Belgian *S. aureus* and other *Staphylococci* NRC Annual report 2022

National Reference Center *S. aureus* and other *Staphylococci*
Laboratoire Hospitalier Universitaire de Bruxelles –
University Laboratory of Brussels (LHUB-ULB)
Service de Microbiologie
Route de Lennik 808
Hôpital Erasme
1070 Brussels
Belgium

Medical staff

Nicolas Yin, MD
Delphine Martiny, PharmD, PhD
Charlotte Michel, MD

Scientific staff

Ariane Deplano, MSc
Benoit Prevost

Technical staff

Genevieve Hay
Christine Thiroux
Pascale Buidin
Nathalie Legros
Raf De Ryck
Farida Ahajjam

LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

Anderlecht
Route de Lennik, 808
Lennikse Baan
1070 Bruxelles-Brussel
T. +32 (0)2 555.66.75

Horta
Place Van Gehuchten, 4
Van Gehuchten Plein
1020 Bruxelles-Brussel
T. +32 (0)2 477.22.99

Porte de Hal - Hallepoort
Rue Haute, 322
Hoogstraat
1000 Bruxelles-Brussel
T. +32 (0)2 435.20.00

Schaerbeek - Schaarbeek
rue du Foyer Schaerbeekois, 36
Schaarbeeksehaardstraat
1030 Bruxelles-Brussel
T. +32 (0)2 477.93.17

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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), *mec*A, *mec*B, *mec*C and *mec*D genes (coding for resistance to oxacillin), *mup*A and *mup*B genes (coding for mupirocin resistance), *cfr*, *cfr*(B), *optr*A and *poxt*A 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, *se*G to *se*I and *se*R to *se*T) and enterotoxin-like (*sel*I, *sel*K to *sel*G 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, 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://www.sciensano.be/fr/nrc-nrl/centre-national-de-reference-cnr-de-staphylococcus-aureus). 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 2022, the NRC identified and/or determined the antimicrobials susceptibility of 53 clinical staphylococcal isolates.

Resistance against glycopeptides was tested for 3 MRSA, 5 MSSA and 7 coagulase negative *Staphylococcus* (CNS). No decreased susceptibility was detected.

Fifty *S. aureus* isolates were received for confirmation of oxacillin/cefoxitin resistance. Among these, 8 (16%) were identified as MRSA, carrying the *mec*A 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 *mec*A 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 *mec*C 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. Seven isolates (14%) were classified as BORSA/MODSA presenting phenotypic resistance to both oxacillin (MIC > 2 µg/mL) and cefoxitin (MIC > 4 µg/mL) (n=3) or to only cefoxitin (MIC > 4 µg/mL) (n=4). These BORSA/MODSA isolates proved to be negative for the presence of *mec*A, *mec*B, *mec*C and *mec*D genes. The remaining *S. aureus* isolates investigated were MSSA (n=32).

Resistance to mupirocin was determined by MIC and *mup*A detection for 21 *S. aureus* isolates. Among these, 8 (38%) showed a high level resistance to mupirocin (MIC > 512 µg/mL) and carried the *mup*A gene.

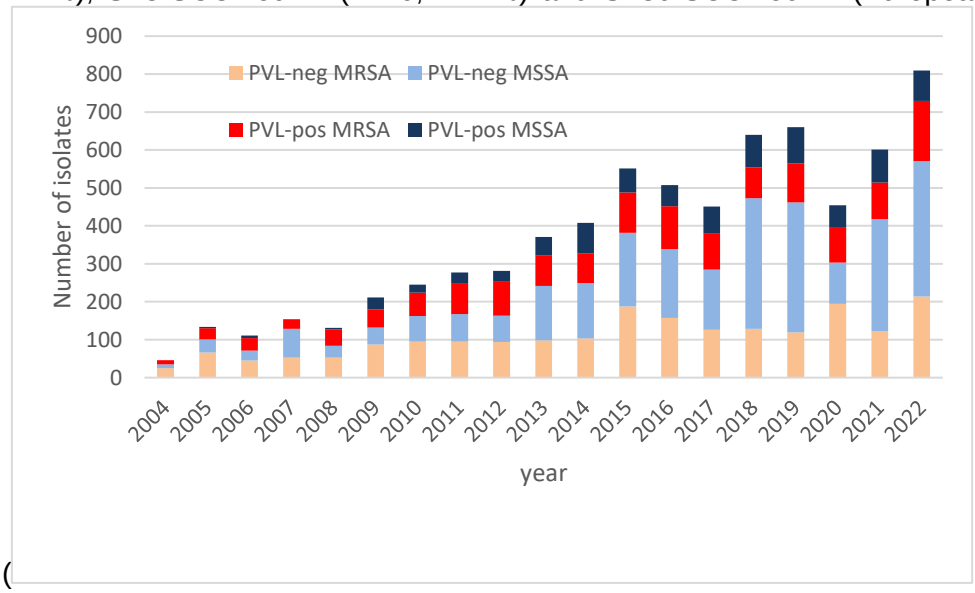
Resistance to linezolid was determined by MIC for 3 *S. aureus* isolates, 2 were found resistant to linezolid (MIC > 8 µg/mL) but the *cfr* gene was not detected.

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 2022, 809 isolates of *S. aureus* including 372 *mec*A positive MRSA and 437 MSSA were sent to the NRC for exotoxins (PVL, TSST-1, *eta*, *etb*) detection.

A total of 158 (42%) 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=75, 47.5%) but also from deep fluids (n=23, 14.6%), screenings (n=6, 3.8%), blood cultures (n=3, 1.9%) or other sites (n=51, 32.3%).

By molecular typing, 73/158 (46%) PVL-positive MRSA isolates belonged to one of the four following clones: ST30-SCC*mec* IV (Southwest Pacific clone) (n=29, 18.3%), ST1-SCC*mec* IV (USA-400) (n=20, 12.7%), ST8-SCC*mec* IV (n=20, 12.7%) and ST80-SCC*mec* IV (European clone) (n=4, 2.6%), (



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1030 Bruxelles-Brussel
T. +32 (0)2 477.93.17

Figure 2). Twelve of the 20 (60%) 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: CC772 (n=4), CC398 (n=4) and other clonal complexes or non-typeable (n=77). Since 2015, we observe a great diversification of CA-MRSA circulating clones with a decrease of the European ST80 CA-MRSA clone.

Eighty (18.3%) 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: CC1 (n=7, 8.7%), CC121 (n=7, 8.7%), CC30 (n=5, 6.2%), CC22 (n=1, 1.2%), or to 28 "other" *spa* types (n=57, 71.2%). The number of PVL-positive strains remains stable since 2014.

Figure 1: *Number of MRSA and MSSA isolates received for PVL detection, 2004-2022*

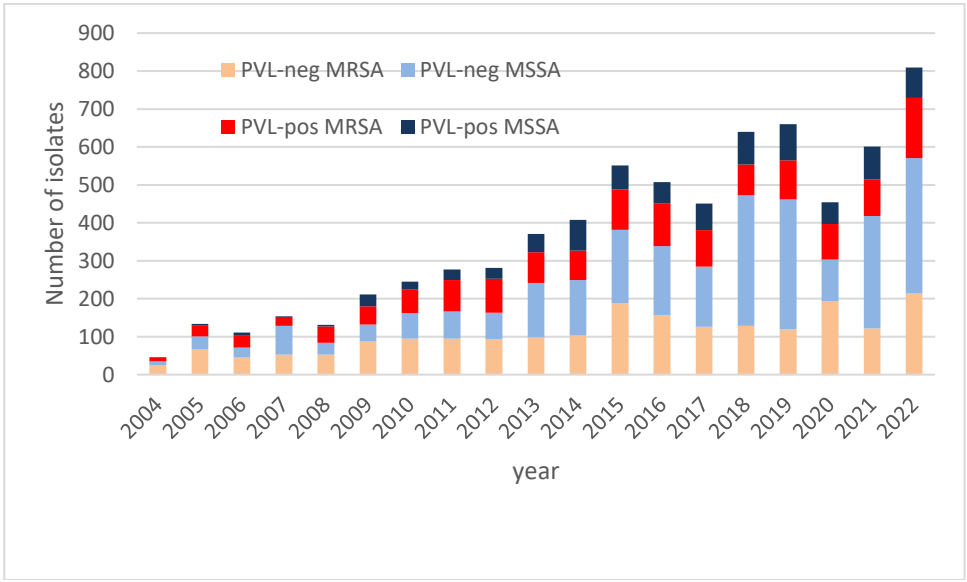
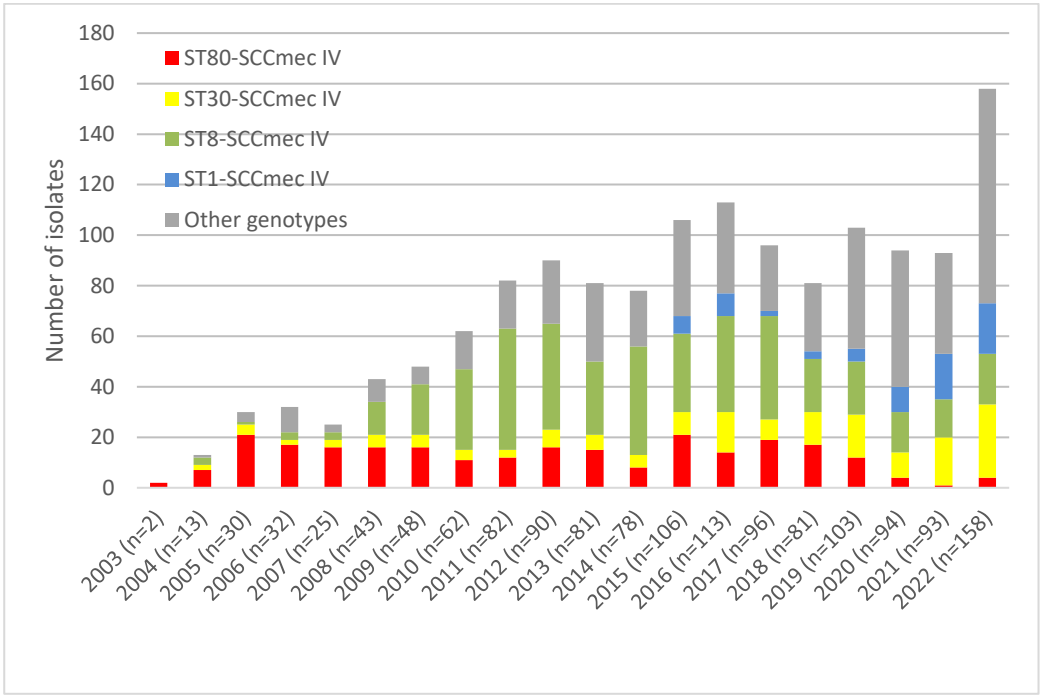


Figure 2: *Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2022*



TSST-1 toxin was detected in 32 MRSA (8.6%) and 47 MSSA (10.5%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=27, 34%), screenings (n=5, 6.3%), deep fluids (n=16, 20.3%), blood cultures (n=6, 7.6%), or other sites (n=25, 31.6%). Molecular typing showed that majority of TSST-1 positive isolates belonged to CC30 (n=16, 20%) or CC22 (n=11, 14%).

Eleven (2.9%) MRSA and 4 (0.9%) MSSA strains carried both TSST-1 and PVL. Molecular typing revealed great genetic diversity among these strains.

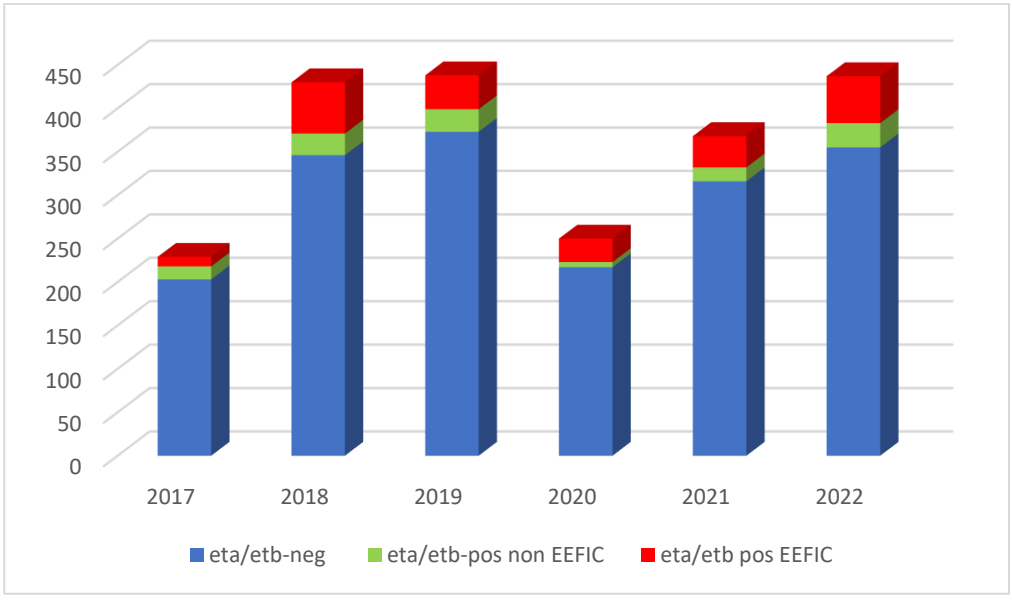
During recent years, strains causing toxic shock syndrome received special attention due the decease of young women suffering this shock after using tampons. In 2022, presence of TSST-1 was detected from vaginal sample in 3 women with a toxic shock syndrome (in 2 girls aged 15 and a young woman aged 24). From 2015 to 2022, the NRC has received 50 requests related to strains causing toxic shock and showing presence of TSST-1 gene (**Table 1**). Thirteen of these (26%) 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 8 years (from 2015 to 2022, ~10% of the ~600-900 strains analysed per year).

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

Year	Confirmed TSST-1 positive isolates
2022	9
2021	5
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 82 MSSA isolates. A total of 58/82 (70%) 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 (**Figure 3**). Among these EEFIC MSSA, 15 showed co-resistance to mupirocin (mupirocin-R EEFIC or M-EEFIC). These 15 strains were clonal, belonging to the ST121 and exhibiting the gene *mupA* in addition to the virulence gene harboured by EEFIC (*eta* and/or *etb*).

Figure 3: Proportion of *eta* and/or *etb* positive and EEFIC among MSSA isolates received for toxin detection, 2017-2022



Antimicrobial resistance percentages according to EUCAST clinical breakpoints[1] of MRSA and MSSA isolates received for toxin detection are summarized in **Table 2** and **Table 3**.

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

Antimicrobials	Antimicrobial resistance of MRSA isolates (%)		
	PVL positive (n=158) N (%)	PVL negative (n=214) N (%)	Total (n=372) N (%)
Erythromycin	83 (52.5)	63 (29.4)	146 (39.2)
Clindamycin[a]	38[b] (24.0)	58 (27.1)	96 (25.8)
Ciprofloxacin	64 (40.5)	101 (47.2)	165 (44.3)
Kanamycin	104 (65.8)	89 (41.6)	193 (51.9)
Tobramycin	73 (46.2)	68 (31.8)	141 (37.9)
Gentamycin	63 (39.9)	40 (18.7)	103 (27.7)
Minocycline	-	26 (12.0)	26 (6.7)
Tetracycline	69 (43.7)	80 (37.4)	149 (40.0)
Cotrimoxazole	5 (3.1)	5 (2.3)	10 (2.7)
Fusidic acid	39 (24.7)	109 (51)	148 (39.8)
Mupirocin	3 (1.9)	29 (13.6)	32 (8.6)
Linezolid	-	-	-

[a] Included both inducible and constitutive resistance.
[b] 29/38 with inducible resistance, 9/38 with 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=80) N (%)	PVL negative (n=357) N (%)	Total (n=437) N (%)
Erythromycin	10 (12.5)	97 (27.2)	107 (24.5)
Clindamycin[a]	5[b] (6.2)	88 (24.6)	93 (21.3)
Ciprofloxacin	6 (7.5)	15 (4.2)	21 (4.8)
Kanamycin	3 (3.7)	33 (9.2)	36 (8.2)
Tobramycin	2 (2.5)	25 (7.0)	27 (6.1)
Gentamycin	2 (2.5)	5 (1.4)	7 (1.6)
Minocycline	-	4 (1.1)	4 (0.9)
Tetracycline	5 (6.2)	21 (5.9)	26 (5.9)
Cotrimoxazole	-	1 (0.3)	1 (0.2)
Fusidic acid	9 (11.2)	99 (27.7)	108 (24.7)
Mupirocin	2 (2.5)	18[c] (5.0)	20 (4.6)
Linezolid	-	2 (0.6)	2 (0.4)

[a] Included both inducible and constitutive resistance.
[b] 3/5 with inducible resistance, 2/5 with constitutive resistance.
[c] all were co-resistant to Fusidic acid
N: number of resistant isolates -: absence of resistant isolates

Antibiotics were considered as resistant if intermediate or resistant according to EUCAST 2019 i.e. if considered resistant-only according to EUCAST 2022 with the exception of Mupirocin for which intermediate results according to EUCAST 2019 were not considered as resistant.

3. Typing for epidemiological investigations

In 2022, molecular typing using *spa*-typing was performed on 422 *S. aureus* isolates including 211 MRSA (all *mec*A-positive) and 211 MSSA. Among these, 5 MRSA, 4 MSSA and 2 *S. epidermidis* isolates were sent for epidemiological investigation.

Molecular typing allowed confirmation of horizontal transmission of MRSA and MSSA isolates in 2 of the 4 clusters investigated (all involving hospitals). Nevertheless, in one outbreak more than one strain were involved.

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

Among the 211 MRSA strains typed in 2022, 8 (3.8%) ST398 MRSA isolates, called livestock-associated (LA-) MRSA, were observed. These 8 isolates came from hospitalised or ambulant patients of 5 hospitals from Flanders and one from Brussels. These MRSA were isolated from screenings (n=2), deep fluids (n=2), wound (n=1) or other sites (n=3). Six ST398 MRSA strains carried PVL but had other markers suggesting they were LA-MRSA (tetracycline and erythromycin/clindamycin resistance).

None MSSA isolates belonging to clone ST398 were identified.

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

In 2022-2023, 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 will be produced and analysed during 2023. Details and results of this surveillance will be available in a separate report on the Sciensano website (https://www.sciensano.be/fr/nrc-nrl/centre-national-de-reference-cnr-de-staphylococcus-aureus).

6. Conclusions

In 2022, 50 *S. aureus* isolates were received for confirmation of oxacillin resistance. Among these, one isolate was cryptic (also named heterogeneous) MRSA and 2 oxacillin and/or cefoxitin resistant isolates lacked the *mec*A gene but carried the *mec*C gene. Seven *S. aureus* isolates were classified as BORSA/MODSA.

The number of PVL-positive strains among MRSA received by the NRC for exotoxin detection seems to be increasing (158 in 2022 versus 93-103 from 2019 to 2021), their antimicrobial resistance profile remains stable. Nevertheless, the PVL-positive MRSA isolates that are resistant to fusidic acid is increasing (39% in 2022 versus 19-30% from 2019 to 2021). Increase of resistance to fusidic acid and mupirocin were also observed in PVL-negative MRSA.

The proportion of CA-MRSA belonging to the ST8-SCC*mec* IV clone and USA-300 clone within the CA-MRSA ST8 population has drastically decreased these recent years. We observe a great diversification of CA-MRSA circulating clones with emergence of ST1-SCCmec IV (USA-400) MRSA clone and decrease of European ST80 CA-MRSA clone.

The number of MSSA isolates received for toxin detection per year continued to increase (n=437) compared with 2021 (n=383) and 2020 (n=166). However, the proportion of PVL-positive MSSA cases remains stable (~20-30%). A wide diversity of genotypes was observed as usual.

Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC) were frequently recovered among *eta* and/or *etb* positive MSSA isolates sent for toxin detection. Fusidic-acid resistance seems to be a good marker for detecting the EEFIC clone. Emergence of a mupirocin resistant subpopulation of EEFIC (M-EEFIC) was observed. This is of concern, as fusidic acid and mupirocin are the only topical agents for treating impetigo available in Belgium.

Finally, the proportion of CC398 (3.8%) was stable compared to 2021 (4.9%).

7. NRC publications 2019-2022

Deplano A., Hallin M., Bustos Sierra N., Michel C., Prevost B., Martiny D., Yin N**.** Persistence of the *Staphylococcus aureus* epidemic European fusidic acid-resistant impetigo clone (EEFIC) in Belgium. J Antimicrob Chemother 2023. doi.org/10.1093/jac/dkad204

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Timmermans M, Bogaerts B, Vanneste K, De Keersmaecker SCJ, Roosens NHC, Kowalewicz C, Simon G., Argudin M.A, Deplano A., Hallin M. et al. Large diversity of linezolid-resistant

isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019. J Antimicrob Chemother. 2021 24;77(1):49–57.

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 Oct;47(5):853–6.