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ORIGINAL RESEARCH

Emergence and spread of worldwide *Staphylococcus aureus* clones among cystic fibrosis patients

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Background: The aim of this study was to assess the relatedness of molecular types of *Staphylococcus aureus* isolates colonizing cystic fibrosis (CF) patients with their antimicrobial resistance and prevalence of toxin genes.

Methods: A total of 215 isolates from the airways of 107 patients with CF were tested for *spa* and SCC*mec* type, antimicrobial resistance and carriage of toxin genes.

Results: t015, t084, t091, t700 and t002 were the largest group (approximately 25%) among all 69 identified spa types. Five new spa types, t14286, t14287, t14288, t14289 and t14290, were identified and registered. Isolates from CF patients were clustered into 11 multi-locus sequence typing clonal complexes, with CC30, CC22, CC97, CC45, CC15 and CC5 being the most frequent ones. Twelve (5.6%) methicillin-resistant *S. aureus* (MRSA) isolates and 102 (47.7%) multidrug-resistant isolates were identified, along with three SCC*mec* types (I, III and V). All isolates (both MRSA and methicillin-sensitive *S. aureus*) were Panton–Valentine leucocidin-negative, and 56.7% harbored egc genes. This was the first study documenting the presence of ST398-V-t571 livestock-associated MRSA in a European patient with CF.

Conclusion: These findings imply that individuals with CF can also be colonized with animal-related ST398 MRSA, and justify constant monitoring of staphylococcal colonization and identification of epidemic *S. aureus* clones in this group.

Keywords: *Staphylococcus aureus*, cystic fibrosis, ST398 MRSA, Panton–Valentine leukocidin, *spa* typing, MRSA

Introduction

Staphylococcus aureus is a primary pathogen colonizing the airways and causing respiratory infections in cystic fibrosis (CF) patients. It is isolated in approximately one-third of the patients, usually shortly after diagnosis. In some cases, staphylococcal colonization persists till the end of patient's life. Respiratory colonization with *S. aureus* strains is common during the first decade of life and plays a vital role in morbidity and mortality in most CF patients. *S. aureus* strains may affect the respiratory function of the lungs in CF patients, which is associated with a high risk of life-threatening infections.[1]

The growing prevalence of *S. aureus* in patients with CF observed recently in Europe and the US may result from an increase in the number of isolates resistant to antimicrobial agents, in particular methicillin-resistant *S. aureus* (MRSA).[2] Epidemiological surveillance of staphylococcal colonization is essential to reduce the incidence of epidemic *S. aureus* clones and to prevent the spread thereof. Molecular typing is a very helpful and important instrument that can be used to study the relatedness, genetic diversity and clonal distribution of *S. aureus* isolates. Although a number of various

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techniques have been used to identify *S. aureus* strains, *spa* typing and multi-locus sequence typing (MLST) are the most popular methods for the epidemiological analysis of staphylococcal isolates from different sources.[3–6] Available evidence suggests that the results of *spa* typing correlate strongly with the outcomes of clonal grouping obtained with other methods.[7]

The incidence of staphylococcal colonization in CF patients is still poorly recognized, and only few previous studies have analyzed the clonal structure of *S. aureus* strains isolated from this group of subjects.

The aim of this study was to assess the relatedness of molecular types of *S. aureus* isolates colonizing CF patients with their antimicrobial resistance and prevalence of toxin genes.

Materials and methods

Bacterial isolates

The study included material from 107 CF patients (age 1 month to 47 years) treated at the Outpatient Cystic Fibrosis Clinic at "Polanki" Children Hospital in Gdansk between 2012 and 2014. The number of collected swabs varied depending on the course of the treatment, from at least one per patient to two or even five. Collection of swabs for laboratory testing was a part of routine clinical practices. A total of 215 *S. aureus* isolates from throat swabs, sputum and bronchoalveolar lavage were studied. Throat swabs were obtained after provoking a cough (deep swabs). The material was subcultured onto Columbia blood agar and incubated at 35°C for 24 hours. Suspected staphylococcal isolates were identified on the basis of colony characteristics, pigment production, Gram staining, hemolysis and Staphyslide agglutination test (BioMérieux, Marcy-l'Étoile, France). Additionally, all strains identified as *S. aureus* were examined for the presence of species-specific thermostable nuclease gene (*nuc*SA), as described by Baron et al.[8] The isolates were stored at −80°C in trypticase soy broth (Oxoid, Basingstoke, UK) supplemented with 15% glycerol.

spa typing

spa typing was performed according to Harmsen et al.[9] Nucleotide sequencing of the repeat-containing region of the *spa* gene was conducted on both DNA strands of the PCR product by GenoMed (St Louis, MO, USA), using BigDye Terminator Ready Reaction Cycle Sequencing Kit. The *spa* types were identified with Ridom StaphType software v.2.1.1 (Ridom GmbH, Münster, Germany),[9] and grouped using BURP, Ridom StaphType software. *spa* types were clustered if the cost between members of a given group was less than or equal to four. The BURP algorithm was used to assign *spa* types into *spa* clonal complexes (*spa*-CCs) with defined default parameters "exclude *spa* types shorter than five repeats" and "cluster *spa* types into the same group if cost distances are less than four".[10] Since the results of *spa* typing and MLST are highly concordant,[7] the *spa* typing data could be easily mapped on the MLST types using SpaServer database (http://spaserver.ridom.de/).

Preparation of bacterial DNA

Total DNA of *S. aureus* isolates was purified using Genomic Mini DNA Kit (A&A Biotechnology, Gdynia, Poland), in line with the manufacturer's instructions.

Detection of methicillin resistance and determination of SCCmec cassette type

The isolates were screened for their resistance to oxacillin on the basis of growth of blue colonies in the selective medium (ORSAB; Oxoid). Suspected MRSA isolates were further examined for the presence of *S. aureus mec*A gene, as described elsewhere.[11] Each PCR contained *mec*A-positive (*S. aureus* ATCC 43300) and *mec*A-negative (*S. aureus* ATCC 29213) strains as controls. All *mec*A-negative *S. aureus* isolates able to grow on ORSA plates were tested for the carriage of *mec*C gene using primers described by Cuny et al.[12] Typing of the staphylococcal chromosomal cassette *mec* (SCC*mec*) was carried out as described previously by Milheirico et al.[13] The PCR products were electrophoretically resolved in 1.5% agarose gel containing 0.5 μg/mL ethidium bromide.

Antimicrobial resistance

The resistance of *S. aureus* isolates to antimicrobial agents was determined by disk diffusion and interpreted according to the CLSI document no. M02-A11.[14] The following drugs were used for the test: penicillin, erythromycin, azithromycin, roxithromycin, clindamycin, lincomycin, ciprofloxacin, ofloxacin, levofloxacin, tetracycline, amikacin, tobramycin, netilmicin, gentamicin, fusidic acid, sulfamethoxazole/trimethoprim, chloramphenicol, vancomycin (all from Becton Dickinson, Franklin Lakes, NJ, USA) and mupirocin (Oxoid). Multidrug resistance was defined as resistance to antimicrobial agents from at least three various classes. For isolates identified as resistant to erythromycin but susceptible to clindamycin, D-test was performed to detect inducible clindamycin resistance. Minimal inhibitory concentration (MIC) for vancomycin was determined by E-tests, in line with the manufacturer's instructions (AB Biodisk, Solna, Sweden).

Detection of toxin genes

Genes for enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seu*), exfoliative toxins (*eta*, *etb*), toxic shock syndrome toxin-1 (*tst*) and Panton–Valentine leukocidin (*lukS*-PV/*lukF*-PV) were detected by means of multiplex PCR, as described elsewhere.[15]

Statistical analysis

Distributions of the study variables were presented as numbers and percentages, and compared between groups with Pearson's chi-squared test or Fisher's exact test. All calculations were carried out with Statistica 10 software (StatSoft, Tulsa, OK, USA), with the threshold of statistical significance set at $p<0.001$.

Results

spa typing

The 215 *S. aureus* isolates from CF patients represented 69 *spa* types, primarily t015 (n=14, 6.5%), t084 (n=11, 5.1%), t091 (n=11, 5.1%), t700 (n=11, 5.1%) and t002 (n=8, 3.7%), which constituted approximately one-fourth of all examined isolates (n=55, 25.6%). Only one isolate was *spa* non-typeable. The examined material included five new *spa* types: t14286 (n=2), t14287 (n=1), t14288 (n=2), t14289 (n=5) and t14290 (n=1); all of them had been registered in the international database, Ridom SpaServer (http://spaserver.ridom.de/) (Table 1). MRSA isolates were assigned to four *spa* types: t2029, t073, t151 and t571.

The identified *spa* types were clustered into 11 *spa*-CCs by BURP repeat analysis. Ten isolates (4.7%) represented *spa* types that were excluded from BURP cluster analysis due to the presence of less than five *spa* repeats. Another 43 isolates (20%) belonged to *spa* types with a repeat pattern not associated with other detected staphylococci (i.e. singletons). The majority of isolates represented five clusters, namely *spa*-CC 021 belonging to ST-CC30, *spa*-CC 005 belonging to ST-CC22, *spa*-CC 267/359 belonging to ST-CC97, *spa*-CC 065 belonging to ST-CC45 and *spa*-CC 4096/091 belonging to ST-CC15 and ST-CC7. The newly described *spa* types were distributed across various clusters and singletons (Figure 1; Table 1).

Antimicrobial resistance

The examined isolates showed resistance to penicillin (83.3%), azithromycin (50.7%), erythromycin (49.8%), roxithromycin (49.8%), clindamycin (43.7%), lincomycin (43.7%), ciprofloxacin (13%), ofloxacin (13%), levofloxacin (9.3%), tetracycline (7.9%), amikacin (8.8%), tobramycin (6.1%), netilmicin (6.1%) and gentamicin (5.6%). The D-test demonstrated that 10.7% of isolates from CF patients represented the inducible phenotype of clindamycin resistance ($MLSB_i$). Resistance to fusidic acid, sulfamethoxazole/trimethoprim and chloramphenicol was found in 4.2%, 3.3% and 1.9% of the isolates, respectively (Table 2). All isolates were sensitive to vancomycin and mupirocin, with MICs for the former antimicrobial agent ranging between 0.25 μg/mL and 1 μg/mL.

Twelve (5.6%) *S. aureus* isolates were identified as MRSA. All these isolates were *mec*A-positive. All MRSA isolates displayed multidrug resistance. The prevalence of

Table 1 *spa* types and *spa* clonal complexes of *Staphylococcus aureus* isolated from cystic fibrosis patients

Cluster	*spa*	*spa*-CC	MLST-CC	Predicted ST	N
1	t700	*spa*-CC 021	CC30	ST30	11
1	t012	*spa*-CC 021	CC30	ST30	5
1	t338	*spa*-CC 021	CC30	ST30	4
1	t2029	*spa*-CC 021	CC30	ST30/ST239	4
1	t019	*spa*-CC 021	CC30	ST30	3
1	t234	*spa*-CC 021	CC30	ST30/ST239	2
1	t913	*spa*-CC 021	CC30	ST30	2
1	t021	*spa*-CC 021	CC30	ST30	1
1	t342	*spa*-CC 021	CC30	ST30	1
1	t726	*spa*-CC 021	CC30	ST30/ST1901	1
1	t3285	*spa*-CC 021	CC30	ST30	1
2	t005	*spa*-CC 005	CC22	ST22	7
2	t14289	*spa*-CC 005	CC22	ST22	5
2	t709	*spa*-CC 005	CC22	ST22	3
2	t223	*spa*-CC 005	CC22	ST22	1
2	t474	*spa*-CC 005	CC22	ST22	1

(*Continued*)

Table 1 (*Continued*)

Cluster	*spa*	*spa*-CC	MLST-CC	Predicted ST	N
2	t891	*spa*-CC 005	CC22	ST22	1
2	t2618	*spa*-CC 005	CC22	ST22	1
2	t4585	*spa*-CC 005	CC22	ST22	1
3	t359	*spa*-CC 267/359	CC97	ST97	5
3	t1236	*spa*-CC 267/359	CC97	ST97	3
3	t267	*spa*-CC 267/359	CC97	ST97	2
3	t521	*spa*-CC 267/359	CC97	ST97	2
3	t865	*spa*-CC 267/359	CC97	ST97	2
4	t880	*spa*-CC 065	CC45	ST45/ST1914	7
4	t330	*spa*-CC 065	CC45	ST45	3
4	t065	*spa*-CC 065	CC45	ST45	2
4	t715	*spa*-CC 065	CC45	ST45	1
5	t084	*spa*-CC 4096/091	CC15	ST15	11
5	t091	*spa*-CC 4096/091	CC7	ST7	11
5	t2932	*spa*-CC 4096/091	CC7/CC15	ST7	2
5	t4096	*spa*-CC 4096/091	CC15	ST15	1
6	t002	*spa*-CC 5213	CC5	ST5	8
6	t1228	*spa*-CC 5213	CC5	ST5	3
6	t6991	*spa*-CC 5213	CC5	ST5	2
6	t5213	*spa*-CC 5213	CC5	ST5	1
7	t078	*spa*-CC 078	CC25	ST25	7
7	t14286	*spa*-CC 078	CC25	ST25	2
7	t167	*spa*-CC 078	CC25	ST25	1
8	t094	*spa*-CC 2636	CC15	ST15	2
8	t547	*spa*-CC 2636	CC15	ST15	1
8	t2636	*spa*-CC 2636	CC15	ST15/ST1905	1
9	t11263	*spa*-CCa	CC30	ST34	3
9	t166	*spa*-CCa	CC30	ST34	1
10	t015	*spa*-CCb	CC45	ST45	14
10	t073	*spa*-CCb	CC45	ST45	4
11	t645	spa-CCc	CC121	ST121	2
11	t14288	spa-CCc	CC121	ST121	2
Singleton	t127	Singleton	CC1	ST1	6
Singleton	t571	Singleton	CC398	ST398	6
Singleton	t151	Singleton	CC5	ST5/ST225	5
Singleton	t209	Singleton	CC9	ST109	5
Singleton	t4771	Singleton	–	–	4
Singleton	t056	Singleton	CC101	ST101	3
Singleton	t156	Singleton	CC12	ST12	3
Singleton	t008	Singleton	CC8	ST8	2
Singleton	t230	Singleton	CC45	ST45	2
Singleton	t148	Singleton	–	ST72/ST1434/ST1723	1
Singleton	t647	Singleton	–	ST1027	1
Singleton	t924	Singleton	CC30	ST30	1
Singleton	t4428	Singleton	–	–	1
Singleton	t4992	Singleton	–	–	1
Singleton	t14287	Singleton	–	–	1
Singleton	t14290	Singleton	–	–	1
Excluded	t693	Excluded	CC9	ST9	4
Excluded	t362	Excluded	CC45	ST45	2
Excluded	t748	Excluded	CC30	ST30	2
Excluded	t026	Excluded	CC45	ST45	1
Excluded	t1509	Excluded	CC15	ST15	1
NT	NT	NT	NT	NT	1

Abbreviations: spa-CC, *spa* clonal complex; MLST, multi-locus sequence typing; NT, non-typeable.

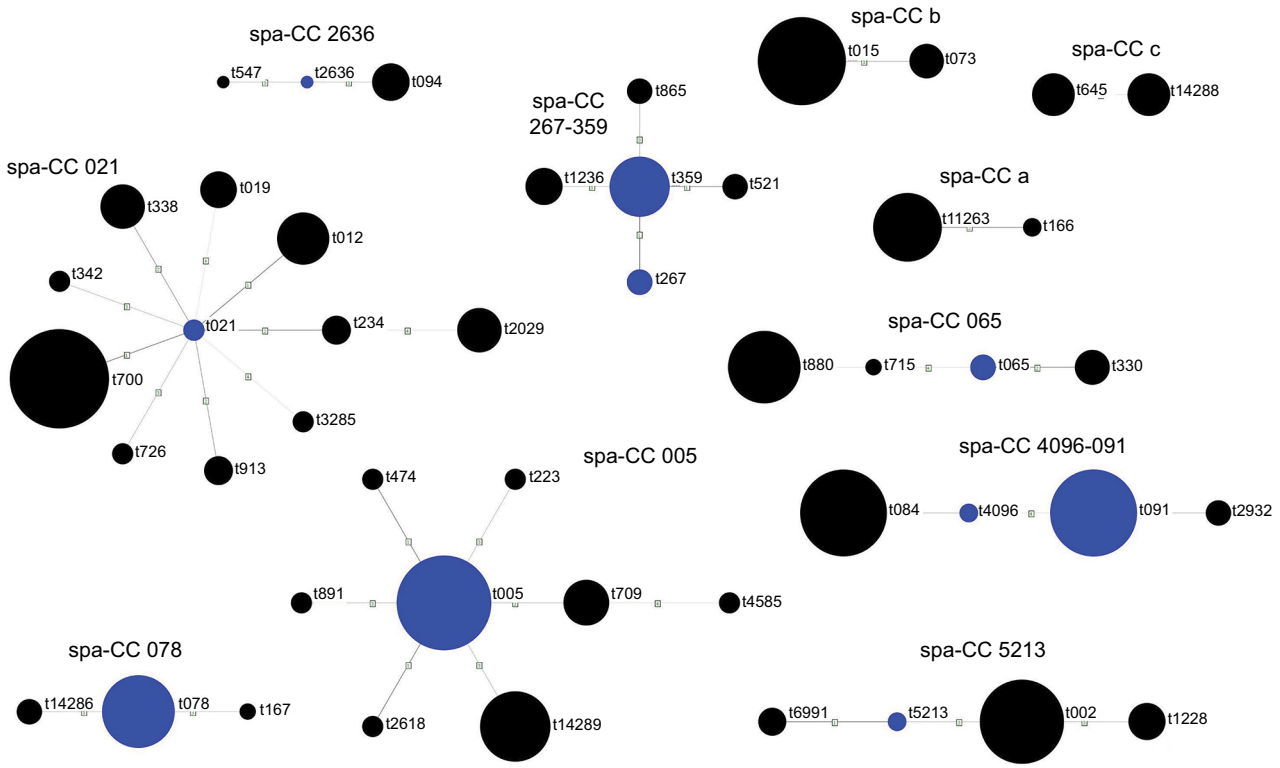

Figure 1 Cluster analysis of *spa* types of *Staphylococcus aureus* isolates from cystic fibrosis patients. Blue circles represent group founders and circle sizes are proportional to the frequency of a given *spa* type.
Abbreviation: spa-CC, spa clonal complex.

Table 2 Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from cystic fibrosis patients

Antibiotics	MRSA % (n=12)	MSSA % (n=203)	*p*-value	Total *S. aureus* % (n=215)
Penicillin	100 (n=12)	82.3 (n=167)	0.110	83.3 (n=179)
Azithromycin	100 (n=12)	47.8 (n=97)	<0.001	50.7 (n=109)
Erythromycin	100 (n=12)	46.8 (n=95)	<0.001	49.8 (n=107)
Roxithromycin	100 (n=12)	46.8 (n=95)	<0.001	49.8 (n=107)
Clindamycin	100 (n=12)	40.4 (n=82)	<0.001	43.7 (n=94)
Clindamycin$_{ind}$*	91.6 (n=11)	5.9 (n=12)	<0.001	10.7 (n=23)
Lincomycin	100 (n=12)	40.4 (n=82)	<0.001	43.7 (n=94)
Ciprofloxacin	50 (n=6)	10.8 (n=22)	<0.001	13 (n=28)
Ofloxacin	41.6 (n=5)	11.3 (n=23)	0.002	13 (n=28)
Levofloxacin	41.6 (n=5)	7.4 (n=15)	<0.001	9.3 (n=20)
Tobramycin	50 (n=6)	3 (n=6)	<0.001	6.1 (n=13)
Amikacin	25 (n=3)	6.4 (n=13)	0.017	8.8 (n=19)
Netilmicin	25 (n=3)	4.9 (n=10)	0.005	6.1 (n=13)
Gentamicin	41.6 (n=5)	4.4 (n=9)	<0.001	5.6 (n=12)
Tetracycline	33.3 (n=4)	6.4 (n=13)	0.001	7.9 (n=17)
Fusidic acid	16.6 (n=2)	3.4 (n=7)	0.026	4.2 (n=9)
Sulfamethoxazole/trimethoprim	16.6 (n=2)	2.5 (n=5)	0.007	3.3 (n=7)
Chloramphenicol	0	2 (n=4)	0.623	1.9 (n=4)
Vancomycin	0	0		0
Mupirocin	0	0		0
Multidrug-resistant	100 (n=12)	41.9 (n=90)	<0.001	47.4 (n=102)

Note: *Clindamycin$_{ind}$: inducible clindamycin resistance.
Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

multidrug-resistant (MDR) staphylococci among MRSA isolates turned out to be significantly higher than among methicillin-sensitive *S. aureus* (MSSA) ($p<0.001$). All MRSA isolates were resistant to macrolides and lincosamides, and 91.6% showed inducible phenotype of clindamycin resistance ($MLSB_i$). The latter proportion was significantly higher than the percentage of MSSA isolates with inducible resistance to clindamycin ($p<0.001$) (Table 2). MRSA isolates represented three distinct SCC*mec* types: SCC*mec* I, III and V. Isolates SCC*mec* V belonged to ST398-t571 (Table 3).

Prevalence of toxin genes

The list of virulence factors harbored by the examined CF isolates included *sea* (16.7%), *seb* (3.7%), *sec* (15.8%), *sed* (0.5%), *seg* (56.7%), *seh* (4.7%), *sei* (57.6%), *sej* (2.8%), *sek* (36.7%), *sel* (27%), *sem* (56.7%), *sen* (57.2%), *seo* (57.6%), *seu* (28.8%), *eta* (4.7%) and *tst* (14.9%) (Table 4). Enterotoxin gene cluster (*egc*)-positive isolates (56.7%) turned out to be the largest group among all examined staphylococci.

Discussion

Using *spa* typing, we demonstrated a substantial diversity of *S. aureus* isolates from the airways of CF patients (a total of 69 various *spa* types were isolated from 107 patients). Even greater diversity has been recently reported by Masoud-Landgraf et al[16] who identified up to 48 *spa* types within only 58 isolates from Austrian patients with CF. Comparison of our hereby presented findings with recent data from other European countries shows that *spa* types isolated from CF patients are similar to those from individuals with other staphylococcal colonization or from the carriers.[17,18] Thus, CF patients do not seem to be colonized with any unique *spa* type(s), but rather colonized with the types being currently spread in a given population.

spa types identified in this study formed clonal complexes with a pattern typical for Polish hospitals.[19,20]

Table 4 Prevalence of toxin genes among *Staphylococcus aureus* isolated from cystic fibrosis patients

Toxin genes*	Number of CF isolates (%)
sea	36 (16.7)
seb	8 (3.7)
sec	34 (15.8)
sed	1 (0.5)
see	0
seg	122 (56.7)
seh	10 (4.7)
sei	124 (57.6)
sej	6 (2.8)
sek	79 (36.7)
sel	58 (27)
sem	122 (56.7)
sen	123 (57.2)
seo	124 (57.6)
seu	62 (28.8)
eta	10 (4.7)
etb	0
tst	32 (14.9)
lukS-PV/*lukF*-PV	0

Note: *Genes encoding staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seu*), exfoliative toxins (*eta*, *etb*), toxic shock syndrome toxin-1 (*tst*) and Panton–Valentine leukocidin (*lukS*-PV/*lukF*-PV).
Abbreviation: CF, cystic fibrosis.

Table 3 Characteristics of methicillin-resistant *Staphylococcus aureus* isolates from cystic fibrosis patients

Patient	Number of isolates	Cluster	*spa*	*spa*-CC	MLST-CC	Predicted ST	SCC*mec*	Toxin genes	Antibiotic resistance
A	2	1	t2029	*spa*-CC 021	CC30	ST30/ST239	III	*sea*	FOX, AZ, ERY, ROX, L, CLIN, TET
A	1	1	t2029	*spa*-CC 021	CC30	ST30/ST239	III	*sea*	FOX, AZ, ERY, ROX, L, CLIN, TET, GEN, AM, NET, TO, SXT
B	1	10	t073	*spa*-CCb	CC45	ST45	II	*seg*, *sei*, *sem*, *sen*, *seo*	FOX, AZ, ERY, ROX, L, CLIN, CIP
C	1	Singleton	t151	Singleton	CC5	ST5/ST225	II	None	FOX, AZ, ERY, ROX, L, CLIN, CIP, OF, LEV, GEN, TO
C	2	Singleton	t151	Singleton	CC5	ST5/ST225	II	None	FOX, AZ, ERY, ROX, L, CLIN, CIP, OF, LEV, GEN, TO
C	2	Singleton	t151	Singleton	CC5	ST5/ST225	II	None	FOX, AZ, ERY, ROX, L, CLIN, TET, CIP, OF, LEV, GEN, TO, NET, AM
D	3	Singleton	t571	Singleton	CC398	ST398	V	None	FOX, AZ, ERY, ROX, L, CLIN, SXT

Abbreviations: *spa*-CC, *spa* clonal complex; MLST, multi-locus sequence typing; FOX, cefoxitin; AZ: azithromycin; ERY, erythromycin; ROX, roxithromycin; L, lincomycin; CLIN, clindamycin; TET, tetracycline; GEN, gentamicin; AM, amikacin; NET, netilmicin; TO, tobramycin; SXT, sulfamethoxazole/trimethoprim; CIP, ciprofloxacin; OF, ofloxacin; LEV, levofloxacin.

Clonal complexes CC30 and CC22 from clusters 1 and 2 for many years have been predominating in many European countries, including Poland.[21,22] It should be emphasized that the predominance of CC30 complex has also been recently reported by Masoud-Landgraf et al[16] in Austrian CF patients. In contrast to studies that have been conducted a decade ago, cluster 1 (CC30) did not include any t037/ST239 isolates, previously widespread in Polish hospitals.[19,20]

Despite a huge diversity of *spa* types (eight out of 20 isolates), the second cluster with CC22 complex showed high homogeneity in terms of sequence type (ST22), which distinguished this cluster from other clusters (especially cluster 5) in which an opposite phenomenon was observed.

The third cluster, with CC97 complex, included less isolates than the remaining two and showed greater genetic stability, representing only one sequential type. This complex can be represented by isolates with CA-MRSA phenotype, originating from both humans and cattle.[22,23] In our study, staphylococci belonging to CC97 complex turned out to be sensitive to methicillin (MSSA); likewise, more than 90% of *S. aureus* isolates from our material were sensitive to methicillin.

Although some recently published papers documented a dramatic increase in the proportion of MRSA isolated from CF patients, even up to 25%,[24] we found MRSA in only four study subjects; thus, MRSA isolates represented merely 5.6% of all staphylococci examined in this study. Low prevalence of MRSA documented in this study is generally consistent with the results of previous studies of CF patients from other European countries,[16,25] but stays in opposition to frequent occurrence of these isolates reported from the US.[24]

It is colonization with MDR isolates, rather than resistance to methicillin, which seems to be a primary concern in CF patients; MDR isolates constituted nearly a half of staphylococci identified in this study, including all MRSA isolates. Aside from beta-lactams, MDR strains most often showed resistance to macrolides, lincosamides and fluoroquinolones, which is not surprising owing to current standards of antibacterial treatment in CF patients.

MRSA isolates identified in this study belonged to four different clonal complexes: CC30, CC45, CC5 and CC398. SCC*mec* types I–III carry additional genes that provide resistance to antibiotics other than beta-lactams; in contrast, SCC*mec* types IV and V usually do not carry additional drug resistance genes. ST5-t151 MRSA isolates identified in our study showed a pattern band characteristic for SCC*mec* type II, and were characterized by the highest rate of antimicrobial resistance. Previously, this type was associated with an *spa*-CC found primarily in a hospital setting, and corresponded to one of the most prevalent hospital clones, ST5, or to its single-locus variant, Rhine-Hesse clone ST225. Both these STs have been recently identified among epidemic hospital clones from Poland and neighboring countries.[20,26] The first CC5-ST5 pediatric clone was described in Portugal.[27]

Isolation of a single MRSA isolate ST45, with SCC*mec* type II, was previously reported in Poland, the US, Hong Kong and Finland.[20,28,29] This isolate was resistant to macrolides, lincosamides and quinolones, and harbored *egc*.

SCC*mec* type III was related to ST239-III-t2029 type which involved isolates resistant to many antibiotics (macrolides, lincosamides, aminoglycosides, cotrimoxazole) and carried *sea* gene. This MRSA type has been previously isolated in a Malaysian hospital, also from the respiratory tract, and aside from the antibiotics mentioned above, also showed resistance to rifampicin and fusidic acid.[30]

Six of our isolates represented ST-389 type which may cause various human infections. A number of patients with soft tissue and skin infections caused by this type were reported.[31] However currently, ST-398 is considered to be a noteworthy zoonotic pathogen commonly found in livestock and persons being in close contact with ST398-positive animals. This livestock-associated MRSA (LA-MRSA) clone is widespread in Europe, Asia and North America.[5] LA-MRSA have been isolated from various human infections, including folliculitis, osteomyelitis, endocarditis and skin and soft tissue infections.[31] To the best of our knowledge, the only published report documenting isolation of this clone from a CF patient originates from Brazil.[5] Moreover, Chinese authors reported isolation of *S. aureus* ST398-V-t571 from a student's nasal cavity.[3] Our isolate originated from a 1-year-old boy whose mother practiced horse riding. According to the most recent report published in 2017, ST398 is a predominant staphylococcal isolate from equine infections caused by MRSA.[4] In a recent study conducted by Mroczkowska et al,[4] ST398 turned out to be a predominant clone isolated from animals in Poland. To the best of our knowledge, our current study is the first to demonstrate the colonization with LA-MRSA CC398 clone in a CF patient from Europe.

ST398 is commonly associated with the presence of Panton–Valentine leukocidin.[32] However, both ST398-t571 and other isolates found in our patients were PVL-negative. The role of PVL in staphylococcal infections is still a matter of discussion.[33,34] On the one hand, presence of PVL genes in MRSA isolates from CF patients was shown to be associated with invasive lung infection.[35] On the other hand, either in our present study or in previous studies conducted in Europe

and on other continents, all staphylococci from individuals with CF turned out to be PVL-negative.[5,16,36] This implies that PVL is not necessarily a key virulence factor involved in staphylococcal infections in CF patients.

According to some previously published reports, CC22 may also include PVL-harboring *S. aureus*.[37] However, although we have isolated staphylococci belonging to this complex, none of them harbored PVL-encoding genes. This implies that isolates belonging to the same clonal lineage may display diverse toxin gene profiles.

Enterotoxin genes *seg*, *sei*, *sem*, *sen*, *seo* and *seu* are organized within the *egc*. This cluster is located on the genomic island nSAb and considered to be the "nursery" of staphylococcal enterotoxins.[38] Many staphylococcal isolates from our CF patients were *egc*-positive, which is consistent with previous reports.[2,39] No relatedness with any specific molecular type was found in *egc*-positive isolates. In line with these findings, *egc* may be a virulence factor promoting colonization with *S. aureus* in CF patients.[40]

Staphylococcal superantigens were shown to nonspecifically activate proliferation of T lymphocytes, binding to VbTCR-2 on these cells and to MHC class II molecules. Indeed, some *S. aureus* isolates from our CF patients harbored genes for superantigens, including among them enterotoxins *sea* and *sec*, and *tst*. Also, Liu et al[2] detected diverse superantigens genes in more than 50% of staphylococcal isolates from patients with CF. Taken all together, these findings imply that presence of superantigens genes may be linked with the pathogenesis of staphylococcal infections in this group.

To summarize, this study demonstrated that *S. aureus* isolates from CF patients vary considerably in terms of *spa* types, clonal complexes and carriage of toxin genes. We did not find any PVL-positive isolates, which implies that this toxin is not necessarily a key virulence factor involved in staphylococcal infections in CF patients. To the best of our knowledge, this is the first Polish study documenting genetic structure of *S. aureus* isolates from patients with CF, as well as the first European report describing the presence of ST398-V-t571 LA-MRSA in a person with this condition. This implies that patients with CF can also be colonized with ST398 MRSA, and justifies constant monitoring of staphylococcal colonization and identification of epidemic *S. aureus* clones in this group.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. *Respir Care*. 2009;54(5):595–605.
2. Liu Y, Zhang J, Zhong D, et al. Characterization of Staphylococcus aureus isolates from pediatric patients with cystic fibrosis. *World J Microbiol Biotechnol*. 2016;32(10):162.
3. Du J, Chen C, Ding B, et al. Molecular characterization and antimicrobial susceptibility of nasal Staphylococcus aureus isolates from a Chinese medical college campus. *PLoS One*. 2011;6(11):17.
4. Guerin F, Fines-Guyon M, Meignen P, et al. Nationwide molecular epidemiology of methicillin-resistant Staphylococcus aureus responsible for horse infections in France. *BMC Microbiol*. 2017;17(1):104.
5. Lima DF, Cohen RW, Rocha GA, Albano RM, Marques EA, Leao RS. Genomic information on multidrug-resistant livestock-associated methicillin-resistant Staphylococcus aureus ST398 isolated from a Brazilian patient with cystic fibrosis. *Mem Inst Oswaldo Cruz*. 2017;112(1):79–80.
6. Mroczkowska A, Zmudzki J, Marszalek N, et al. Livestock-associated Staphylococcus aureus on Polish pig farms. *PLoS One*. 2017;12(2):e0170745.
7. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, Witte W. Assignment of Staphylococcus isolates to groups by spa typing, SmaI macrorestriction analysis, and multilocus sequence typing. *J Clin Microbiol*. 2006;44(7):2533–2540.
8. Baron F, Cochet MF, Pellerin JL, et al. Development of a PCR test to differentiate between Staphylococcus aureus and Staphylococcus intermedius. *J Food Prot*. 2004;67(10):2302–2305.
9. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442–5448.
10. Mellmann A, Weniger T, Berssenbrugge C, et al. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. *BMC Microbiol*. 2007;7:98.
11. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol*. 1991;29(10):2240–2244.
12. Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in humans in Germany. *PLoS One*. 2011;6(9):8.
13. Milheirico C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in Staphylococcus aureus. *Antimicrob Agents Chemother*. 2007;51(9):3374–3377.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. Document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
15. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Von Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of Staphylococcus aureus isolated from blood and nasal specimens. *J Clin Microbiol*. 2003;41(4):1434–1439.
16. Masoud-Landgraf L, Johler S, Badura A, et al. Genetic and phenotypic characteristics of Staphylococcus aureus isolates from cystic fibrosis patients in Austria. *Respiration*. 2015;89(5):390–395.
17. Becker K, Schaumburg F, Fegeler C, Friedrich AW, Kock R. Staphylococcus aureus from the German general population is highly diverse. *Int J Med Microbiol*. 2017;307(1):21–27.

18. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW. Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med*. 2010;7(1):1000215.
19. Krzyszton-Russjan J, Empel J, Leski T, Gniadkowski M, Hryniewicz W. Clonal structure of the methicillin-resistant Staphylococcus aureus (MRSA) population in Poland: revision and update. *Microb Drug Resist*. 2005;11(2):127–136.
20. Luczak-Kadlubowska A, Sulikowska A, Empel J, et al. Countrywide molecular survey of methicillin-resistant Staphylococcus aureus strains in Poland. *J Clin Microbiol*. 2008;46(9):2930–2937.
21. Grundmann H, Schouls LM, Aanensen DM, et al. The dynamic changes of dominant clones of Staphylococcus aureus causing bloodstream infections in the European region: results of a second structured survey. *Euro Surveill*. 2014;19(49):20987.
22. Holtfreter S, Grumann D, Balau V, et al. Molecular epidemiology of Staphylococcus aureus in the general population in Northeast Germany: results of the Study of Health in Pomerania (SHIP-TREND-0). *J Clin Microbiol*. 2016;54(11):2774–2785.
23. Locatelli C, Cremonesi P, Caprioli A, et al. Occurrence of methicillin-resistant Staphylococcus aureus in dairy cattle herds, related swine farms, and humans in contact with herds. *J Dairy Sci*. 2017;100(1):608–619.
24. Rosenthal VD, Bijie H, Maki DG, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 36 countries, for 2004-2009. *Am J Infect Control*. 2012;40(5):396–407.
25. Campana S, Cocchi P, Döring G, Taccetti G, Moroney SM. Emergence of an epidemic clone of community-associated methicillin-resistant Panton-Valentine leucocidin-negative Staphylococcus aureus in cystic fibrosis patient populations. *J Clin Microbiol*. 2007;45(9):3146; author reply 3146–3147.
26. Kasprzyk J, Piechowicz L, Wisniewska K, Dziewit L, Bronk M, Swiec K. [Differentiation of spa types and staphylococcal cassette chromosome mec (SCCmec) in clinical methicillin-resistant Staphylococcus aureus isolated in medical sites of Gdansk region]. *Med Dosw Mikrobiol*. 2015;67(2):79–88. Polish [with English abstract].
27. Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin-resistant Staphylococcus aureus: identification of two ancestral genetic backgrounds and the associated mec elements. *Microb Drug Resist*. 2001;7(4):349–361.
28. Ip M, Yung RW, Ng TK, et al. Contemporary methicillin-resistant Staphylococcus aureus clones in Hong Kong. *J Clin Microbiol*. 2005;43(10):5069–5073.
29. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother*. 2003;47(12):3926–3934.
30. Lim KT, Teh CS, Yusof MY, Thong KL. Mutations in rpoB and fusA cause resistance to rifampicin and fusidic acid in methicillin-resistant Staphylococcus aureus strains from a tertiary hospital in Malaysia. *Trans R Soc Trop Med Hyg*. 2014;108(2):112–118.
31. van Belkum A, Melles DC, Peeters JK, et al. Methicillin-resistant and -susceptible Staphylococcus aureus sequence type 398 in pigs and humans. *Emerg Infect Dis*. 2008;14(3):479–483.
32. Zhao C, Liu Y, Zhao M, et al. Characterization of community acquired Staphylococcus aureus associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. *PLoS One*. 2012;7(6):6.
33. Bubeck Wardenburg J, Palazzolo-Ballance AM, Otto M, Schneewind O, DeLeo FR. Panton-Valentine leukocidin is not a virulence determinant in murine models of community-associated methicillin-resistant Staphylococcus aureus disease. *J Infect Dis*. 2008;198(8):1166–1170.
34. Labandeira-Rey M, Couzon F, Boisset S, et al. Panton-Valentine leukocidin causes necrotizing pneumonia. *Science*. 2007;315(5815):1130–1133.
35. Elizur A, Orscheln RC, Ferkol TW, et al. Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus lung infection in patients with cystic fibrosis. *Chest*. 2007;131(6):1718–1725.
36. Mimica MJ, Berezin EN, Damaceno N, Carvalho RB. SCCmec type IV, PVL-negative, methicillin-resistant Staphylococcus aureus in cystic fibrosis patients from Brazil. *Curr Microbiol*. 2011;62(2):388–390.
37. Masiuk H, Kopron K, Grumann D, et al. Association of recurrent furunculosis with Panton-Valentine leukocidin and the genetic background of Staphylococcus aureus. *J Clin Microbiol*. 2010;48(5):1527–1535.
38. Monday SR, Bohach GA. Genes encoding staphylococcal enterotoxins G and I are linked and separated by DNA related to other staphylococcal enterotoxins. *J Nat Toxins*. 2001;10(1):1–8.
39. Ormerod KL, George NM, Fraser JA, Wainwright C, Hugenholtz P. Comparative genomics of non-pseudomonal bacterial species colonising paediatric cystic fibrosis patients. *PeerJ*. 2015;3:e1223.
40. Nowrouzian FL, Dauwalder O, Meugnier H, et al. Adhesin and superantigen genes and the capacity of Staphylococcus aureus to colonize the infantile gut. *J Infect Dis*. 2011;204(5):714–721.