ORIGINAL ARTICLE

MICROBIOLOGICAL FEATURES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RESPIRATORY TRACT OF CHILDREN WITH CYSTIC FIBROSIS

DOI: 10.36740/WLek202109112

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ABSTRACT

The aim: To determine the prevalence rate of *Staphylococcus aureus* infection among children with Cystic Fibrosis in the Dnieper region, to provide microbiological characteristics of the isolates and to elevate their susceptibility to antimicrobials.

Materials and methods: Sputum, tracheobronchial lavage waters and/ or deep smear from the posterior pharyngeal wall were taken from children with genetically confirmed Cystic Fibrosis. Bacteriological method was the main. The first screening for small colony variants of *Staphylococcus aureus* was carried out after 48 hours of incubation. The antimicrobials susceptibility testing was determined by disk-diffusion method according to the EUCAST 2019. Microsoft Office Excel 2010 was used for statistical data processing.

Results: Twenty one children were enrolled in the survey. The culture of *Staphylococcus spp*. was obtained from all patients with 40.8% positive for *Staphylococcus aureus*. Small colony variants appeared with the prevalence rate 21.6% after 48 hours of incubation. The frequency of associations between *Staphylococcus aureus* with auxotroph phenotype with the presence of *Pseudomonas aeruginosa* was significantly higher than with wild-type group. The 3d-generation aminoglycosides, the 3d-generation fluoroquinolones, linezolid, rifampicin and tetracyclines showed the best antimicrobial activity, however, resistance to cefoxitin and gentamicin was significantly higher in auxotroph-modified group.

Conclusions: Infection *Staphylococcus aureus* is common among children. The appearance of auxotrophs registered after treatment with aminoglycosides and/ or co-trimoxazole and co-infection *Pseudomonas aeruginosa*. Isolates of *Staphylococcus aureus* showed good chemotherapeutic sensitivity, but tendency in increasing resistance registered for auxotroph-modified phenotype.

KEY WORDS: cystic fibrosis, *Staphylococcus aureus*, small colony variants, chronic respiratory tract infection

Wiad Lek. 2021;74(9 p.I):2094-2099

INTRODUCTION

Polymicrobial infection of a lower respiratory tract in people with Cystic fibrosis (CF) is responsible for the development of chronic obstructive pulmonary disease and, consequently, for poor outcomes. *Staphylococcus aureus* is the most common pathogen isolated from the respiratory tract of such patients [1-4]. According to the European Cystic Fibrosis Society Patient Registry in 2016, the overall prevalence rate of *S. aureus* infection in Europe region was about 38% with the predominance among children, up to 45% in the senior school age group [1]. It is speculated that chronic infection *S. aureus* initiates such specific changes in the lungs that create background for the establishment of *Pseudomonas aeruginosa* infection designating morbidity and mortality in patients with CF [4].

It was shown that in CF-models internalization of *S. aureus* by CFTR-deficient macrophages (Cystic Fibrosis transmembrane conductance regulator) is impaired and the microorganism became able to switch to fermentative type of existence, so chronical pulmonary infection *S. aureus* rises possible to establish. Small colony variants (SCVs) have altered metabolic activity, they are able to avoid the effectors of the immune system, mechanical clearance and harmful effects of antimicrobial agents [2-6]. The follow factors responsible for induction of auxotrophic phenotype selection are hypoxia, antibiotic treatment (aminoglycosides, sulfonamides, fluoroquinolones) and *P. aeruginosa* co-infection. The microorganism *P. aeruginosa* affects *S. aureus* through long-term exposure of 2-n-heptyl-4-hydroxyquinoline-N-oxide and siderophores that causes auxotrophy to menadione and hemin. In contrast, auxotrophy to thymidine is usually associated with the long-term sulfonamides that competitively inhibit the enzyme responsible for the formation of folates [4, 7]. Such adaptive properties are the way to avoid the biocide effects of antimicrobials, including tobramycin, trimethoprim-sulfamethoxazole etc.

It should be mentioned, that in their most SCVs of *S. aureus* are unstable and can be reversed into a fast-growing and dangerous wild-type [7, 8].

Resistant bacteria threaten the exceptional health benefits that have been achieved with antimicrobials. The crisis is global, reflecting the worldwide overuse of these drugs; consequently, patients with regular needs in antibiotic therapy are vulnerable. Antibiotic-resistant infections place a substantial health and economic burden on the health care system and population [9]. Basically, rational use of antimicrobials is extremely important, so the knowledge of

regional resistance stands necessary for benefit treatment and improvement clinical outcomes in CF.

THE AIM

The aim of the study is to determine the prevalence rate of *S. aureus* infection among children with CF in the Dnieper region, to provide microbiological characteristics of the obtained isolates and to check their susceptibility to antimicrobials.

MATERIALS AND METHODS

The study was conducted in the scientific laboratory of the Department of Microbiology, Virology, Immunology and Epidemiology» from January 2019 to April 2020. Children aged from 0 to 17 years 11 months 29 days with geneticall-ly confirmed diagnosis of CF were enrolled in the study. Sputum and/ or deep smear from the posterior pharyngeal wall were taken, and we didn`t rule out the possibility of collecting of tracheobronchial lavage waters. The planned minimum frequency of visits was 1 time per 3 months, unscheduled visit in a case of deteriorated clinical condition. Parents or healthcare proxies signed the Patient Informed Consent Form. Previously, the study was approved by the Ethics Committee of the institution.

The main method of the research was bacteriological. The samples were taken in the morning before hygiene practices. Biological material from the posterior pharyngeal wall was collected by sterile cotton swab soaked in a saline; sputum from children with expectoration was collected in sterile containers. The obtained biological material *ex tempore* was inoculated on a standard set of growth mediums. The salt agar (Farmaktiv, Ukraine) with egg-yolk emulsion (Hi-Media, India) used for selective isolation of *Staphylococcus spp*. For selective isolation of *P. aeruginosa* Cetrimide agar with glycerol (BioLife, Italy) was used. The Colombian agar with 5% sheep blood (bioMerieux, France) was used for cultivation of *Staphylococcus spp*., *P. aeruginosa* and assessment of its hemolytic activity.

The Petri dishes were incubated in a thermostat at 37 °C for 24-72 hours. The Petri dishes supposed for isolation of *Staphylococcus spp*. additionally were left at room temperature for up to 5 days [2, 7]. The number of microorganisms was evaluated in CFU/ml. The yielded cultures were stained by Gram.

Colonies of *Staphylococcus spp*. were evaluated by its cultural characteristics, lecitovitelase activity, ability to coagulate rabbit`s plasma (Pharmstandart-BIOLEK, Ukraine) and by catalase activity (10% hydrogen peroxide solution, NICF, RF). *Staphylococcus spp*. were identified with using a commercial kit STAPHY test 16 (ErbaLachema, Czech Republic), which contains the following indicators of biochemical activity: urease, arginine, ornithine, β-galactosidase, β-glucuronidase, esculin, nitrates, phosphatase, galactose, sucrose, trehalose, mannitol, xylose, maltose, mannose, lactose).

The first screening for SCVs of *S. aureus* was carried out after 48 hours of incubation. Small (less than 1 mm), unpigmented, non-hemolytic colonies, sometimes like «fried eggs» on blood agar, and all colonies on yolk-salt agar were considered to be suspicious for SCVs of *S. aureus* [2, 8].

Identification of *P. aeruginosa* was performed by its cultural characteristics followed by NEFERM test 24 (ErbaLachema, Czech Republic).

The susceptibility of isolates to antimicrobials was determined on Mueller-Hinton agar (SRE IFR NAAS, Ukraine) by a standardized disc-diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019, version 9.0 [10, 11]. We used the follow disks with antibiotics (Hi-Media, India): benzylpenicillin 1 U, cefoxitin 30 mcg, norfloxacin 10 mcg, ciprofloxacin 5 mcg, levofloxacin 5 mcg, moxifloxacin 5 mcg, ofloxacin 10 mcg, amikacin 30 mcg, gentamycin 10 mcg, tobramycin 10 mcg, erythromycin 15 mcg, clindamycin 2 mcg, tetracycline 30 mcg, tigecycline 15 mcg, minocycline 30 mcg, linezolid 10 mcg, rifampicin 5 mcg, trimethoprim/ sulfamethoxazole 25 mcg. The obtained data were included to the answer sheet and consequently into the table of results.

The Microsoft Office Excel 2010 was used for statistical data processing. Normality data distribution was checked using the Shapiro-Wilk test, the hypothesis of normality was rejected at $p>.05$. To express the incidence rate per 100 determination for data with non-normality distribution Median,% and its 95% confidence interval – Me (95% CI) were used; for the proportion expressed with extensive indicator – %. The Mann-Whitney U-test was used for estimation of the statistically significant differences between quantitative quantities; Pearson's The chi-square test was used for qualitative binary data. The results of the experiments were considered to be statistically significant at $p\leq.05$ [12].

RESULTS

Twenty-one children (10 boys and 11 girls) aged from 1 year 8 months to 18 years with an average age of 9.1 (3.7) years were enrolled in the study. Within 16 months, 119 samples were taken. We had 105 planned and 14 unscheduled visits. Samples included sputum (n = 77), mucus from a deep smear from the posterior pharyngeal wall (n = 38), and lavage water after tracheobronchial lavage (n = 4).

All samples, 100%, were positive for pathogenic microbiota, and monoculture was found only in 18.5% of cases. The culture of *Staphylococcus spp.* (n = 126) were yielded from all patients. The composition of *Staphylococcus spp*. isolated from respiratory samples is shown in Fig. 1. Worth noting, that 40.8% of the collected samples were positive for *S. aureus*. It was estimated that among 51 isolates of *S. aureus*, a mixed culture was obtained in 47 samples. The isolates in their most (n = 78) were obtained from children of the 6-11-year-old group.

It is worth noting that the youngest child in the study at the age of 1 year 8 months had a positive result for *S. aureus* and had the same result in repeated investigations. Furthermore, *S. aureus* yielded from pathological material

in the etiologically important amount of 10^5-10^7 CFU/ml. This data indicates the initial colonization of the airways by aggressive pathogens that play a critical role in the pathogenesis of the disease. A total of thirteen children (61.9%) were positive for *S. aureus* at minimum three times for at least 6 months, suggesting a chronic infection.

Usually, all isolates had typical cultural, morphological and biochemical properties. Due to lipochromic pigment, *S. aureus* on yolk-salt agar with egg emulsion formed cloudy, round, creamy colonies, the colour is yellow or orange (Fig. 2). On blood agar wild-type *S. aureus* produced a zone of β-hemolysis (Fig. 3).

Isolates of *S. aureus* with the phenotype of SCVs we had in 21.6% of cases. Such cultures in 81.8% of cases were yielded from children treated with aminoglycosides and/or sulfonamides. The frequency of associations of SCVs with *P. aeruginosa* was 100%, and significantly higher than in the group with the wild phenotype ($\chi 2$ = 8,516; df=1; p = 0.007). *S. aureus* with the phenotype of SCVs appeared on Petri dishes after 48-72 hours of cultivation and was less than 1 mm in diameter, they didn`t produce pigments and didn`t perform a visible hemolytic activity, sometimes they had an appearance as «fried eggs» on blood agar (Fig. 4).

The antimicrobial susceptibility of *S. aureus* isolates was determined in 100% of cases. All isolates were phenotypically tested for methicillin resistance. It is known that the test with a cefoxitin disk 30 mcg is a reliable predictor of the existence of the *mecA* or *mecC* genes, and screening for BORSA (borderline oxacillin resistant *S. aureus*) from oxacillin minimal inhibitory concentration (MIC) is not recommended by the EUCAST committee for routine use [9]. There were 5 yields among 51 isolates of *S. aureus* resistant to cefoxitin, that is, methicillin-resistant by phenotype. All of them were sequentially obtained from one child aged 9 years 3 months, she was positive for SCVs too; the average diameter of growth retardation was 10.8 (1.1)

Table I. Results of the antimicrobials susceptibility testing of *Staphylococcus aureus* isolates obtained from children with Cystic Fibrosis (disc-diffusion method; n=51)

№	Disk with antibiotic	Proportion of sensitive isolates, %	The diameter of the growth retardation zone – Me (95% CI), mm
	Phenotype of resistance to beta-lactams		
1	*Benzylpenicillin 1U*	66,67	26 (20-29)
2	*Cefoxitin 30 mcg*	90,20	29 (28-32)
	Phenotype of resistance to fluoroquinolones		
3	*Norfloxacin 10 mcg*	78,43	21 (18-22)
4	*Ciprofloxacin 5 mcg**	94,1	34 (12-36)
5	*Ofloxacin 5 mcg**	96,1	32 (13-36)
6	*Levofloxacin 5 mcg**	100,0	32 (28-34)
7	*Moxifloxacin 5 mcg**	100,0	32 (28-34)
	Phenotype of resistance to aminoglycosides		
8	*Gentamicin 10 mcg*	94,1	22 (21-22)
9	*Tobramycin 10 mcg*	100,0	22 (21-23)
10	*Amikacin 30 mcg*	98,1	22 (21-23)
11	*Netilmicin 10 mcg*	100,0	21 (21-22)
	Phenotype of resistance to macrolides		
12	*Erythromycin 15 mcg*	70,6	22 (21-24)
	Phenotype of tetracycline resistance		
13	*Tetracycline 30 mcg*	98,4	23 (22-24)
14	*Minocycline 30 mcg **	100,0	26*
15	*Tigecycline 15 mcg*	100,0	23 (23-25)
	Phenotype of resistance to oxazolidones		
16	*Linezolid 10 mcg*	100,0	28 (28-35)
	Phenotype of resistance to other antibiotics		
17	*Chloramphenicol 30 mcg*	74,5	21 (18-23)
18	*Rifampicin 5 mcg*	100,0	31 (29-32)
19	*Trimethoprim/ sulfamethoxazole 25 mcg*	85,4	22 (21-22)

* Values refer to the growth retardation zone only for those isolates that were resistant to the appropriate screening

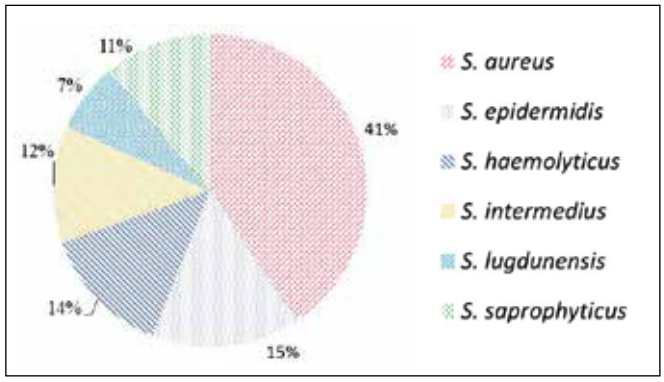

Fig. 1. The composition of *Staphylococcus spp.* isolated from children with Cystic Fibrosis

mm. Such isolates also showed resistance to erythromycin, gentamicin, norfloxacin and ciprofloxacin, chloramphenicol and trimethoprim-sulfamethoxazole.

The results of the chemotherapeutic sensitivity of *S. aureus* are shown in the Table. I. The best data were registered for aminoglycosides of the third generation, fluoroquinolones of the third generation, linezolid, rifampicin and tetracyclines.

In our study, microorganisms susceptible to all beta-lactams (including unprotected penicillins) were only 66.67 (95%CI 53.73-79.60) %, however, it should be mentioned that even in such case the expected efficacy of oral drugs *in vivo* remains questionable. Those *S. aureus* that showed resistance to penicillin, which is known to be due to the ability to produce penicillinases, were resistant to benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin, piperacillin and ticarcillin.

Sensitivity to cefoxitin was shown in 96.5% of isolates. Such cultures were susceptible to penicillins in combination with beta-lactamase inhibitors (if they did not show sensitivity to penicillin), isoxazolylpenicilinins (oxacillin, cloxacillin, dicloxacillin and flucloxacillin) and nafcilin, most cephalosporins (cephalosporin, cephaloximlor, cefoxitin, cefpodoxime, ceftriaxone, cefpodoxime) and carbapenems (ertapenem, imipenem, meropenem, meropenem-vaborbactam).

The sensitivity to screening with erythromycin was 74.1%, and such isolates were directly susceptible to erythromycin, azithromycin, clarithromycin and roxithromycin. Resistant isolates were found to be sensitive to clindamycin; the appearance of the D-phenomenon was not registered.

The sensitivity to screening with norfloxacin was 82.8%; resistant isolates were individually tested for susceptibility to ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin and showed a positive result in most cases.

The sensitivity to screening with tetracycline was 98.4%; such isolates were susceptible directly to tetracycline, as well as to doxycycline and minocycline. The only isolate that showed resistance to tetracycline was found to be sensitive to minocycline. It is known that strains resistant to tigecycline are extremely rare or not reported, and there were no such isolates in our study.

Note that testing for sensitivity to macrolides and some tetracyclines, as well as a confirmation of resistance to tetracycline, are not described due to the MIC method, which was not intended for use in the report. MIC is also a method for determining susceptibility to vancomycin, therefore here we do not present such data either.

We understand that standard methods for determining the sensitivity of microorganisms to antibiotics *in vitro* have been developed and approved for testing of fast-growing bacteria, and statistical processing requires a sufficient sample, then since SCVs have changed properties, and the sample is only 11 isolates, the results should be interpreted with caution [10, 11].

When comparing the chemotherapeutic sensitivity of wild-type *S. aureus* and auxotrophic modification, similar results were obtained, but there was a tendency towards an increase in resistance to beta-lactams and gentamicin. Among these isolates were both methicillin-susceptible and methicillin-resistant.

The median of the growth retardation zone for cefoxitin for isolates with a normal phenotype (n = 50) was 30 (95%

Fig. 2. Wild-type *Staphylococcus aureus* produces typical colonies with zones of lecithovitelase activity on yolk-salt agar, 18 h.

Fig. 3. Wild-type *Staphylococcus aureus* produces a zone of β-hemolysis on blood agar, against the light, 24 h

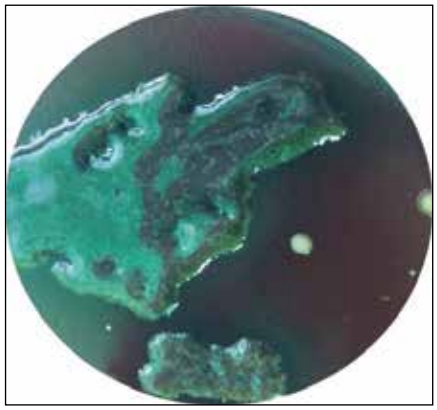

Fig. 4. Mixed culture of mucoid *Pseudomonas aeruginosa* and small colony variants of *Staphylococcus aureus* («fried eggs») on blood agar, 72 h.

CI 28-32) mm, and for isolates with an altered phenotype (n = 11) – 26 (95% CI 10-28) mm. The difference between the median value is 5 (95% CI 2-9) mm and is statistically significant (p = .001 according to the Mann-Whitney test). Similar results were obtained for gentamicin sensitivity and the difference between the median value was 4 (95% CI 2-7) mm and was also statistically significant (p = .0000005 according to the Mann-Whitney test). However, there was no significant difference in the frequency of isolation of sensitive isolates between both phenotypes for cefoxitin and gentamicin: $\chi 2 = 1.753$; df=1; p = .370 and $\chi 2 = 2.648$; df=1; p = .207, respectively.

When comparing the diameters of growth inhibition zones and the frequency of isolation of sensitive isolates concerning other antibiotics, no statistically significant results were obtained (p> 0.05), which may be due to the small sample size of the auxotrophic phenotype.

DISCUSSION

Pulmonary *S. aureus* infection in CF often occur early in childhood and prior to colonization with other pathogens, in particular *P. aeruginosa* [1, 4, 8, 13]. Our results demonstrate similar data.

Under antibiotic pressure, *S. aureus* acquires the ability to switch to SCVs. These SCVs invade epithelial cells, overcome antibiotic therapy inside the cells and serve as a starting point for extracellular recolonization. The relation between the presence of SCVs and a poor clinical outcome has already been reported in several studies, with patients harbouring SCVs variants having lower blood oxygen levels and a significantly lower FEV1 score [2].

Long-term treatment with trimethoprim/sulfamethoxazole or aminoglycosides favours the emergence of thymidine-dependent SCVs in CF patients [2, 14]. This is in concordance with our data, as positive patients had been previously treated with aminoglycosides and/ or sulfonamides.

Some studies also show that the sensitivity of *S. aureus* to antimicrobials might be changed due to exposure to *P. aeruginosa* in a polymicrobial microenvironment.

It was shown experimentally that 2-n-heptyl-4-hydroxyquinoline-N-oxide and siderophores are involved in multiple *P. aeruginosa – S. aureus* interactions and provide *P. aeruginosa*-mediated killing and protection of *S. aureus* from antibiotics. *P. aeruginosa*-mediated killing of *S. aureus* occurs in a few hours after exposure, whereas *P. aeruginosa*-mediated tolerance of *S. aureus* to vancomycin can occur in the early stage of co-culturing. Additionally, *S. aureus* cells could be experiencing these *P. aeruginosa* exoproducts at a distance, resulting in a gradient of *P. aeruginosa*-mediated protection of *S. aureus* from antibiotics [3]. The presence of *P. aeruginosa* in our study was associated with the occurrence of SCV of *S. aureus* that had changed chemotherapeutical susceptibility.

In our study, above 21% of *S. aureus* isolates belonged to SCVs, but we are concerned that the real prevalence was lower than previously reported from CF studies in other countries [6, 14]. The prevalence of MRSA also was low in our centre. This could be related to either limited sample size or the young age of participants that have not acquired resistance yet; multiple antibiotic-resistant *S. aureus* SCVs were not common in this study also.

The suggested inductive effect on *S. aureus* SCVs via *P. aeruginosa* co-infection could be verified in our study. This data occurred in contrast to some previously described [14].

Recent publications e.g., *Melter et al.* reported that SCV variants are much more resistant to antibiotics than the non-SCV *S. aureus* isolates [2, 5]. Similarly, we found differences in the susceptibility rates between wild-type and SCV *S. aureus* isolates, in part of beta-lactams and aminoglycosides.

In conclusion, this first report on *S. aureus* SCVs from the Dnipro region (Ukraine) revealed a high number of *S. aureus* positive CF children but rather low SCV prevalence. The last might be due to cultivation features that were not considered unintentionally.

CONCLUSIONS

Infection *Staphylococcus spp*. among children of the Dnieper region is common in all age groups. The main pathogenic species *S. aureus* is also found in all age groups; more isolates were obtained in the 11-17 age group.

Most isolates of *S. aureus* obtained from children with Cystic Fibrosis have typical cultural properties. The proportion of isolates with phenotype of SCVs was 37.3%. Auxotrophs are characterized by the appearance after 42-72 hours of cultivation, small size (less than 1 mm), the absence of pigment and hemolysis, as well as the appearance of the colonies like «fried eggs». These points were the biggest features that distinguished SCVs of *S. aureus* from wild-type *S. aureus* in our study.

The appearance of auxotrophs in about 82% of cases was recorded from children who received aminoglycosides and/ or co-trimoxazole. All isolates of SCVs were obtained in a mixed culture with *P. aeruginosa*, the frequency in this group is significantly higher than in the group with the wild phenotype.

Isolates of SCVs belonged to both methicillin-sensitive and methicillin-resistant (n = 2 and n = 9, respectively).

In general, *S. aureus* has shown good chemotherapeutical sensitivity. The best results have been registered for the third generation aminoglycosides, the third generation fluoroquinolones, linezolid, rifampicin and tetracyclines. However, the isolation of methicillin-resistant isolates (phenotypically) among children is alarming, because it has been proven that successful antibiotic therapy is a prognostic factor for the outcomes [4, 8, 13].

Auxotrophs showed similar results to wild-type isolates in chemotherapeutic susceptibility, however, resistance to cefoxitin and gentamicin was significantly higher. There was no statistically significant difference in the frequency of isolation of susceptible isolates to individual antibiotics between both phenotypes.

Infection *S. aureus* as well as the presence of SCVs phenotype is predictive, consequently, must be monitored. This research demands the co-working between clinicians and laboratory specialists.

REFERENCES

1. Hatziagorou E., Orenti A., Drevinek P. et al. Changing epidemiology of the respiratory bacteriology of patients with Cystic Fibrosis – data from the European Cystic Fibrosis Society Patient Registry. J Cyst Fibros. 2019. doi: 10.1016/j.jcf.2019.08.006 .
2. Masoud-Landgraf L., Zarfel G., Kaschnigg T. et al. Analysis and Characterization of Staphylococcus aureus Small Colony Variants Isolated From Cystic Fibrosis Patients in Austria. Curr Microbiol. 2016; 72 (5): 606-611. doi: 10.1007/s00284-016-0994-z.
3. Orazi G., O'Toole G.A. Pseudomonas aeruginosa Alters Staphylococcus aureus Sensitivity to Vancomycin in a Biofilm Model of Cystic Fibrosis Infection. mBio. 2017; 8 (4): e00873-17. doi: 10.1128/mBio.
4. Li C., Wu Y., Riehle A. et al. Staphylococcus aureus survives in cystic fibrosis macrophages, forming a reservoir for chronic pneumonia. Infect Immun. 2017; 85: e00883-16. doi: 10.1128/IAI.00883-16.
5. Melter O., Radojevič B. Small Colony Variants of Staphylococcus aureus – review. Folia Microbiol. 2010; 55 (6): 548–558.
6. Wolter D.J., Onchiri F.M., Emerson J. et al. Prevalence and clinical associations of Staphylococcus aureus small-colony variant respiratory infection in children with cystic fibrosis (SCVSA): a multicentre, observational study. Lancet Respir Med. 2019; 7(12): 1027-1038. doi: 10.1016/S2213-2600(19)30365-0.
7. Kahl B.C. Small colony variants (SCVs) of Staphylococcus aureus – A bacterial survival strategy. Infect. Genet. Evol. 2013; 21: 515-522. doi: 10.1016/j.meegid.2013.05.016 .
8. Blanchard A.C., Waters V.J. Microbiology of Cystic Fibrosis Airway Disease. Semin Respir Crit Care Med. 2019; 727–736. doi: 10.1055/s-0039-1698464 .
9. Ventola C.L. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015; 40 (4): 277-283.
10. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, 2019. http://www.eucast.org.
11. The European Committee on Antimicrobial Susceptibility Testing. Aminoglycoside in vitro susceptibility test interpretation criteria evaluations. Version 1.3, 2019. https://app.box.com/s/un3-r9bjyulkecbeu3uji34i5c9mbkii7 .
12. van Belle G., Lloyd D. et al. Biostatistics: A Methodology for the Health Sciences. 2-nd Edition. John Wiley & Sons, Inc; 2002.
13. Chadha A.D., Thomsen I.P., Jimenez-Truque N. et al. Host Response to Staphylococcus aureus Cytotoxins In Children with Cystic Fibrosis. J Cyst Fibros. 2016; 15 (5): 597–604. doi:10.1016/j.jcf.2015.12.023.
14. Wolter D.J., Emerson J.C., McNamara S. et al. Staphylococcus aureus small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis. 2013; 57: 384–391. doi: 10.1093/cid/cit270.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 21.09.2020
Accepted: 02.06.2021

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article