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Persistence of the *Staphylococcus aureus* epidemic European fusidic acid-resistant impetigo clone (EEFIC) in Belgium

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Objectives: In August 2018, a public health alert was issued in Belgium regarding clusters of impetigo cases caused by the epidemic European fusidic acid-resistant impetigo clone (EEFIC) of *Staphylococcus aureus*. As a result, the Belgian national reference centre (NRC) was commissioned to update the epidemiology of *S. aureus* causing community-onset skin and soft tissues infection (CO-SSTI) to assess the proportion of EEFIC among them.

Methods: For 1 year, Belgian clinical laboratories were asked to send their first three *S. aureus* isolated from CO-SSTI each month. Isolates were tested for antimicrobial susceptibility to oxacillin, mupirocin and fusidic acid. Resistant isolates were also *spa* typed and tested for the presence of the genes encoding the Panton–Valentine leucocidin, the toxic shock syndrome toxin and the exfoliatins A and B. MLST clonal complexes were deduced from the *spa* types.

Results: Among the 518 *S. aureus* strains analysed, 487 (94.0%) were susceptible to oxacillin. Of these, 79 (16.2%) were resistant to fusidic acid, of which 38 (48.1%) belonged to the EEFIC. EEFIC isolates were mostly isolated from young patients with impetigo and showed a seasonal late summer peak.

Conclusions: These results suggest the persistence of EEFIC in Belgium. Furthermore, its prevalence may lead to reconsideration of the treatment guidelines for impetigo.

Introduction

In August 2018, a signal was sent to the Flemish region public health agency regarding several clusters of impetigo cases in the Kempen area. The reported cases presented extensive lesions, yellow crusts, high rate of recurrence, and risks of scarring. Analysis performed by the *Staphylococcus aureus* national reference centre (NRC) revealed the majority of cases were due to the so-called epidemic European fusidic acid-resistant impetigo clone (EEFIC), a fusidic acid-resistant MSSA clone, harbouring genes encoding the exfoliatins A and/or B (*eta* and/or *etb*) and belonging to the clonal complex MLST CC121.[1] This clone was first described as spreading in Europe in the early 2000s,[2,3] then as declining in Norway 10 years after.[4] From January to September 2018, 47 out of 460 (10.2%) isolates sent to the NRC for toxin gene detection belonged to the EEFIC. Most EEFIC cases (70.2%) were reported in August and September 2018, affecting children aged from 3 to 12 years. The increasing prevalence of fusidic acid-resistant (FA-R) *S. aureus* strains in skin infections is of concern because topical fusidic acid is the empirical choice for treatment of impetigo in many countries.[5,6]

The aim of the present study was to collect representative microbiological data to assess the prevalence of EEFIC, and more generally, to evaluate the global epidemiology of *S. aureus* causing community-onset skin and soft tissue infection (CO-SSTI).

Materials and methods

From February 2020 to January 2021, all Belgian clinical laboratories were invited to collect the first one to three non-duplicate, consecutive CO-SSTI-causing *S. aureus* per month. Skin samples of both adult and paediatric outpatients and patients hospitalized for less than 48 h were eligible. Samples from chronic or surgical wounds were excluded. Participating laboratories sent their strains along with a case report form detailing the type of specimen, the patient age and sex and the

type of CO-SSTI (among abscess, cellulitis, epidermolysis bullosa, impetigo, infected wound, infected eczema and furunculosis).

For each isolate received, species identification was confirmed by MALDI-TOF with a MALDI Biotyper® sirius IVD system (version 4.1.100, Bruker Daltonics, Bremen, Germany). Susceptibility to oxacillin (inferred by cefoxitin), fusidic acid and mupirocin was assessed by disc diffusion according to the EUCAST 2019 norm. The mechanism of resistance to oxacillin was further studied by PCR detection of the *mecA* gene,[7] while the mechanism of resistance to mupirocin was studied by PCR detection of the *mupA* gene.[8]

All confirmed *S. aureus* isolates showing resistance to oxacillin, fusidic acid or mupirocin were further analysed for: (i) disc diffusion susceptibility testing to gentamicin, kanamycin, tobramycin, co-trimoxazole, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, linezolid, minocycline, rifampicin and tetracycline according to the EUCAST 2019 norm; (ii) PCR detection of *eta*, *etb*, the toxic shock syndrome toxin (TSST-1) and the Panton–Valentine leucocidin (PVL)-encoding genes (respectively *tst* and *luk*SF-PV);[9] and (iii) *spa* typing (sequencing of the protein A gene polymorphic X region).[10]

MLST clonal complexes (CCs) were assigned when possible by deduction from *spa*-typing data and single repeat variants, using the *spa* typing website[11] (http://spaserver.ridom.de/) developed by Ridom GmbH and curated by SeqNet.org (http://www.SeqNet.org/) as well as previously published data.[1–3,12] Isolates were defined as 'EEFIC' if they were FA-R MSSA carrying *eta* and/or *etb* and harbouring a *spa* type related to CC121.

A subset of randomly selected fusidic acid-susceptible MSSA isolates were also analysed by PCR for toxin detection and *spa* type as described above.

Results

Participation and strains collected

Twenty-four of the 47 Belgian laboratories (51.1%), located in 6 of the 10 provinces, agreed to participate. The number of *S. aureus* isolates collected reached 543, sampled from January 2020 to March 2021, of which 518 were analysed (Figure 1).

Antimicrobial susceptibility testing

The majority of *S. aureus* ($n=487$; 94.0%) were MSSA. Thirty-one (6.0%) were MRSA, all carrying the *mecA* gene. Of the 518 isolates, 81 (15.6%) were FA-R, including 79 (16.2%) MSSA. Among the FA-R MSSA, four showed a high level of resistance to mupirocin (MIC > 256 mg/L) combined with resistance to tobramycin and amikacin, and harboured the *mupA* gene. These four isolates were susceptible to all the other antimicrobials tested.

Toxin detection and molecular typing

Of the 79 FA-R MSSA, 38 (48.1%) carried *eta* and/or *etb* (33 carried both, 3 *eta* only, and 2 *etb* only), 2 (2.5%) carried the TSST-1-encoding gene, and 1 (1.3%) carried the PVL-encoding genes. The FA-R MSSA carrying *eta* and/or *etb* ($n=38$) harboured 12 different *spa* types, all CC121-related, and therefore were considered as EEFIC. The most frequent *spa* type (17/38) was t408.

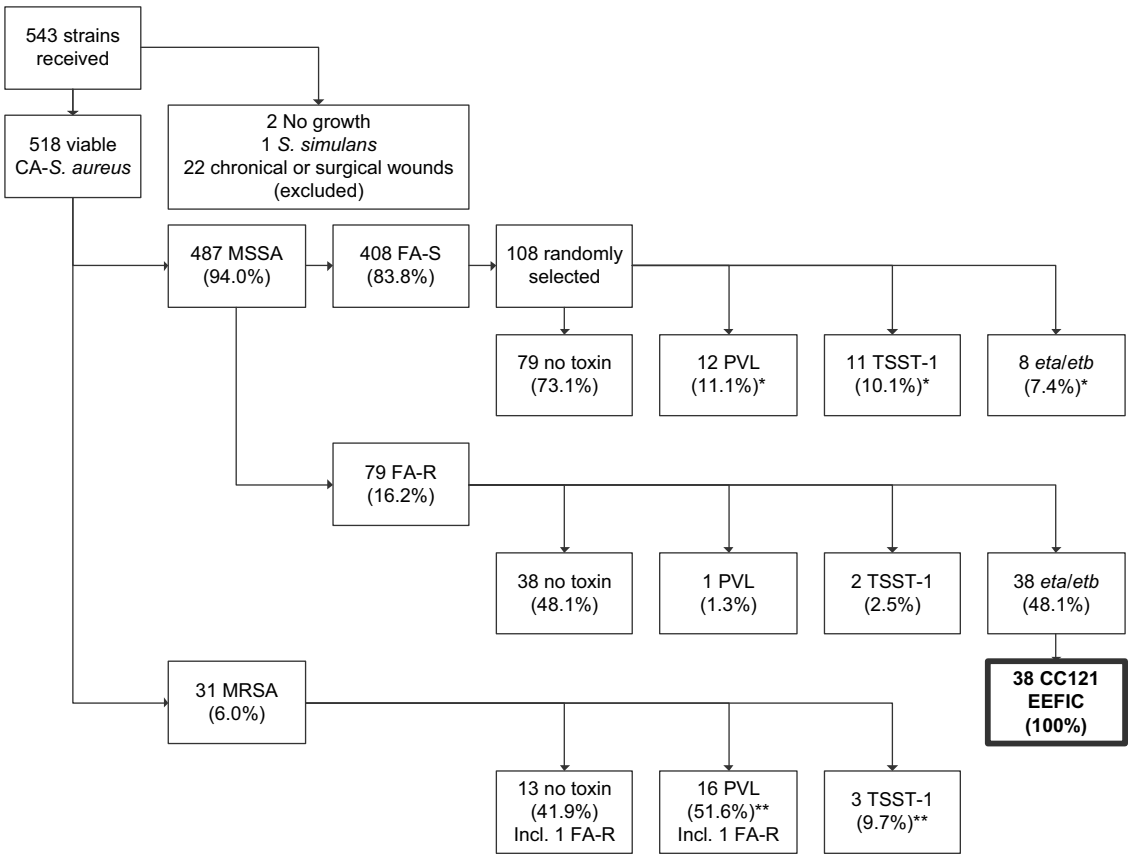

Figure 1. Toxin gene detection and resistance to oxacillin and fusidic acid in the 518 *S. aureus* strains collected. CA: community-acquired; FA-S: fusidic acid susceptible. *One MSSA exhibited PVL and TSST-1 genes; one MSSA exhibited PVL genes and *eta*. **One MRSA exhibited PVL and TSST-1 genes.

Four EEFIC isolates were co-resistant to mupirocin, tobramycin and amikacin. One EEFIC isolate was only co-resistant to tobramycin and amikacin. Other EEFIC isolates ($n=33$) remained susceptible to all the other antimicrobials tested.

Of the 31 MRSA, 16 (51.6%) carried the PVL-encoding genes, and 3 (9.7%) carried the TSST-1-encoding gene.

Among a random subset of 108 fusidic acid-susceptible MSSA, 12 (11.1%) carried the PVL-encoding genes, 11 (10.1%) carried the TSST-1-encoding gene, and 8 (7.4%) carried *eta* and/or *etb*.

Demographics and clinical data

Demographics and clinical data collected during the survey are displayed in Table 1. EEFIC was more frequently isolated from young patients (median age of 9.5 years, 79.0% were less than 16 years old), from lesions originating from the head and neck (41.2%). It was mainly isolated from impetigo (85.7%). In comparison, other MSSA were isolated from older patients (median age of 54 years), abscesses (33.0%) and infected wounds (31.0%), frequently from the limbs (63.4%). MRSA were isolated from older patients (median age of 43 years) from abscesses (61.1%), the pelvo-perineum (45.8%) and the limbs (41.7%).

Geographical and time distribution of the EEFIC

The 38 EEFIC strains were sent by laboratories located in the Flemish region while none was identified from the MSSA sent by the four participating laboratories of Wallonia. Although only

Table 1. Clinical data and demographics of 518 *S. aureus* CO-SSTI

Characteristics	MRSA ($n=31$)	Non-EEFIC MSSA ($n=449$)	EEFIC ($n=38$)
Number of *spa* types	20	77[a]	12
Number of assigned CC	8	17[a]	1
Age (years)			
Median (range)	43 (1–101)	54 (0–98)	9.5 (1–68)
Mean	47	50	15
Gender, *n*/*N* (%)			
Female	17/31 (54.8)	207/445 (46.5)	18/38 (47.4)
Male	14/31 (45.2)	238/445 (53.5)	20/38 (52.6)
Infection site			
Head & neck, *n*/*N* (%)	1/24 (4.2)	72/407 (17.7)	14/34 (41.2)
Face, *n*	1	36	10
Scalp, *n*	—	28	4
Neck, *n*	—	8	—
Trunk, *n*/*N* (%)	2/24 (8.3)	46/407 (11.3)	4/34 (11.8)
Thorax, *n*	—	21	—
Abdomen, *n*	2	13	2
Back, *n*	—	12	2
Limbs, *n*/*N* (%)	10/24 (41.7)	258/407 (63.4)	12/34 (35.3)
Underarm, *n*	2	12	1
Arm, *n*	—	31	1
Hand, *n*	1	31	1
Leg, *n*	4	98	6
Foot, *n*	3	86	3
Pelvo-perineum, *n*/*N* (%)	11/24 (45.8)	31/407 (7.6)	4/34 (11.8)
Genitals, *n*	1	13	—
Inguinal creases, *n*	3	9	—
Buttock, *n*	7	9	4
Number of lesions, *n*/*N* (%)			
Unique	22/27 (81.5)	381/407 (93.6)	35/38 (92.1)
Multiple	5/27 (18.5)	26/407 (6.4)	3/38 (7.9)
Type of SSTI, *n*/*N* (%)			
Abscess	11/18 (61.1)	85/258 (33.0)	—
Infected wound	3/18 (16.7)	80/258 (31.0)	3/28 (10.7)
Infected eczema	—	32/258 (12.4)	1/28 (3.6)
Impetigo	1/18 (5.6)	25/258 (9.7)	24/28 (85.7)
Cellulitis	1/18 (5.6)	20/258 (7.8)	—
Furunculosis	2/18 (11.1)	15/258 (5.8)	—
Epidermolysis bullosa	—	1/258 (0.4)	—

[a]Among 149 *spa*-typed MSSA.

15.0% of all isolates originated from the province of Antwerp, almost half of the EEFIC cases (47.4%) were located there. Eighteen of the 38 EEFIC strains were isolated in August and September 2020 compared with no more than four isolates per month for the other months. A peak was reached in September 2020, where 10/38 (26.3%) of all the *S. aureus* strains were EEFIC.

Discussion

This survey was conducted to assess the epidemiology of *S. aureus* causing CO-SSTI in the Belgian community. FA-R was detected in 2 (6.5%) MRSA and in 79 (16.1%) MSSA. FA-R seems to be a good marker for detecting the EEFIC, as around half of these FA-R MSSA were indeed belonging to this clone. The large majority of *S. aureus* causing CO-SSTI remained susceptible to oxacillin (94.0%). Among the 6.0% of MRSA collected, more than half showed the presence of PVL. This finding is in line with Vandenesch *et al.*[13] previously showing that PVL represented a stable genetic marker of the community MRSA strains, explaining its higher rate compared with MSSA. Thirty-eight (7.8%) EEFIC were recovered among the 487 CO-SSTI-causing MSSA collected during this survey. The vast majority of EEFIC were recovered from impetigo (85.7%), from patients less than 16 years old (79.0%), and from the province of Antwerp (47.4%). The vast majority of EEFIC remained susceptible to other antimicrobials tested but four strains (10.5%) showed co-resistance to mupirocin and aminoglycosides, which can lead to difficulties in topical antibiotic therapy. A peak was observed during the August–September period with EEFIC representing 26.3% of all the CO-SSTI-causing *S. aureus* collected in September 2020. Moreover, the summer of 2020 was particularly hot in Belgium with temperatures above 30°C for 8 consecutive days. With climate change, hot spells might lead to more CO-SSTIs.

In a review,[14] Koning concludes that topical mupirocin or fusidic acid were equally or more effective than oral treatment for impetigo. In Belgium[5] and the Netherlands,[6] topical fusidic acid is a first-line treatment for impetigo. However, 27 of the 50 impetigo-causing strains studied here were FA-R and some were co-resistant to mupirocin. There are currently no topical treatments recommended in Belgium to cover these FA-R and mupirocin-resistant strains. In comparison, health authorities in France recommend the use of topical mupirocin only for localized forms of impetigo and in addition to hygiene care[15] while the UK recommends considering an antiseptic as first line (hydrogen peroxide 1% cream).[16]

The survey was intended to cover a 1 year period, which is of importance due to the seasonal character of impetigo clusters.[17] Unfortunately, the social distancing and restrictions that were successively in application during the COVID-19 pandemic, combined with the clinical laboratories' work overload during the study period, have certainly generated biases that are difficult to fully apprehend. Another limitation is the heterogeneity of participation rate between the different regions, as well as the lack of data concerning the overall number of cultures positive for *S. aureus* by region. Finally, CO-SSTIs are usually not sampled if not complicated, which leads to a probable overestimation of resistance and toxin rates. Nevertheless, this survey has allowed prospective collection of a large nationwide microbiological dataset regarding *S. aureus*-related CO-SSTI.

A retrospective analysis of isolates sent for toxin detection to the NRC from 2013 to 2022 showed an increase in the proportion of EEFIC among MSSA isolated from skin smears from 2017. Although based on strains sent on a voluntary basis, no more than 3.0% of MSSA isolated from skin smears per year were identified as EEFIC before 2017. This proportion increased to reach 12.6% in 2017 and 18.4% in 2022, showing the persistence of EEFIC in Belgium.

This study showed persistence of the EEFIC in the Belgian paediatric population with a seasonal peak in late summer. This persistence could be favoured by the wide use of topical fusidic acid and be responsible for treatment failures. This is particularly of concern since a public health alert was issued by the Netherlands on EpiPulse in February 2023 to report an ongoing outbreak of impetigo among young children of a community-associated *eta*- and *etb*-positive CC121 FA-R MRSA clone,[18] thus presenting resistance to oxacillin on top of all the characteristics of the EEFIC (2023-ARH-00002). All these data taken together highlight the need to set surveillance of *S. aureus* CO-SSTI to follow the emergence and spread of invasive and/or resistant strains and to allow dynamic adaptation of the recommendations for first-line treatment. In this frame, mupirocin or retapamulin[19] (currently unavailable in Belgium and not tested here) could be better topical treatments than fusidic acid for localized forms of impetigo. Further studies focused on impetigo should be undertaken, as impetigo is recognized as an understudied area both in terms of resistance to antibiotics and treatment efficacy.[20]

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Transparency declarations

The authors have no competing interests to declare. This study is an epidemiological observational study; therefore, no ethical approval was required.

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