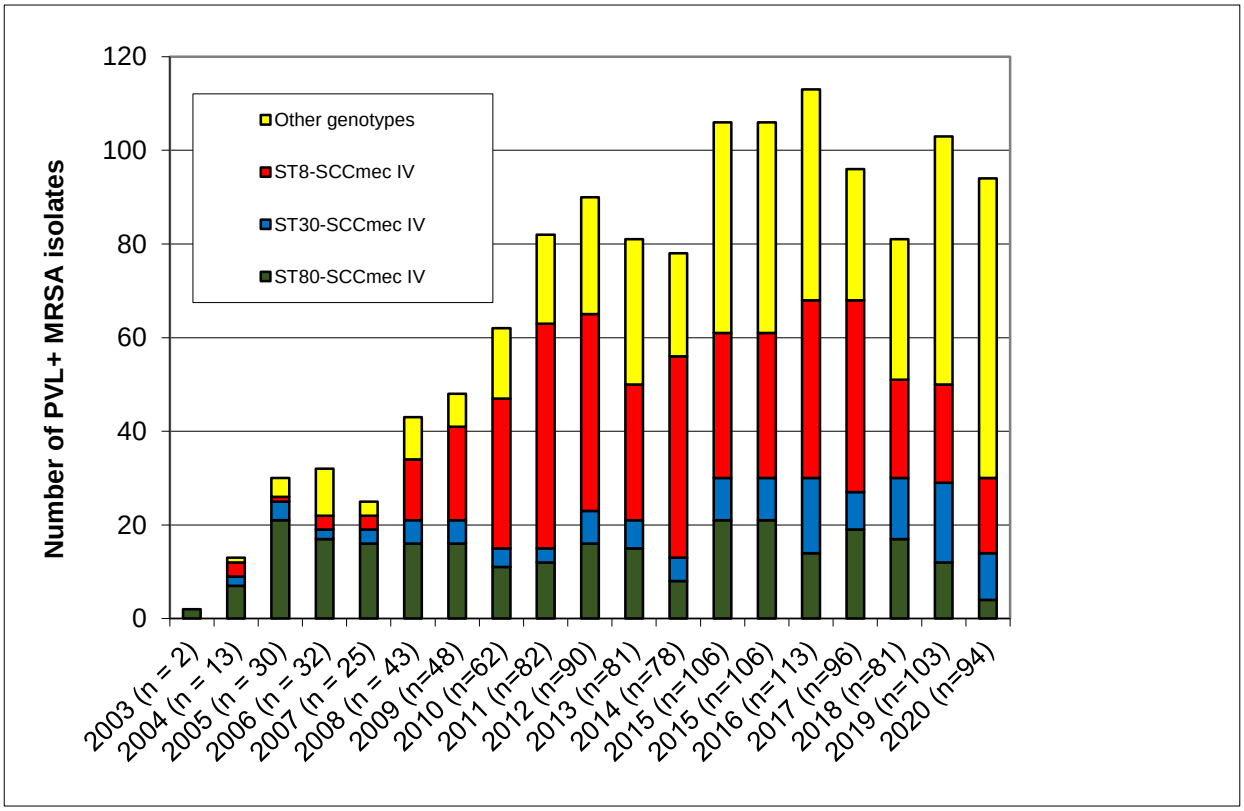
**Figure 2:** Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2020




TSST-1 toxin was detected in 31 MRSA (15.4%) and 25 MSSA (10%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=17), screenings (n=12), deep fluids (n=5), blood cultures (n=2), or other sites (n=20). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC22 (n=16, 28.6%), CC30 (n=9, 16%), and CC5 (n=8, 14%). CC22 MRSA (n=7) and CC30 MSSA (n=3) carried both TSST-1 and PVL.

During recent years, strains causing toxic shock syndrome received 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 2020, the NRC has received 36 requests related to strains causing toxic shock and showing presence of TSST-1 gene (**Table 1**). Nine of these (25%) mentioned the use of tampons. 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). The number of TSST-1 positive strains has been stable these last 6 years (from 2015 to 2020, ~11% of the ~500-600 strains analysed per year).

**Table 1:** Number of toxic shock diagnosis with TSST-1 positive per year.

| Year | Confirmed TSST-1 positive isolates |
|---|---|
| 2020 | 4 |
| 2019 | 2 |
| 2018 | 7 |
| 2017 | 6 |
| 2016 | 8 |
| 2015 | 9 |

Genes coding for exfoliatin A (*eta*) and/or B (*etb*) were found in 33 MSSA isolates. Both genes (*eta* and *etb*) were found in 21 MSSA isolates, all of them belonging to CC121. A total of 27 MSSA isolates did possess 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 11 MSSA isolates (belonging to CC5, CC15 and CC121). The gene coding for exfoliatin B was recovered 'alone' in one MSSA isolates belonging to CC15.

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=94) N (%) | PVL negative (n=107) N (%) | Total (n=201) N (%) |
| Erythromycin | 52 (55.3) | 43 (40.1) | 95 (47.3) |
| Clindamycin[a] | 32 (34.0) | 38 (35.5) | 70 (34.8) |
| Ciprofloxacin | 34 (36.1) | 28 (26.2) | 62 (30.8) |
| Kanamycin | 68 (72.3) | 41 (38.3) | 109 (54.2) |
| Tobramycin | 38 (40.4) | 31 (29.0) | 69 (34.3) |
| Gentamycin | 33 (35.1) | 18 (16.8) | 51 (25.4) |
| Minocycline | - | 22 (20.6) | 22 (11..0) |
| Tetracycline | 45 (47.8) | 46 (43.0) | 91 (45.3) |
| Cotrimoxazole | 1 (1.0) | - | 1 (0.5) |
| Fusidic acid | 18 (19.1) | 18 (16.8) | 36 (17.9) |
| Mupirocin | - | - | - |

[a] Included both inducible and constitutive resistance.
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=57) N (%) | PVL negative (n=193) N (%) | Total (n=250) N (%) |
| Erythromycin | 9 (15.8) | 51 (26.4) | 60 (24) |
| Clindamycin[a] | 7 (12.3) | 44 (22.8) | 53 (21.2) |
| Ciprofloxacin | 3 (5.3) | 2 (1.0) | 5 (2.0) |
| Gentamycin | - | - | - |
| Tobramycin | 1 (1.8) | 6 (3.1) | 7 (2.8) |
| Kanamycin | 1 (1.8) | 7 (3.6) | 8 (3.2) |
| Minocycline | - | 3 (1.5) | 3 (1.2) |
| Tetracycline | 6 (10.5) | 7 (3.1) | 13 (5.2) |
| Rifampicin | 1 (1.8) | - | 1 (0.4) |
| Cotrimoxazole | 1 (1.8) | 2 (1.0) | 3 (1.2) |
| Fusidic acid | 3 (5.3) | 46 (23.8) | 49 (19.6) |
| Mupirocin | 1 (1.8) | 5 (2.6) | 6 (2.4) |

[a] Included both inducible and constitutive resistance.
N, number of resistant isolates; -, absence of resistant isolates.

## 3. Typing for epidemiological investigations

In 2020, molecular typing using *spa*-typing was performed on 292 *S. aureus* isolates including 164 MRSA (all *mecA*-positive) and 128 MSSA. Among these, 35 MRSA isolates and 10 MSSA isolates were sent for epidemiological investigation of local outbreaks in 2020 (7 MRSA outbreaks and 1 MSSA outbreak).

Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 5 of the 7 clusters investigated (all involving hospitals). Nevertheless, in one outbreak more than one strain were involved. Globally, the most frequently recovered genotypes were ST45, ST5 and ST8.

Molecular typing revealed genotypically unrelated isolates in the MSSA cluster investigated.

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

Among the 164 MRSA strains typed in 2020, 13 (7.9%) ST398 MRSA isolates, called livestock-associated (LA-) MRSA, were observed. These 13 isolates came from hospitalised or ambulant patients of 7 hospitals from Flanders, two from Wallonia and one from Brussels. These MRSA were isolated from screenings (n=3), skin lesions (n=3), blood cultures (n=2), or other sites (n=5). Among these, available data showed that 3 patients had direct contact with animals (farmers, vets). Eight ST398 MRSA strains carried PVL but had other markers suggesting they were LA-MRSA (tetracycline and erythromycin/clindamycin resistance).

Four (3.1%, 4/128) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from screenings (n=3) or other sites (n=1). These ST398 MSSA strains were not associated with livestock contacts and were all associated to outbreaks.

## 5. National microbiological surveillance of *S. aureus* and coagulase negative *Staphylococci* in Belgian Hospitals

During 2019, 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 2020. Details and results of this surveillance are available in a separate report "Microbiologic Survey of *Staphylococcus* in Belgian Hospitals, 2019-2020" on the Sciensano website (https://nrchm.wiv-isp.be/fr/centres_ref_labo/staphylococcus_aureus/default.aspx).

## 6. National microbiological surveillance of the Epidemic European Fusidic-acid Resistant Impetigo Clone (EEFIC).

From March 2020 to March 2021, a national surveillance of *S. aureus* causing community-acquired (CA) skin infections was conducted to determine the proportion of CA- *S. aureus* strains causing impetigo in Belgium that are resistant to fusidic-acid and the prevalence of the Epidemic European Fusidic-acid Resistant Impetigo Clone (EEFIC) in Belgian community. Data were produced and analysed during 2020. Details and results of this surveillance are available in a separate report "Microbiologic survey of *S. aureus* causing community-acquired skin infections in Belgian community" on the Sciensano website (https://nrchm.wiv-isp.be/fr/centres_ref_labo/staphylococcus_aureus/default.aspx).

## 7. Conclusions

In 2020, a total of 50 *S. aureus* isolates were received for confirmation of oxacillin resistance. Among these, one isolate was cryptic (also named heterogeneous) MRSA and 6 oxacillin and/or cefoxitin resistant isolates lacked the *mecA* gene. From these 6 isolates, two isolates carried the *mecC* gene and the remaining 4 were classified as BORSA/MODSA.

Investigation of resistance against glycopeptides was requested for 6 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 (94 in 2020 versus 103 in 2019), as well as their antimicrobial resistance profile. Nevertheless, since 2016, the PVL-positive MRSA isolates that are resistant to tetracycline is increasing (2016-2017: 25%, 2018: 36%, 2019 and 2020: 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%, 2020: 17%). The percentage of the USA-300 clone within the CA-MRSA ST8 population has also decreased these recent years (74% in 2017, 38% in 2018, 50% in 2019 and 37% in 2020). The proportion of CA-MRSA isolates belonging to the ST80-SCC*mec* IV clone (4%) has also decreased compared with 2017-2018 (20%) and 2019 (12%). In fact, we have observed a great diversification of circulating CA-MRSA clones since 2015.

The number of MSSA isolates received for toxin detection per year has decreased (n=166) compared with 2019 (n=438). But the proportion of PVL-positive MSSA cases remains stable (~20-30%). Wide diversity of genotypes was observed as usual.

Genotyping of MRSA from local outbreaks in hospitals showed that these were mainly due to the nosocomial epidemic clones ST5, ST45 and ST8.

Finally, the proportion of CC398 (7.9%) decreased compared to 2019 (13%), these isolates were recovered from persons living in Flanders and with direct contact with animals.

## 8. NRC publications 2019-2020

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.

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.

Abdelbary MMH, Feil EJ, Senn L, Petignat C, Prod'hom G, Schrenzel J, François P, Werner G, Layer F, Strommenger B, Pantosti A, Monaco M, Denis O, Deplano A, Grundmann H, Blanc DS. Phylogeographical Analysis Reveals the Historic Origin, Emergence, and Evolutionary Dynamics of Methicillin-Resistant *Staphylococcus aureus* ST228. Front Microbiol. 2020 Aug 26; 11:2063. doi: 10.3389/fmicb.2020.02063. PMID: 32983046; PMCID: PMC7479193.