National microbiological surveillance of the epidemic European fusidic-acid resistant impetigo clone (EEFIC)

This work is an un-refereed preprint version of a report accepted for publication in Journal of Antimicrobial Chemotherapy (https://doi.org/10.1093/jac/dkad204)

National Reference Centre for *S. aureus* and other species
Laboratoire Hospitalier Universitaire de Bruxelles
- Universitair Laboratorium Brussel (LHUB-ULB)
Service de Microbiologie
Route de Lennik 808
1070 Brussels, Belgium

Medical staff
Marie Hallin MD, PhD
Delphine Martiny, PharmD, PhD
Charlotte Michel, MD
Nicolas Yin, MD

Scientific staff
Ariane Deplano, MSc
Claire Nonhoff, MSc
Benoit Prevost, PhD

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

With the participation of Sciensano
Natalia Bustos Sierra, MSc

March 2023

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1. Context

In August 2018, a signal was sent to the Flemish Agenschap Zorg en Gezondheid regarding several clusters of impetigo cases in the Kempen. The reported cases presented extensive lesions, yellow crusts, high rate of recurrence and risks of scarring, but no general complications. Analysis performed by the *Staphylococcus aureus* National Reference Centre (NRC) revealed that the majority of cases were due to the so-called Epidemic European Fusidic acid-resistant Impetigo Clone (EEFIC), a fusidic acid-resistant (FA-R), community-acquired, methicillin-susceptible, *S. aureus* (CA-MSSA) clone, harbouring genes encoding for exfoliatin A and/or B (*eta* and/or *etb*) and belonging to MLST CC121 [1]. From January to September 2018, among 305 MSSA sent to the NRC for toxins genes detection, 47 (15.4%) to the EEFIC. Thirty-six cases were clearly identified as coming from skin smears. Most EEFIC cases were reported in August and September 2018 affecting children from 3 to 12 years.

The increasing prevalence of FA-R *S. aureus* from wound infections is of concern because topical fusidic acid treatment is the empirical choice for the treatment of impetigo in Belgium [2]. Moreover, pharmacies in Belgium can dispense fusidic acid cream without prescription.

Our aim was to collect representative microbiological data to assess the situation, and more generally, to evaluate the global epidemiology of *S. aureus* in Belgium causing community onset skin and soft tissue infections (CO-SSTI).

2. Objectives

This national surveillance of *S. aureus* causing CO-SSTIs was conducted:

- to collect nationally representative data regarding the proportion of EEFIC among *S. aureus* causing CO-SSTIs;
- to update data on the proportion community-acquired methicillin-resistant *S. aureus* (CA-MRSA) harbouring the genes encoding the Panton-Valentine leukocidin (PVL) among *S. aureus* causing skin infections;
- more generally, to assess the epidemiology of *S. aureus* causing CO-SSTIs.

The survey funded by Sciensano was organized to cover a one-year period, which is of importance due to the seasonal character of impetigo [3] but happened during the COVID-19 pandemic.

3. Collection of bacterial strains

From February 2020 to January 2021, all Belgian clinical laboratories were invited to collect the first one to three, consecutive CO-SSTI causing *S. aureus* per month. Skin samples of both adult and pediatric outpatient or patients hospitalized for less than 48 hours were eligible. Samples from chronic or surgical wounds were excluded.

Participating laboratories sent their strains along with a case report form detailing the following demographic and clinical items: referring laboratory address, type of specimen, sampling date, if the sample was from an outpatient or taken during hospitalization but less than 48 hours after admission, the patient age and sex, and the indication of any eventual antibiotic therapy at the time of sampling. Laboratories were also asked to provide the aggregate number of skin samples positive for *S. aureus* per month.

4. Methods

For each isolate received, species identification was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF). Susceptibility to oxacillin (inferred by cefoxitin), fusidic acid and mupirocin was assessed by disk-diffusion according to the EUCAST 2019 norm. Resistance to oxacillin was confirmed by PCR detection of the *mec*A gene. Resistance to mupirocin was confirmed by PCR detection of the *mup*A gene.

All confirmed *S. aureus* isolates showing resistance to oxacillin, fusidic acid or mupirocin were further analyzed for:

1. Disk-diffusion susceptibility testing to gentamicin, kanamycin, tobramycin, cotrimoxazole, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, linezolid, minocycline, rifampicin and tetracycline according to the EUCAST 2019 norm;

2. PCR detection by of *eta*, *etb*, the toxic shock syndrome toxin (TSST-1) and the Panton-Valentine leukocidin (PVL) encoding genes;

3. *spa*-typing (sequencing of the protein A gene polymorphic X region).

Multilocus sequence typing (MLST) clonal complexes (CC) were assigned by deduction from *spa*-typing data. Isolates were defined as “EEFIC” if FA-R MSSA carrying *eta* and/or *etb* and harboring a *spa*-type related to the CC121.

A subset of randomly selected fusidic acid susceptible MSSA isolates (n ≈ 100) were also analyzed by PCR for the toxins detection and *spa*-type as described above.

5. Results

5.1. *Participation*

Twenty-four of the 47 Belgian laboratories (51%), 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 (**Table 1** and **Figure 1**). About 4.6% of the isolates (n = 25) were excluded for the following reasons: 2 isolates did not grow after culture, 1 isolate was identified as a *Staphylococcus simulans*, 1 MRSA and 21 MSSA were excluded because isolated from infections of chronic or surgical wounds. Hence, 518 community-acquired *S. aureus* were analyzed.

Table 1: Repartition by province of the laboratories collecting the *S. aureus* isolates during the national survey, January 2020 to March 2021.

Province	Number of isolates	Number of laboratories
East Flanders	168	7
West Flanders	105	4
Limburg	82	4
Antwerp	78	5
Hainaut	70	3
Liège	15	1
Total	**518**	**24**

5.2. *Antimicrobial susceptibility testing*

The majority of the *S. aureus* strains (n = 487, 94.0%) were MSSA (**Figure 1** and **Table 2**). The remaining 31 (6.0%) were MRSA carrying the *mec*A gene. Over the 518 isolates, 81 (15.4%) were FA-R including 79 (16.2%) among MSSA and 2 (6.5%) among MRSA. Among the FA-R MSSA, 4 showed a high level of resistance to mupirocin (minimal inhibitory concentration (MIC) > 2014 µg/ml) combined with a resistance to aminoglycosides and harbored the *mup*A gene.

Table 2: Antimicrobials resistance of *S. aureus* isolates collected during the national survey, January 2020 to March 2021

Resistance to:	MRSA (n=31)	MSSA (n=487)	Total (n=518)
Fusidic acid	2	79	81
Mupirocin	0	4[a]	4

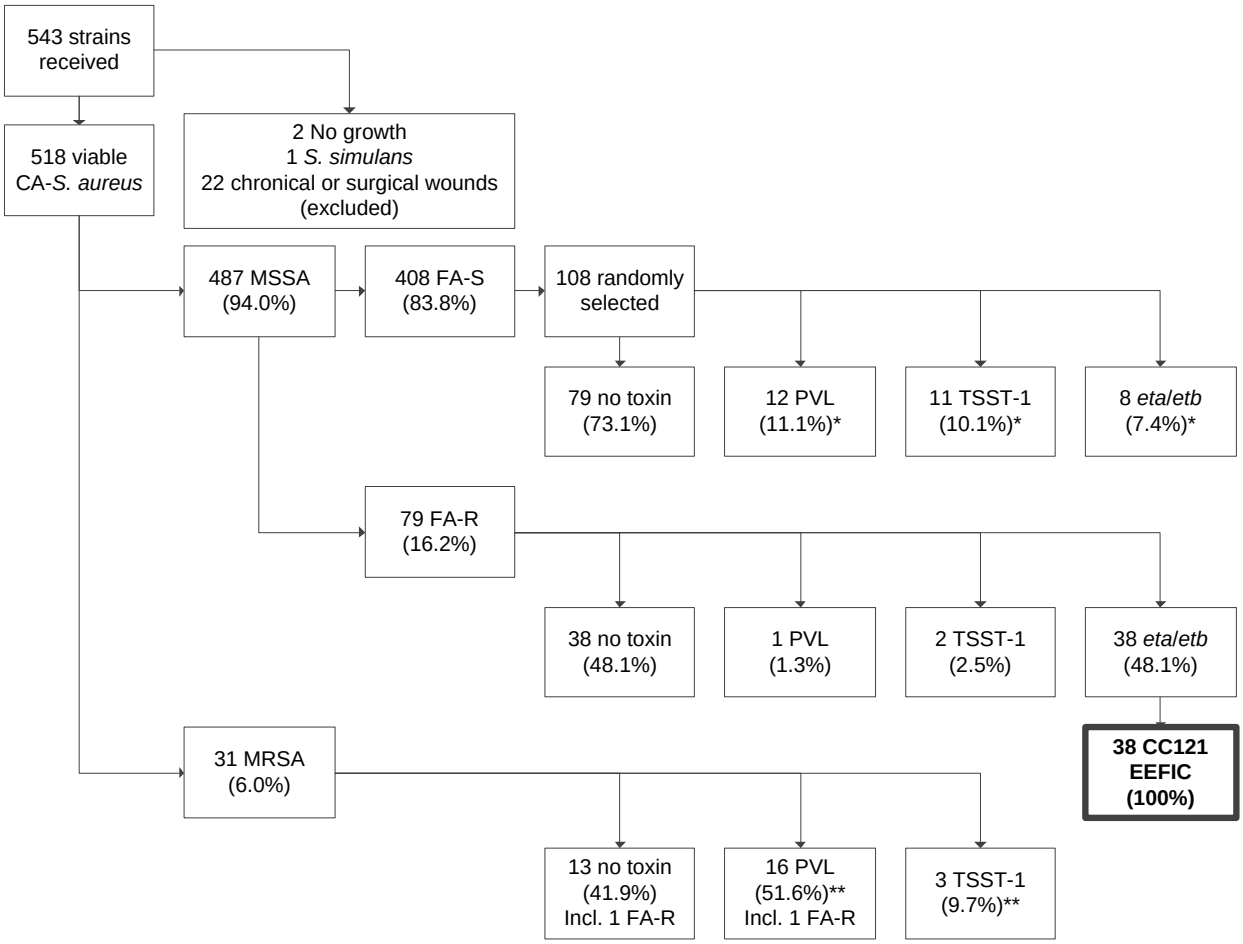
[a]Co-resistance to fusidic acid and aminoglycosides

5.3. *Toxins detection and molecular typing*

Of the 79 FA-R MSSA, 38 (48.1%) carried *eta* and/or *etb*, 2 (2.5%) carried the TSST-1 encoding gene, 1 (1.3%) carried the PVL encoding genes (**Figure 1**). All the 38 FA-R MSSA carrying *eta* and/or *etb* harbored CC121-related *spa*-types, and therefore were considered as EEFIC, including the 4 co-resistant to mupirocin and aminoglycosides. One EEFIC isolate was only co-resistant to aminoglycosides. 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, 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, 8 (7.4%) carried *eta* and/or *etb*.



CA: community-acquired; MSSA: methicillin-susceptible S. aureus; MRSA: methicillin-resistant S. aureus; FA-S: fusidic acid-susceptible; FA-R: fusidic acid-resistant; PVL: Panton-Valentine leukocidin; TSST-1: toxic shock syndrome toxin; eta/etb: exfoliatin A and/or B.
*1 MSSA exhibited PVL and TSST-1 genes, 1 MSSA exhibited PVL genes and *eta*
**1 MRSA exhibited PVL and TSST-1 genes

Figure 1: Toxins genes detection, resistance to oxacillin and fusidic acid in the 518 *S. aureus* strains collected during the national survey, January 2020 to March 2021.

5.4. *Demographics and clinical data*

Demographics and clinical data collected during the survey are displayed in **Table 3**. While demographic data were usually populated, clinical information was more often missing. For example, the type of SSTI was missing in 41.3% of report forms.

EEFIC was more frequently isolated from young patients (median age of 9.5 years - **Figure 2**), from lesions originating from the head and neck (41.2%) and from the limbs (35.3%). It was mainly isolated from impetigo (85.7%).

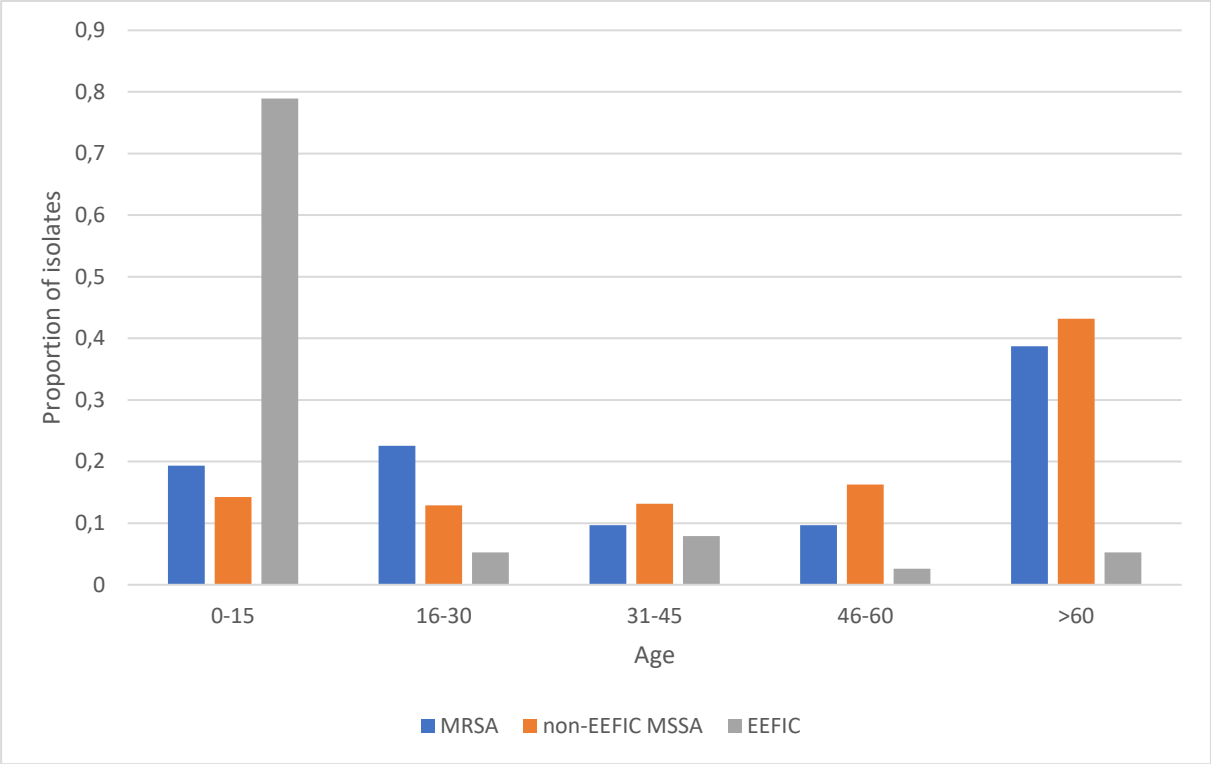
Table 3: Clinical data and demographics of 518 community-onset *S. aureus* skin and soft tissues infections, January 2020 to March 2021

Characteristics	MRSA	Non-EEFIC MSSA	EEFIC
Age (in years)			
Median (range)	43 (1-101)	50 (0-99)	9.5 (1-68)
Mean	47	50	15
Gender			
Female	17 (54.8%)	207 (46.6%)	18 (46.2%)
Male	14 (45.2%)	237 (53.4%)	21 (53.8%)
Infection site			
Head & neck	1 (4.2%)	72 (17.7%)	14 (41.2%)
- Face	1	36	10
- Scalp	-	28	4
- Neck	-	8	-
Trunk	2 (8.3%)	46 (11.3%)	4 (11.8%)
- Thorax	-	21	-
- Abdomen	2	13	2
- Back	-	12	2
Limbs	10 (41.7%)	258 (63.4%)	12 (35.3%)
- Underarm	2	12	1
- Arm	-	31	1
- Hand	1	31	1
- Leg	4	98	6
- Foot	3	86	3
Pelvo-perineum	11 (45.8%)	31 (7.6%)	4 (11.8%)
- Genitals	1	13	-
- Inguinal creases	3	9	-
- Buttock	7	9	4
Number of lesions			
Unique	22 (81.5%)	381 (93.6%)	35 (92.1%)
Multiple	5 (18.5%)	26 (6.4%)	3 (7.9%)
Type of SSTI			
Abscess	11 (61.1%)	85 (33.0%)	-
Infected wound	3 (16.7%)	80 (31.0%)	3 (10.7%)
Infected eczema	-	32 (12.4%)	1 (3.6%)
Impetigo	1 (5.6%)	25 (9.7%)	24 (85.7%)
Cellulitis	1 (5.6%)	20 (7.8%)	-
Furunculosis	2 (11.1%)	15 (5.8%)	-
Epidermolysis bullosa	-	1 (0.4%)	-

EEFIC: epidemic European fusidic acid-resistant impetigo clone; MRSA: methicillin-resistant *S. aureus*; Non-EEFIC MSSA: methicillin-susceptible *S. aureus*; SSTI: skin and soft tissue infection.

In comparison, other MSSAs were isolated from older patients (median age of 54 years). They were isolated from abscesses (33.0%) and infected wounds (31.0%), mainly from the limbs (63.4%).

MRSAs were isolated from older patients as well (median age of 43 years). They were isolated mainly from abscesses (61.1%), the pelvo-perineum (45.8%) and the limbs (41.7%).



EEFIC: epidemic European fusidic acid-resistant impetigo clone ; Non-EEFIC MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*.

Figure 2: Age distribution of patients with a community-onset skin and soft tissues infection due to an EEFIC MSSA as compared to those with a non-EEFIC MSSA or a MRSA, January 2020 to March 2021

5.5. *Geographical and time distribution of the EEFIC*

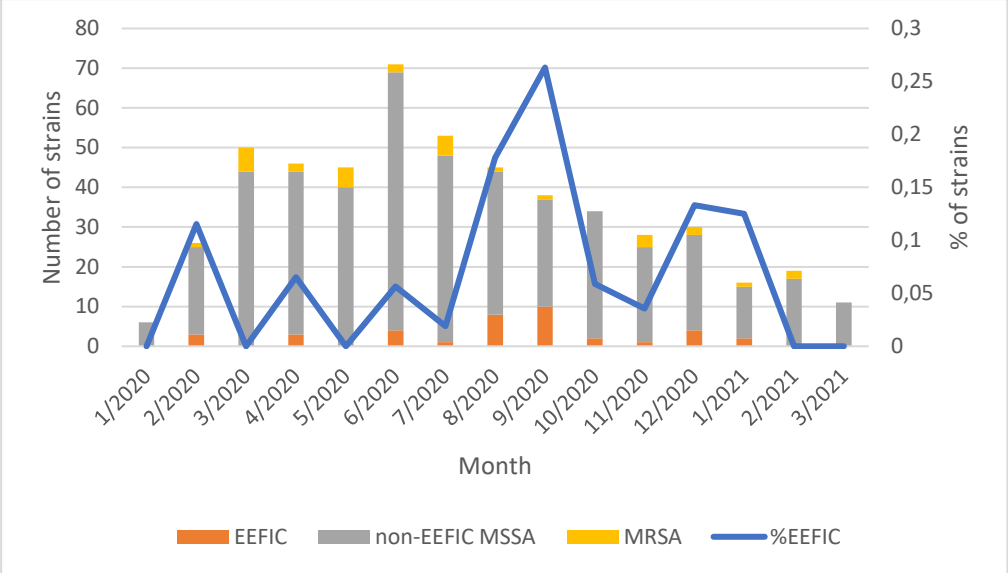
The 38 EEFIC strains were sent by laboratories located in the Flemish region while none were identified from the MSSA sent by the 4 participating laboratories located in Wallonia. Almost half of the cases were originating from the province of Antwerp (**Table 4**).

Table 4: Geographical distribution of EEFIC compared to all MSSA strains received, January 2020 to March 2021

Province	Participating lab	All MSSA	EEFIC	% EEFIC/MSSA
East Flanders	7	160	10	6.3%
West Flanders	5	101	5	5.0%
Limburg	4	76	5	6.6%
Antwerp	5	75	18	24.0%
Hainaut	3	61	-	-
Liege	1	14	-	-
Total	25	487	38	7.8%

EEFIC: epidemic European fusidic acid-resistant impetigo clone ; MSSA: methicillin-susceptible *S. aureus*.

The survey intended to cover a one-year period, which is of importance due to the seasonal character of impetigo clusters. As expected, a peak in the proportion of EEFIC strains collected was observed in the August-September period (**Figure 3**). Unfortunately, and (most probably) due to work overload linked to the COVID-19 pandemic, a drastic drop-out in the participation rate was observed as from September 2020. By October 2020, the participation rate had dropped to 72% and to end with 28% in January 2021. Additionally, 35% of the data regarding the number of skin sample positive for *S. aureus* per lab and per month was also missing over the whole study period. There was no seasonal trend observed in the distribution of *S. aureus* carrying the genes encoding for PVL or TSST-1.



EEFIC: epidemic European fusidic acid-resistant impetigo clone; Non-EEFIC MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*.

Figure 3: Monthly distribution of received *S. aureus* and proportion of EEFIC among them, January 2020 to March 2021

6. Conclusions

This national surveillance was conducted to assess the epidemiology of *S. aureus* causing SSTI in the Belgian community. More specifically, the aims were to determine the proportion of EEFIC and to update data on the proportion of CA-MRSA among them. The survey intended to cover a one-year period, which is of importance due to the seasonal character of impetigo clusters. 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, and in particular the absence of participant laboratories for Brussels. Finally, CO-SSTIs are usually not sampled if not complicated, which leads to a probable overestimation of resistance and toxins rates. Nevertheless, this surveillance has allowed, for the first time, to prospectively collect large nation-wide microbiological data on *S. aureus*-related CO-SSTIs.

The large majority of *S. aureus* strains causing SSTIs in the community remain susceptible to oxacillin (94.0%). Among the 6.0% of CA-MRSA collected, more than half showed the presence of the PVL, suggesting that, in cases of CA-MRSA SSTI, the presence of PVL should always be highly suspected.

Among the 31 MRSA and 487 MSSA strains collected, FA-R was detected in 2 (6.5%) MRSA and in 79 (16.1%) MSSA strains, suggesting that FA-R among CA-*S. aureus* should be closely monitored. FA-R seems to be a good marker for detecting the EEFIC clone, as around half of FA-R MSSA were indeed belonging to this clone.

Thirty-eight (7.8%) EEFIC strains were recovered from the 487 SSTI-causing MSSA strains collected during this survey. The vast majority of EEFIC strains were recovered from impetigo (85.7%), from patients less than 16 years old (79.0%), and from the province of Antwerp (47.4%). A peak was observed during the August-September period with EEFIC being involved in 26.3% of all *S. aureus* collected in September 2020. The vast majority of EEFIC strains remained susceptible to other antimicrobials tested but four strains (10.2%) showed co-resistance to mupirocin (MIC > 1024 µg/ml) and aminoglycosides which can lead to difficulties in topical antibiotic therapy.

In Belgium [2] and in the Netherlands [5], topical fusidic acid is a first-line treatment for impetigo. However, 27 of the 50 impetigo-causing strains studied here were FA-R. In comparison, health authorities in France recommend the use of topical mupirocin only for localized form in addition of hygiene care [6] while the United Kingdom recommends to consider an antiseptic as first line (hydrogen peroxide 1% cream) instead of topical antibiotics [7].

This study showed the persistence of the EEFIC in Belgium, particularly in the paediatric population with a seasonal peak in late summer. This dissemination could be favoured by the wide use of topical fusidic acid in general practice and be responsible for treatment failures. It might be necessary to reconsider the current recommendations for first-line treatment of impetigo accordingly. Further studies focused on impetigo should be undertaken, as impetigo is recognized as an understudied area in terms of both resistance to antibiotics and treatment efficacy [8].

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