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PULMONARY BACTERIAL PATHOGENS IN PATIENTS WITH CYSTIC FIBROSIS

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Abstract

The aim of the research is to perform microbiological analysis of respiratory tract samples in patients with cystic fibrosis and to verify the aetiology, prevalence and antibiotic sensitivity of the isolated pathogens.

Sixteen patients with cystic fibrosis aged from 3 months to 30 years were included. Twenty-nine respiratory and one digestive tract samples were tested. The identification of isolated pathogens was performed using conventional methods and automated miniAPI and VITEK 2 systems (bioMerieux, France). Antibiotic sensitivity of all isolates was determined by a disk diffusion test or the minimum inhibitory concentration method.

A total of 34 microbial strains from 15 patients were isolated: Gram-positive bacteria 12 (35.29%), Gram-negative bacteria 20 (58.82%) and yeast-like fungi 2 (5.88%). *S. aureus* is the dominant pathogen among Gram-positive bacteria. Most of the Gram-negative bacteria are identified as *P. aeruginosa* and other non-fermenting glucose bacteria and the rest as *H. influenzae* and *E. coli*. *P. aeruginosa* was isolated from patients in different age groups. The strains showed good sensitivity to antibiotics.

Microorganisms isolated from the respiratory tract in patient with cystic fibrosis vary over the period of follow-up. Gram-negative bacteria, especially *P. aeruginosa*, are dominant but showed good sensitivity to antimicrobial agents and no multidrug-resistant strains were isolated.

Key words: cystic fibrosis, infections, aetiology, *S. aureus*, *P. aeruginosa*

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Introduction. Cystic fibrosis is one of the most common autosomal recessive diseases within the Caucasian population. In Bulgaria the disease occurs in 1:2500 new-borns [1, 2]. The most common cause of complications and mortality among patients with cystic fibrosis is chronic bacterial infection [1]. The results from microbiological examination of sputum in patients with cystic fibrosis have changed significantly due to the improved treatment and prolonged life expectancy. Increasingly, atypical and multi-resistant microorganisms are isolated, including the mucoid strain of *P. aeruginosa*, *Burkholderia cepacia,* and others. A number of studies [3] show variability in the control of initial infections, the possibility of early treatment of exacerbations, isolation of bacterially infected patients, and optimization of antibiotic treatment [4]. The purpose of this study is to determine the aetiology of bacterial infection and specific phenotypic features of the most common pathogens in patients with cystic fibrosis, as well as their sensitivity to antibacterial drugs.

Materials and methods. ***Patients.*** For the period from September 2018 to July 2019, microbiological examination was carried out on 16 patients with cystic fibrosis treated in the Clinic for Pediatric Diseases of the University Hospital in Pleven. The mean age of the patients was 9 years and 4 months (from 3 months to 30 years) distributed in age groups as presented in Fig 1. Seven of the patients were female and the other nine were male. The diagnosis cystic fibrosis was made on the basis of clinical manifestation, a twofold increase in sweat chlorides and the presence of mutations in the CFTR gene.

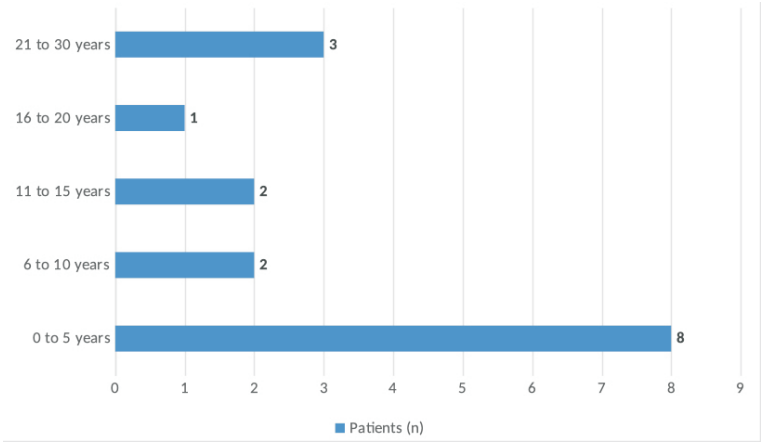

Fig. 1. Patients distributed in age groups

Materials. A total of 30 samples were examined, 29 of which were from the respiratory system and one from the digestive system (faeces). For the study period in five patients' samples were examined once, in 8 cases – two samples and in three patients – three samples at different time intervals. The average interval between samples was 3.6 months (from 1 to 8 months).

Methods. The samples from respiratory system (sputum, tracheal aspirates) were cultured on chocolate agar in 10–12% CO_2 atmosphere, blood agar (5% sheep blood) and aerobic Levin setting. The digestive system material was cultured on apocholate-citrate agar, Levin setting and selenite broth. The identification of the isolated microorganisms was performed according to international standards using conventional biochemical methods and automated miniAPI and VITEK 2 systems (bio Merieux, France). The sensitivity analysis of all isolated microorganisms to antibiotics was performed by the disk diffusion test or the method of determining minimum inhibitory concentration.

Results. From 28 samples, 34 microbial strains were isolated as presented in Fig. 2. In only two of the samples normal flora of the upper respiratory tract was isolated.

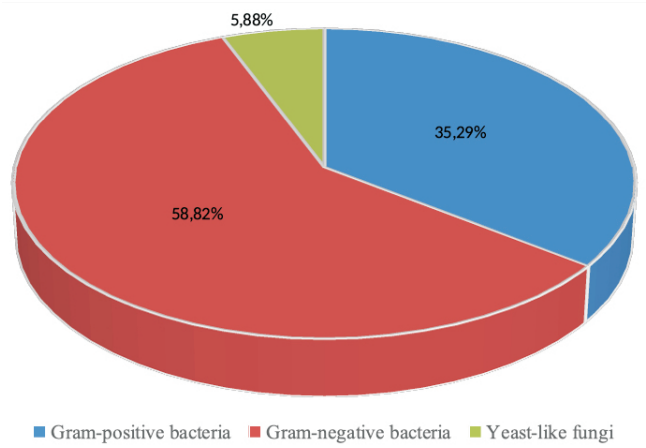

Fig. 2. Isolates in the study group

The dominant pathogen among the isolated Gram-positive bacteria was *Staphylococcus aureus* – 11 (91.6%). In only one case it was *Streptococcus pyogenes* – from sputum. *S. aureus* strains were isolated from 8 patients, in three cases *S. aureus* was isolated twice from two different sputum samples taken at intervals of 1 to 5 months. In seven cases, *S. aureus* was isolated in pure culture and in the other four samples in association with Gram-negative bacteria. *S. aureus* was isolated from patients of different age (3 months to 30 years), with a mean age of 12 years and 5 months. The pathogens showed sensitivity to antimicrobials (penicillin, methicillin, erythromycin, clindamycin, vancomycin, teicoplanin, ciprofloxacin and trimethoprim/sulfamethoxazole), seven of them being resistant only to penicillin and two on both erythromycin and clindamycin.

Among the Gram-negative bacterial strains the non-fermenting glucose Gram-negative bacteria was dominant – 15 (75%), followed by *Haemophillus influenzae* – 3 strains and *Escherichia coli* – 2 strains. Among the non-fermenting glucose Gram-negative bacteria, the dominant pathogen was *Pseudomonas aeruginosa* 13

(86.6%), followed by *Ochrobactrum anthropi* and *Alcaligenes faecalis* – one strain isolated from different patients. The thirteen *P. aeruginosa* strains were isolated from 9 patients, 4 of them being isolated twice at different time intervals – from 1 month in one patient and 7 months' intervals in the other three. The isolated strains showed extremely phenotypic diversity both in terms of the colour of pigments formed and in the mucosal appearance of the colonies. Mucoid phenotypes were dominant, with a tendency for mucoid strains to revert to non-mucoid phenotypes upon repeated subculturing or refreshment after freezing. The Gram preparations of the mucoid phenotypes exhibited an extremely strong capsule-like substance around the cells, exceeding the thickness of the cell itself.

Some of the strains grew with a pronounced zone of beta-haemolysis around the colonies on blood agar. All isolated *P. aeruginosa* strains showed good sensitivity to antibiotics (piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, tobramycin, trimethoprim/sulfamethoxazole, ciprofloxacin and levofloxacin). Most of the isolated strains were resistant only to trimethoprim/sulfamethoxazole, and 3 of them were with intermediate sensitivity to ciprofloxacin. The *A. anthropi* strain was resistant to beta-lactam antibiotics, including imipenem and meropenem, as well as tobramycin, and the *A. faecalis* strain was resistant only to trimethoprim/sulfamethoxazole.

Candida albicans strains were isolated from two patients in one case as pure culture and in the other in association with *E. coli*.

Discussion. Chronic lung infection in patients with cystic fibrosis is usually associated with a limited number of microorganisms.

The 2019 Consensus Guidelines [4] recommend clear infection control policies to be put in place and implemented. Patients with cystic fibrosis are at particular risk of developing multidrug-resistant organisms, such as methicillin-resistant *S. aureus*, *P. aeruginosa* and *B. cepacia* [5]. Most commonly *S. aureus*, *P. aeruginosa*, patients are divided into groups according to the type of bacterial agent: *B. cepacia*, multiresistant *S. aureus*, *P. aeruginosa*, and non-pseudomonas infections. There is significant difference in the type of the infection by age, i.e. *B. cepacia* occurs in only 9% of children and in 95% of adults [4].

In our study, half of the patients had *S. aureus* in sputum. Our data on the age at which this pathogen was isolated most often is in concordance with the results of other studies. The mean age of our patients was 12 years and 5 months. According to Kiska and Liddell [6], the incidence of colonization with *S. aureus* increases until puberty is completed, and then decreases. According to them, by 2008, *S. aureus* was isolated in 50.9% of patients with cystic fibrosis, with the highest incidence being detected between the ages of 6 and 17 years.

P. aeruginosa is the most common bacteria in patients with cystic fibrosis and it is present in up to 80% in the age between 25–35 years [4]. This leads to faster deterioration of lung function [7, 8], more severe disease [9] and reduced of life expectancy [10].

Respiratory tract colonization with *P. aeruginosa* can occur at any time in the patient's life. Although, in early childhood, *S. aureus* is considered a leading pathogen, according to most authors, between 70% and 80% of patients are colonized with *P. aeruginosa* by the age of majority [11].

Piedrola et al. [8] reported that colonization with this microorganism can reach up to 95% in patients older than 3 years. In 9 of our patients (56.2%), we observed colonization and infection with *P. aeruginosa*. The mean age of the patients was 10 years and 9 months (from 3 months to 30 years), but it is noteworthy that the majority of patients were in the age group of 0 to 5 years – two of the children being 3 and 4 months old, respectively.

Although various toxins, pigments, and enzymes are mentioned among the virulence factors of the strains, the importance of the *P. aeruginosa* mucoid phenotypes is undeniable [8]. If the colonization with *P. aeruginosa* is not prevented in early childhood, this microorganism is isolated permanently and almost always mutates in the mucoid strain. As a result, conversion to the mucoid phenotype is associated with significantly higher morbidity and mortality [11, 12]. In our patients with *P. aeruginosa* infection, there were 4 deceased patients, one within the study period and three one year after the study end, three of them with isolated mucoid phenotypes two or three times.

As reported by other authors, the emergence of the mucoid phenotype is a result of mutations in various genes, including algD, which encodes a guanosine diphosphate-mannose dehydrogenase enzyme and catalyses the last step in the synthesis of one of the alginate precursors [3]. In two independent studies from the United States and Denmark, approximately 80% of *P. aeruginosa* isolates were mucoid. In contrast, in patients without cystic fibrosis, only 3% of isolated *P. aeruginosa* strains from the respiratory tract were mucoid [13].

Although the high percentage of multidrug-resistant *P. aeruginosa* has been reported [14], our results showed good sensitivity of the isolates to antimicrobial agents. We did not isolate multidrug-resistant strains.

In our study, other bacterial pathogens were isolated at a lower frequency – *H. influenzae* and *E. coli*. *H. influenzae* strains were isolated from three different patients aged 14, 23 and 30 years, and in all three cases they were in association with *P. aeruginosa* or other non-fermenting glucose bacteria, so it was difficult to determine their importance as leading pathogens.

Two strains of rare non-enzymatic glucose non-fermenting Gram-negative bacteria were isolated – *Ochrobactrum anthropi* and *Alcaligenes faecalis* from two different patients aged 2 years and 7 months and 23 years – in both cases in association with *S. aureus*. Despite the low incidence, these bacteria are recognized as typical pathogens in patients with cystic fibrosis. *O. anthropi* are Gram-negative non-fermenting glucose rods, widely distributed in the environment but rarely reported as bacterial pathogens. It has been reported as a causative agent of infections in both immunocompromised and immunocompetent patients, including

those with cystic fibrosis [7, 15]. It is characterized by its high antibiotic resistance [16], as shown by the isolated strain, as well as its inability to be precisely identified by conventional methods and the need of using automated systems and genetic methods [16, 17].

The purpose of the treatment of bacterial infection in patients with cystic fibrosis is to prevent colonization with *P. aeruginosa* and *B. cepacia* and to prevent and delay the onset of chronic infection and to prevent lung deterioration.

The microorganisms isolated from the respiratory tract in patients with cystic fibrosis vary over the period of follow-up. Gram-negative bacteria, especially *P. aeruginosa*, dominate. Recent isolates from patients with cystic fibrosis show specific phenotypic features. Periodic monitoring of patients throughout their lives helps the control of colonization and prevents the development of bacterial infections.

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