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Original Article

Emergence of livestock-associated MRSA isolated from cystic fibrosis patients: Result of a Belgian national survey

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Abstract

Background: This study aims to determine the prevalence and characteristics of *Staphylococcus aureus* in Belgian cystic fibrosis (CF) patients.
Methods: Non-duplicate respiratory samples from 510 CF-patients (2012−2013) were examined. One isolate per patient was analysed unless different phenotypes were recovered. Isolates were investigated for *mecA*/*mecC,* toxins presence, *spa*-typing, MLST and SCC*mec*-typing. Potential livestock-associated (LA) isolates were examined for their immune-evasion-cluster (IEC) genes.
Results: S. aureus (n = 380), including 41 small-colony variants (SCVs), were isolated from 66.7% patients. The prevalence of methicillin-resistant *S. aureus* (MRSA) colonization was 4.9%. Two MRSA isolates carried toxic shock syndrome toxin 1 (TSST-1). Most MRSA (65%) belonged to two nosocomial epidemic clones (CC5, CC8) widespread in Belgium. Methicillin susceptible *S. aureus* (MSSA) showed great genetic diversity. Five of 33 isolates belonging to potential LA-lineages were IEC negative, including three methicillin-resistant isolates, suggesting an animal origin.
Conclusions: The MRSA-prevalence in Belgian CF-patients remained constant (2001−2013), but SCV-prevalence increased. Most MRSA belonged to health-care-associated clones. Three patients carrying LA-MRSA were found, requiring further investigation to determine the risk factors for LA-MRSA acquisition.

Keywords: Small colony variant; *spa*-typing; LA-MRSA; CC398

1. Introduction

Staphylococcus aureus is a major pathogen commonly isolated from the respiratory tracts of patients with cystic fibrosis (CF). This bacterium is one of the first bacteria which colonizes and infects the airways of children with CF. The prevalence of

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methicillin-resistant *S. aureus* (MRSA) in CF populations is increasing worldwide [1]. MRSA presence in CF-patients' airways seems to be associated with accelerated lung function decline and worse survival [2]. In addition to the hospital-associated (HA-) MRSA, community-associated (CA-) MRSA, which often carry the Panton-Valentine leucocidin (PVL), were increasingly reported in CF-patients [3]. Recently, a new reservoir of MRSA [livestock-associated (LA-) MRSA] has been reported in food-producing animals and in humans exposed to animals, but rarely described in CF-patients [4,5]. Once detected in the lungs of CF-patients, MRSA will persist in the airways over years and be repeatedly isolated from sputum samples.

The switch to small-colony-variant phenotype (SCV) is observed in numerous chronic infections. SCVs of *S. aureus* can be distinguished phenotypically from normal *S. aureus* by a small, non-pigmented and non-haemolytic colony morphotype [6]. These thymidine, menadione or hemin-dependant slowly growing isolates can be difficult to detect in clinical cultures and are often missed in routine laboratories. Clinically, SCVs are associated with a higher rate of antibiotic resistance and more advanced lung disease in CF-patients [7].

Although the prevalence of MRSA in CF-patients in the United States is >25%, European CF-centres report a lower MRSA-prevalence (<10%) [8,9]. In Belgium, a nationwide study conducted in 2001 showed the prevalence of MRSA-colonization to be 5% in CF-patients (attending nine CF-centres) [10]. Most of the MRSA-strains identified in that survey belonged to predominant Belgian HA-clones. The prevalence of SCV-colonization was 4%, but the recovery of SCVs was only reported in three out of the nine participating centres. In this multicentre study, we updated the prevalence of *S. aureus* and MRSA colonization in Belgian CF-patients; and we characterized the phenotype (SCV versus normal colony phenotype) and clonal distribution of the staphylococcal strains.

2. Methods

2.1. Study design

A multicentre prospective study was conducted during a two-year period (2012–2013) in CF-patients attending four CF-centres (centres 2 to 5) and one rehabilitation CF-centre (centre 1). Samples were collected on each patient's visit for routine microbiological analysis. Sputum samples or deep throat swabs for patient too young or unable to expectorate were cultured on blood agar and selective media for *S. aureus* including mannitol salt or chromogenic agar in the participating laboratories as recommended [11]. All different colony types morphologically consistent with either the normal-colony-phenotype or SCV were subjected to species identification. The collected *S. aureus* isolates were sent to the coordinating laboratory; the National Reference Centre (NRC) for Staphylococci. One *S. aureus* isolate per patient was further analysed unless different phenotypes, including morphological aspect (normal or SCV) or antibiotic resistance profile [MRSA vs methicillin susceptible *S. aureus* (MSSA)] were isolated from a single respiratory sample. Participating laboratories registered the total number of cultures performed per patient and the number of patients for which at least one respiratory culture was obtained during the study period.

2.2. Characterization of isolates and methicillin resistance

S. aureus identification was confirmed using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). SCVs were defined as small greyish or non-pigmented colonies, non-haemolytic and slowly growing on blood agar plates [12]. Methicillin susceptibility was tested by Vitek 2 (bioMérieux, Lyon, France) on isolates showing normal-colony-phenotype according to EUCAST [W1]. Minimal inhibitory concentrations (MICs) to oxacillin and cefoxitin were determined by *E*-test (bioMérieux) for SCVs. Isolates were investigated by PCR for the presence of 16S rRNA, *mecA*, *mecC* and *nuc* genes [13]. Borderline oxacillin resistant *S. aureus* (BORSA) were defined as *mecA*/*mecC* negative isolates showing oxacillin/cefoxitin resistance.

2.3. Antimicrobial susceptibility testing and detection of resistance genes

For the normal-colony-phenotype *S. aureus*, antimicrobial susceptibility was determined by using Vitek2 ASP-P633 (bioMérieux). For the SCV-strains, resistance was tested by disk diffusion and by *E*-test (bioMérieux) for glycopeptides on Mueller-Hinton (MH) agar supplemented with 5% sheep blood (Bio-Rad, Marnes la Coquette, France). For SCVs unable to grow on the enriched media, antimicrobial susceptibility was tested on Schaedler Agar with Vitamin K 1 and 5% Sheep Blood (Becton-Dickinson, Heidelberg, Germany). Linezolid and mupirocin MICs were determined on resistant strains by *E*-test. The presence of *cfr* and *mupA* genes was determined on strains resistant to linezolid or mupirocin, respectively [14,15]. EUCAST breakpoints were used for interpretation [W1].

2.4. Molecular typing and presence of virulence determinants

Molecular typing and assessment of genetic relatedness were performed by *spa*-typing with the Ridom StaphType software [W2] [13]. Isolates were grouped into *spa* clonal complexes (*spa*-CC) using the Based Upon Repeat Pattern (BURP) algorithm using default parameters. A subset of representative *spa*-types from each *spa*-CC (n = 14) were further analysed by Multilocus Sequence Typing (MLST) [W3]. The most prevalent *spa*-type in each *spa*-CC was chosen for MLST analysis. Assignment of *S. aureus* lineages [clonal complexes (CCs)] was based on the combination of *spa*-CCs, *spa*-types and MLST results. For MRSA isolates, SCC*mec* typing was performed [13].

The toxin profile was characterized by PCR for genes encoding toxic shock syndrome toxin (TSST-1) and PVL [13]. The presence of the prophage carrying the immune-evasion cluster (IEC) genes [*sak*, *scn*, *chp*] was determined for isolates belonging to potential LA-lineages (CC9, CC97 and CC398) to determine whether they may have human or animal origin [13].

Table 1
Prevalence of *S. aureus* in CF-patients.

Centre	Number of CF patients with culture	Median age (range in years)	Number of CF patients (%)		
			S. aureus[a]	MRSA	SCV
1	53	16.3 (6–36)	40 (75.5)	0 (0)	3 (5.7)
2	63	9.7 (0–10)	39 (61.9)	3 (4.8)	4 (6.3)
3	60	31 (12–70)	45 (75)	9 (15)	9 (15)
4	162	17.8 (0–51)	81 (50)	7 (4.3)	10 (6.2)
5	172	18.1 (0–52)	135 (78.5)	6 (3.5)	10 (5.8)
Total	510		340 (66.7)	25 (4.9)	36 (7.1)

[a] Number of patients positive for *S. aureus* (including both MRSA and MSSA, normal-colony and SCV phenotype).

2.5. Data collection and statistical analysis

Demographic information was collected with a case report for: age, gender, zip code. The Pearson χ^2 and Fisher's exact test ($p \leq 0.05$) were used for statistical analysis using the R-commander software [W4]. The Mann-Whitney test was used for median comparison (non-Gaussian distribution) using the Graphpad software [W5].

2.6. Ethical consideration

The study protocol was approved by the different institutional ethical committees. A written informed consent was signed by each participant (or both parents for minor children under 16 years old) after explanation of the objectives, relevance and methods of the study by the physician.

3. Results

3.1. Characteristics of patients and S. aureus prevalence

A total of 510 CF-patients was screened for the presence of *S. aureus* in respiratory samples. *S. aureus* was isolated from 340 patients with a mean age of 20 years (ranging from 1 month to 70 years), 185 (54.4%) patients were male (Table 1). Of the 340 respiratory specimens, 80 were deep throat swab samples and 260 were sputum samples. The prevalence of *S. aureus* colonization ranged from 50% to 78.5% by centre (Fig. 1; Table 1). A single strain was characterized in 305 patients and multiple strains with distinct morpho- and/or resistance-types ($n = 75$) were analysed from 35 patients.

3.2. SCV prevalence and characterization

S. aureus SCV ($n = 41$) isolates were recovered from 36 CF-patients. Out of these 36 patients, 20 had only SCV-phenotype and 16 had both SCV and normal-colony-phenotypes (Table 2). When sub-cultured at the NRC, 14 SCVs found by the participating laboratories reverted their SCV-phenotype, and 13, not reported by participating laboratories, were classified as SCVs by the NRC. The SCV-phenotype was more prevalent in one centre where 15% of patients were colonized with at least one *S. aureus* SCV-strain. Similarly, in this centre, the proportion of MRSA among SCVs was greater (50%). The median age of patients carrying SCVs was 19.5 years (ranging from 3 to 53), compared to 17 years (ranging from 0 to 70 years) for patients with normal-colony-phenotype *S. aureus* ($p = 0.09$) (Fig. S1).

3.3. MRSA prevalence

Twenty-nine *S. aureus*, from 25 patients, were MRSA (*mecA*-positive). Among these isolates, six MRSA strains exhibited

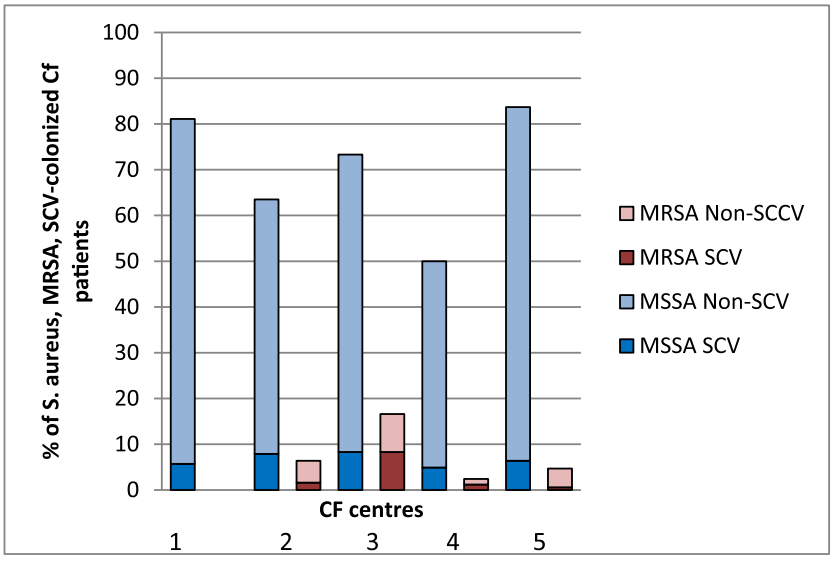

Fig. 1. Patients colonized with MRSA, SCV-MRSA, MSSA, SCV-MSSA in relation of total number of patients tested per centre. The centre 1 corresponds to the rehabilitation centre, while the four CF-centres are numbered for 2 to 5.

susceptibility to oxacillin (MICs 0.25–1 mg/L) and low to middle level resistance to cefoxitin (MICs 4–24 mg/L). This hetero-resistance was observed for four SCV-MRSA, and two non-SCV MRSA. Three strains (including one SCV and two non-SCVs) showing cefoxitin/methicillin resistance not linked to *mec*-genes, were considered BORSA. The MRSA-prevalence rate in CF-patients was 4.9% ranging from 0% to 15% by centres ($p = 0.007$) (Table 1, Fig. 1). The proportion of MRSA among *S. aureus* was significantly different in the five centres (0% to 20%) ($p = 0.005$). The median age of MRSA-colonized patients was significantly higher than MSSA-colonized patients (23 versus 17 years, respectively; $p = 0.003$) (Fig. S1). Out of the 41 SCV-isolates, nine were SCV-MRSA (22%). The proportion of MRSA was higher in the SCV-phenotype than in the normal-colony-phenotype (5.9%) The proportion of SCV-phenotype among MRSA was 31%.

3.4. Antimicrobial resistance

Antimicrobial resistance profiles are shown in Table 3. All isolates were susceptible to glycopeptides. MRSA strains were remarkably resistant to tobramycin-kanamycin, erythromycin-clindamycin and ciprofloxacin. MSSA strains were mainly erythromycin-clindamycin resistant. All MRSA strains were susceptible to linezolid, chloramphenicol and mupirocin; while few MSSA isolates were resistant to these antimicrobials. Two MSSA strains were resistant to linezolid (MIC = 6 mg/L) but did not harbour the *cfr* gene. Three MSSA strains showed low-level resistance to mupirocin ($n = 2$; MICs 64 and 192 mg/L) or high-level resistance ($n = 1$; MIC>1024 mg/L, *mupA*-positive). More than a half (51.1%) of SCV-phenotype strains were resistant to co-trimoxazole ($p < 0.001$). Nineteen percent of SCVs were gentamicin resistant, while only 5.6% of normal-colony-phenotype isolates were gentamicin resistant.

3.5. Molecular typing and virulence profile

The 379 *S. aureus* isolates showed a total of 159 *spa*-types corresponding to 14 *spa*-CC and 22 singletons (Table 4). One MSSA was non-typeable by *spa*-typing. Fifteen *spa*-types were recovered among the 29 MRSA. SCC*mec*–typing showed that MRSA carried SCC*mec* IV ($n = 22$), I ($n = 3$), II (n = 3) and V ($n = 1$). Most MRSA (89.6%) were classified into nine clonal lineages: CC5 SCC*mec* I, CC5 SCC*mec* II, CC5 SCC*mec* IV, CC8 SCC*mec* I, CC8 SCC*mec* IV, CC30 SCC*mec* V, CC45 SCC*mec* II, CC45 SCC*mec* IV, CC398 SCC*mec* IV. The three remaining MRSA were classified into a singleton (t237) or excluded from clustering (t3022). The two most prevalent MRSA clones (58.6%) were CC8-SCC*mec* IV ($n = 13$) and CC398-SCC*mec* IV ($n = 4$). The *spa*-typing of MSSA revealed high diversity (151 *spa*-types), most being distributed in eleven lineages. Thirty-three strains belonged to potential LA-lineages: CC398 (four MRSA and 16 MSSA), CC97 (nine MSSA) and CC9 (four MSSA). Within these 33 isolates, all but one MRSA were IEC-negative, suggesting an animal origin. Conversely, all but two MSSA carried two/three IEC-genes, suggesting a human origin.

Table 2
Molecular typing of SCV-positive isolates.

Patient	SCV *S. aureus* phenotype		Normal-colony *S. aureus* phenotype[a]
	MSSA CC/*spa-type* (n)	MRSA CC/*spa-type*/SCC*mec* (n)	MSSA CC/*spa-type* (n)
1	CC5/t002 (1)	–	–
2	CC5/t2049 (1)	–	–
3	–	CC8/t14009/I (1)	–
4	CC30/t710 (1)	–	CC30/t710 (1)
5	ND/t5314 (1)	–	–
6	CC15/t084 (1)	–	CC15/t084 (1)
7	CC45/t13455 (1)	–	–
8	–	CC398/t011/IV[d] (1)	–
9	CC30/t166 (1)	–	CC30/t166 (1)
10	–	CC5/t1399/IV (1)	–
11	CC15/t289 (1)	–	CC15/t289 (1)
12	–	CC45/t655/IV[e] (1)	–
13	CC97/t4955 (1)	–	–
14	CC8/t008 (1)	–	CC8/t008 (1)
15	CC5/t002 (1)	–	CC5/t002 (1)
16	–	CC45/t038/II (1)	–
17	–	CC8/t008/IV[f] (2)	–
18	CC15/t289 (1)	–	–
19	CC30/t14078 (1)	–	CC30/t14078(1), CC5/t002 (1)
20	CC30/t12911 (1)	–	–
21	CC30/t021 (1)	–	CC30/t021 (1)
22	CC30/t14078 (1)	–	–
23	CC45/t14010[b](2)	–	–
24	CC45/t050[c] (2)	–	–
25	CC5/t002 (1)	–	–
26	–	CC8/t008/IV (1)	–
27	CC45/t015 (1)	–	CC9/t209 (1)
28	CC30/t9489 (1), CC30/t14498 (1)	–	CC30/t9489 (1)
29	CC5/t539 (1)	–	–
30	CC15/t091 (1)	–	ND/t14533 (1)
31	CC1/t127 (1)	–	CC1/t127 (1)
32	CC398/t10686 (1)	CC398/t011/IV (1)	–
33	CC15/t14257 (1)	–	CC15/t094 (1)
34	CC5/t688 (1)	–	CC5/t688[g] (2)
35	ND/t587 (1)	–	ND/t587 (1)
36	CC1/t189 (1)	–	CC45/t230 (1)

N, number of isolates. ND, not done; −, absence of isolate.

[a] MRSA isolates with normal-colony phenotype were not isolated in these patients.

[b] The only difference between the two SCV-MSSA isolates was the cotrimoxazol susceptibility profile (one isolate susceptible and the other resistant).

[c] Both isolates have the same antimicrobial resistance profile. Only one isolate was determined as SCV by the participating centre, but both was defined as SCV by NRC.

[d] Susceptible to oxacillin (MIC = 0.25 mg/L) and cefoxitin (MIC = 4 mg/L), but *mec*A-positive.

[e] Susceptible to oxacillin (MIC = 0.25 mg/L), resistant to cefoxitin (MIC = 6 mg/L), *mec*A-positive.

[f] One of these two SCV-MRSA isolates was susceptible to oxacillin (MIC = 0.125 mg/L), resistant to cefoxitin (MIC = 8 mg/L) and *mec*A-positive.

[g] Both isolates have the same antimicrobial resistance profile except for penicillin (tested by the participating centre, but not by the NRC).

Out of the 16 patients colonized by SCV and normal-colony-phenotype, 12 (75%) had SCV clonally related with normal-colony *S. aureus* (Table 2). Out of these 12 patients, six showed the same antibiotic resistance profile and four had only discrepancy in

Table 3
Antimicrobial resistance profiles of 380 *S. aureus* isolates from 340 CF-patients determined by Vitek2 or disk diffusion method.

Antimicrobial	Percent of isolates (n)					
	MRSA (n = 29)			MSSA (n = 351)		
	S	I	R	S	I	R
Oxacillin						
Gentamicin	83.0 (24)	–	17.0 (5)	94.0 (330)	–	6.0 (21)
Tobramycin	20.7 (6)	–	79.3 (23)	88.0 (309)	–	12.0 (42)
Amikacin [a]	20.7 (6)	–	79.3 (23)	85.2 (299)	0.3 (1)	14.5 (51)
Ciprofloxacin	27.6 (8 [b])	–	72.4 (21)	27.6 (97 [c])	–	15.4 (54)
Co-trimoxazole	62.1 (18)	13.8 (4)	24.1 (7)	94.0 (330)	0.9 (3)	5.1 (18)
Erythromycin	13.8 (4)	–	86.2 (25)	44.7 (157)	0.3 (1)	55.0 (193)
Clindamycin	13.8 (4)	–	86.2 (25)	54.1 (190)	–	45.9 (161)
Tetracycline	65.5 (19)	6.9 (2)	27.6 (8)	92.6 (325)	2.3 (8)	5.1 (18)
Linezolid	100 (29)	–	–	99.4 (349 [d])	–	0.6 (2)
Fusidic acid	89.7 (26)	–	10.3 (3)	97.2 (341)	–	2.8 (10)
Mupirocin	100 (29)	–	–	99.1 (348)	0.6 (2)	0.3 (1 [e])
Rifampicin	79.3 (23)	3.5 (1)	17.2 (5)	97.7 (343)	0.3 (1)	2.0 (7)
Chloramphenicol	100 (29)	–	–	96.3 (338)	–	3.7 (13)
Vancomycine	100 (29)	–	–	100 (351)	–	–
Teicoplanine	100 (29)	–	–	100 (351)	–	–

S, susceptible; I, intermediate; R, resistant.
[a] Resistance to amikacin was determined by testing kanamycin (resistant if MIC >8 mg/L).
[b] Two strains with MIC = 1 mg/L.
[c] Forty-three strains with MIC = 1 mg/L.
[d] Five strains were classified as resistant by the Vitek2. According to *E*-test, only one strain was resistant (MIC = 6 mg/L).
[e] This strain was *mup*A-positive.

resistance to co-trimoxazole for the SCV-phenotype. One patient had closely related *spa*-types for normal-colony and SCV-phenotypes. Finally, three patients harboured different clones.

Most strains were negative for PVL and TSST-1. Only one PVL-positive MSSA was detected. Two MRSA and 33 MSSA harboured the TSST-1 gene (Table 4).

Table 4
Molecular typing of *S. aureus* isolates (n = 380) from CF-patients (n = 340).

Isolates (n)	CC/ST (n) [a]	Number of *spa*-types [b]	SCC*mec* type (n)	Toxins genes (n)	IEC-profile (n)
mecA-positive (29)	CC5/ST5 (4)	4 (t214, t539, t744, t1399)	I (1), II (2), IV (1)	TSST-1 (2)	ND
	CC8/ST8 (15)	5 (t008, t304, t121, t051, t14009)	I (2), IV (13)	–	ND
	CC30/ST30 (1)	1 (t021)	V (1)	–	ND
	CC45/ST45 (2)	2 (t038, t655)	II (1), IV (1)	–	ND
	CC398/ST398 (4)	4 (t011)	IV (4)	–	*sak-scn* (1), − (3)
	ND (3)	2 (t237 t3022)	IV (3)	–	ND
mecA-negative (351)	CC5/ST5 (89)	19 (t002, t688, t062)	ND	–	ND
	CC45/ST45 (41)	21 (t015, t031, t050, t095, t230)	ND	–	ND
	CC30/ST30 (33)	16 (t012, t021)	ND	TSST-1 (18)	ND
	CC30/ST34 (14)	7 (t166, t136, t9489)	ND	TSST-1 (10)	ND
	CC15/ST15 (28)	13 (t084, t346)	ND	TSST-1 (1), PVL (1)	ND
	CC7/ST7 (19)	3 (t091, t289)	ND	–	ND
	CC398/ST398 (16)	10 (t571, t1451, t6587)	ND	–	*scn-chp* (15), − (1)
	CC8/ST8 (16)	6 (t008)	ND	–	ND
	CC1/ST3 (13)	4 (t127, t177)	ND	–	ND
	CC1/ST188 (3)	1 (t189)	ND	–	ND
	CC97/ST97 (9)	5 (t267)	ND	–	*sak-scn* (9)
	CC9/ST109 (4)	1 (t209)	ND	–	*sak-scn-chp* (3), − (1)
	CC121/ST3647 (12)	5 (t645, t159)	ND	–	ND
	ND (54)	41 [c]	ND	TSST-1 (4)	ND

N, number of isolates; ND, not done; (−), absence of positive isolates.
[a] Based on *spa*-CCs and MLST results.
[b] The most prevalent *spa*-types are given in parentheses.
[c] Including *spa*-types grouped in *spa*-CCs without founder, singletons, excluded at BURP analysis, or non-typeable.

4. Discussion

The prevalence of *S. aureus* carriage in CF-patients in Belgium increased from 44% in 2001 to 66.7% in 2012–2013. A better follow-up of laboratory recommendations may explain the improved detection of *S. aureus* in the different centres [W6]. The microbiological workup of respiratory samples from CF-patients remains a challenge, particularly for the recovery of SCVs. As the presence of SCVs is directly related to a poor clinical outcome [16], searching for SCVs is recommended. This monitoring requires the implementation of adapted culture methods in routine diagnostics. In 2001, Vergison et al. [10] reported a 4% prevalence of *S. aureus* SCVs in Belgian CF-patients, but only three out of nine centres involved in the study were actively searching for SCVs. SCV-prevalence in the present study is higher (~11%), but similar to previous reports from other European countries [7,17]. Khal et al. [18] found SCVs at a higher prevalence (33%) among German CF-patients. The increased prevalence of SCVs in Belgian CF-patients may be explained by the fact that all but one of the centres was reporting the presence of SCV-phenotype in respiratory samples from CF-patients. The high proportion of methicillin resistance among SCVs underlines the importance of correct identification of these isolates both for treatment and implementation of infection control procedure. Among positive cultures for SCVs, 55.6% were negative for normal-colony isolates, indicating that no *S. aureus* would have been detected without SCV-surveillance. Forty-four percent of subjects colonized with SCV were also positive for normal-colony *S. aureus*. In most cases (81%), the SCV isolate was clonally related to normal-colony isolate, suggesting that SCVs were generally selected in-vivo rather than transmitted between subjects. Patients with SCVs were not significantly older than patients with only normal-colony *S. aureus* ($p > 0.05$) (Fig. S1). However, the emergence of SCVs has been related with advanced age, and the reason might be the increased consumption of antibiotics with increasing age [7,10].

Reversion to normal-colony-phenotype was observed in 50% of the SCV-strains detected by the participating centres. This phenotype switch has been observed in several in vivo and in vitro models of persistent infection [19]. Revertants might appear susceptible to antibiotics, making it difficult to define optimal therapy for infections due to *S. aureus* SCVs. Moreover, few SCVs were BORSA. The clinical impact of BORSA on CF-patients should be further investigated.

The MRSA-prevalence in respiratory cultures of CF-patients has increased over the past decade, ranging up to 25.9% in the USA [W7]. Nevertheless in Europe it stays below 10% [9,20] and in Belgian CF patients it remained low (5%) and constant from 2001 to 2013. However, a significant shift in the distribution of MRSA clones in Belgium was observed. This could be reflected by the major changes observed in Belgian hospitals in the last two decades with sequential replacement of MRSA lineages: over 15 years the "Iberian MRSA clone CC8/ST247-I" was replaced in the 90s by the CC45-IV clone, itself gradually replaced by MRSA CC8-IV [21]. The MRSA epidemiology in the CF-population evolved according to the epidemiology in Belgian hospitals. However, MRSA isolated in the CF-population harboured more diverse types of SCC*mec* (I, II, IV and V) compared to MRSA in the general population (mainly IV). A wide variation of MRSA clones by centre was observed, partly explained by centre-specific population characteristics ($p = 0.007$). In fact, some centres only take care of infants while others manage older children and adults. MRSA carriers cannot attend the rehabilitation centre. The centre with the highest MRSA-prevalence was the centre with the oldest CF-population. Older patients have an increased risk of carrying MRSA, explained by the increased likelihood of hospitalisation and the requirement for antimicrobial-treatment [10]. Most MRSA in this study belonged to one of the epidemic clones disseminated in Belgian hospitals, suggesting that the health-care system was the main source of contamination. Variability in prevalence by centres might also be influenced by local hospital policies (segregation procedures, barrier nursing, eradication protocols…) and geographic differences in antibiotic resistance. Since 2003, the implementation of more stringent infection prevention and control strategies to control nosocomial MRSA transmission has significantly reduced the MRSA-prevalence in Belgian hospitals [W8]. However, the same trend was not observed in the CF-population, mainly colonized by HA-MRSA. Indeed, eradication of persistent MRSA in CF-patients is more difficult than in the non CF-population. The sustained presence of MRSA in CF-patients respiratory tract despite intermittent antibiotic therapy supports this theory [22]. Although protocols for eradicating MRSA from CF-lungs provide interesting results [8,23], it is more essential to focus on the prevention of acquisition in order to reduce MRSA incidence in CF-patients and to maintain a low MRSA-prevalence in CF-clinics. Moreover, the improvement of culture procedures for a better detection of normal-colony and SCV *S. aureus* may explain the stable MRSA-prevalence in CF-Belgian patients over the last 12 years despite the reduced prevalence of MRSA in Belgian hospitals.

Besides HA-MRSA, the molecular-typing revealed that 12% of MRSA carriers were colonized by LA-MRSA CC398. These three LA-MRSA CC398 isolates, as well as one LA-MSSA CC398, were tetracycline resistant. The absence of IEC-genes, together with the presence of *mecA* and tetracycline resistance, are markers of the animal population as previously established [24]. The three patients were living in Flanders where pig density farming is the highest of the whole country. One was living in a farm, the second had contacts with animals (horse and dogs) and the third one had contact with camels during holidays. Surprisingly, this observation was not correlated with the last epidemiological data on MRSA-carriage among Belgian hospital. Indeed, in 2008, LA-MRSA ST398 frequency was low (<1%) [25]. To our knowledge, this is one of the first reports showing the presence of LA-MRSA in a European CF-population. In two CF-centres in Münster, a 4.8% LA-MRSA prevalence among CF-patients was reported [5]. A case of a CF-patient colonized with a MRSA-ST398 was also reported in Brazil [4]. The emergence of LA-MRSA in CF-population is alarming, and suggests that contact with farming animals and farmworkers could represent new risk factors for CF-patients. Indeed, private farm visits or contact with persons directly exposed to livestock may increase the risk for LA-MRSA

acquisition [26]. This is particularly worrisome for countries with high level of LA-MRSA like The Netherlands, Germany or Denmark [27].

Contrary to the observation made in CF-patients in America, no PVL-positive MRSA was isolated in Belgian CF-patients [28,29]. This is consistent with studies from Spain and Italy that did not report PVL-positive MRSA [30,31].

Half (52%) of the MSSA clustered within the same CCs as the five MRSA-clones found in CF-patients: CC5, CC8, CC30, CC45, CC398. Potential LA-linages (CC9, CC97, CC398) were also observed among MSSA-strains, although most carried IEC-genes indicating a human origin.

In conclusion, we observed a similar prevalence of MSSA and MRSA colonization in CF-patients treated in specialized Belgian centres to that reported elsewhere in Europe. The MRSA-prevalence in Belgian CF-patients remained constantly low from 2001 to 2013 (~5%). We observed a shift in the genotype distribution among MRSA with the replacement of the Iberian MRSA CC8/ST247-SCC*mec* I by the MRSA CC8-SCC*mec* IV. The prevalence of *S. aureus* SCV increased, suggesting an improvement of microbial detection procedures. In contrast with studies conducted in America showing high prevalence of CA-lineages, most MRSA belonged to HA-clones. However, we have witnessed the presence of LA-MRSA in the Belgian CF-population. Careful surveillance is essential for monitoring trends in the evolving epidemiology and the management of *S. aureus* including MRSA and SCV in the CF-population.

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