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 SCCmec 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 SCCmec types (I, III and V). All isolates (both MRSA and methicillin-sensitive S. aureus) were Panton–Valentine leukocidin-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.

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). 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
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. Available evidence suggests that the results of *spa* typing correlate strongly with the outcomes of clonal grouping obtained with other methods. 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 Gdańsk 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’Etoile, France). Additionally, all strains identified as *S. aureus* were examined for the presence of species-specific thermostable nuclease gene (*nucSA*), as described by Baron et al. The isolates were stored at −80°C in trypticoye soy broth (Oxoid, Basingstoke, UK) supplemented with 15% glycerol.

**spa typing**

*spa* typing was performed according to Harmsen et al. 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), 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”. Since the results of *spa* typing and MLST are highly concordant, 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* mecA gene, as described elsewhere. Each PCR contained mecA-positive (*S. aureus* ATCC 43300) and mecA-negative (*S. aureus* ATCC 29213) strains as controls. All mecA-negative *S. aureus* isolates able to grow on ORSA plates were tested for the carriage of *mecC* gene using primers described by Cuny et al. Typing of the staphylococcal chromosomal cassette *mec* (SCCmec) was carried out as described previously by Milheirico et al. 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. 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, 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).

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 | 4  |
| 1       | t019   | spa-CC 021 | CC30 | ST30 | 3  |
| 1       | t234   | spa-CC 021 | CC30 | ST30 | 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 | 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       | t079   | 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/ST79   | 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  |
| 7       | 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      | STS/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.
Clones of Staphylococcus aureus among cystic fibrosis patients

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 inducible                | 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 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). 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 SCCmec types: SCCmec I, III and V. Isolates SCCmec 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%), sey (56.7%), 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. 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. 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.

### 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)                |
| sey         | 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, seh, sei, sej, sek, sel, sem, sen, sey, 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 | SCCmec | Toxin genes | Antibiotic resistance |
|---------|--------------------|--------|-----|--------|---------|--------------|--------|-------------|----------------------|
| A       | 2                  | I      | t2029 | spa-CC | CC30    | ST30/ST239   | III    | sea         | FOX, AZ, ERY, ROX, L, CLIN, TET |
| A       | 1                  | I      | t2029 | spa-CC | CC30    | ST30/ST239   | III    | sea         | FOX, AZ, ERY, ROX, L, CLIN, TET |
| B       | 1                  | 10     | t073  | spa-CCb| CC45    | ST45         | II     | seg, sei, sem, sen, seo | FOX, AZ, ERY, ROX, L, CLIN, CIP |
| 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, CIP, OF, LEV, GEN, TO |
| 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.
Dramatic increase in the proportion of MRSA isolated from sensitive to methicillin (MSSA); likewise, more than 90% of staphylococci belonging to CC97 complex turned out to be antimicrobial resistance. Previously, this type was associated with the results of previous studies of CF patients from other European countries, but stays in opposition to frequent occurrence of these isolates reported from the US.

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. 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%, 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, but stays in opposition to frequent occurrence of these isolates reported from the US.

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. SCCmec types I–III carry additional genes that provide resistance to antibiotics other than beta-lactams; in contrast, SCCmec 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 SCCmec 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. The first CC5-ST5 pediatric clone was described in Portugal.

Isolation of a single MRSA isolate ST45, with SCCmec type II, was previously reported in Poland, the US, Hong Kong and Finland. This isolate was resistant to macrolides, lincosamides and quinolones, and harbored ege.

SCCmec 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.

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. 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. LA-MRSA have been isolated from various human infections, including folliculitis, osteomyelitis, endocarditis and skin and soft tissue infections. To the best of our knowledge, the only published report documenting isolation of this clone from a CF patient originates from Brazil. Moreover, Chinese authors reported isolation of S. aureus ST398-V-t571 from a student’s nasal cavity. 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. In a recent study conducted by Mroczkowska et al, 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. 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. On the one hand, presence of PVL genes in MRSA isolates from CF patients was shown to be associated with invasive lung infection. 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.12 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.

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