Analysis of the frequency of isolation and drug resistance of microorganisms isolated from the airways of adult CF patients treated in the Institute of Tuberculosis and Lung Disease during 2008–2011

Abstract

Introduction: Cystic fibrosis (CF) is the most common genetic autosomal recessive genetic disease. The most serious symptoms are observed in the lungs. Recurrent respiratory infections are the main cause of hospitalization and death among cystic fibrosis patients. Pathogens that commonly infect the airways of adult CF patients include *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The aim of this study was to analyse the microorganisms cultured from the airways of adult CF patients and to perform antimicrobial resistance tests of the most frequently isolated bacteria.

Material and methods: In this study, 1422 isolates of 89 CF patients were collected during a 4-year period. The microorganisms were cultured and identified according to standard microbiological procedures. Identification and drug susceptibility were performed in a Phoenix (BD) automatic system, Vitek2Compact (bioMérieux) and disk-diffusion method by Kirby-Bauer. Among the 1422 strains, the most frequent pathogens were *Pseudomonas aeruginosa* (55.6%) and *Staphylococcus aureus*. A total of 482 (61%) strains of 790 isolates of *P. aeruginosa* were identified as *P. aeruginosa* of mucoid phenotype. The isolates with mucoid phenotypes were more susceptible than non-mucoid. Eighty-six strains of *S. aureus* showed resistance to methicillin (MRSA), which accounted for 16.0% of all strains of *S. aureus*.

Conclusions: The analysis of microbiological materials from adult CF patients treated in IGiChP allowed us to determine the prevalence of potentially pathogenic microorganisms. The data obtained are consistent with literature.

Key words: antimicrobial resistance, cystic fibrosis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*
Owing to the inflammatory process caused by developing bacterial infection, bronchi and lung tissue are destroyed to a different degree of intensification, and finally, chronic respiratory failure occurs [1, 9–11].

The aetiology of bacterial infections in CF patients changes with age and depends on the degree of progression of the disease [5, 12]. *Staphylococcus aureus* and *Haemophilus influenzae* are isolated mainly from paediatric subjects. Along with the development of the disease, in adult patients, *Pseudomonas aeruginosa* strains are isolated predominantly, rarely — *Stenotrophomonas maltophilia, Burkholderia cepacia complex* (Bcc) and *Achromobacter* spp. [2, 9, 13–16].

In cystic fibrosis vital clinical importance is attributed to infections caused by *P. aeruginosa* [7, 9, 13]. During the initial stage of the disease the strains of this species without a mucous capsule, colonize the airways. In chronic infection, conversion of *P. aeruginosa* strains into mucous form is observed; this phenotype is connected with increased secretion of exocellular polysaccharides [17–19]. These strains demonstrate the change of the colony’s morphology, the change of lipopolysaccharide (LPS) from smooth form into a rough one, resulting from shortened O-specific chains, and a limited proteolytic activity. Many mucoid *P. aeruginosa* strains are deprived of the possibility of production of cilia and pili. Mucoid phenotype of *P. aeruginosa* in adult patients is a factor deepening the bronchi and lung damage [8, 18, 20]. Complete eradication of the pathogen from the patient’s organism is not possible; there is only a chance to reduce the number of bacteria in the place of infection and local limitation of inflammatory process. Early diagnosis of *P. aeruginosa* enables the start of intensive treatment in order to delay transition of infection into a chronic form [10, 13, 19–22].

Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are also observed in cystic fibrosis patients. According to data from the Cystic Fibrosis Foundation Patient Registry of 2010, MRSA strains have been cultivated from 25.7% of patients with cystic fibrosis [13, 23, 24].

Bacterial infections in this group of patients pose a serious diagnostic and therapeutic problem. In order to provide patients with the best care, there is the need for close cooperation of a clinician and a microbiologist. The result of microbiological examination enables therapy to be initiated directed at an appropriate aetiological factor of infection. An intensive, appropriate for a given infection, antibiotic therapy reduces the frequency of aggravations, inhibits progression of the disease and prolongs the life of CF patients [7, 9, 16]. The reliability of the result depends on the proper taking up of material and the time of transportation to the laboratory. Too long time between taking up of material and running microbiological examination, and improper conditions of storage and transport of the samples, may cause the lack of growth of some microorganisms [8, 25, 26].

Evaluation of microbiological material from the airways is extremely difficult due to the presence of a rich bacterial flora therein, which hinders the proper identification of any aetiological factor of infection.

Intensively proliferating mucoid *P. aeruginosa* prevents isolation of Gram-positive bacteria such as *S. aureus* and more challenging Gram-negative *H. influenzae* and *B. cepacia* complex. The use of selective media that inhibit the growth of *P. aeruginosa* is helpful in isolating *S. aureus* and *H. influenzae*, and necessary to isolate *B. cepacia* complex [5, 7, 11, 26].

It should be emphasized that in microbiological diagnosis of cystic fibrosis it is important to extend culture to 48 hours due to slow-growing mucoid *P. aeruginosa* and *S. aureus* SCVs (small colony variants). The culture of Bcc may be extended even to 5 days [5, 7, 11, 26].

Evaluation of drug-resistance of *P. aeruginosa* strains still arouses much controversy. It is caused by the presence of different colony morphotypes and diversified antibiotic susceptibility within one strain’s genotype isolated from diagnostic material. The guidelines of the UK Cystic Fibrosis Trust Microbiology Laboratory Standards Working Group of September 2010 [26] recommend the assessment of drug-resistance of *P. aeruginosa* strains by using the disk-diffusion method and/or determination of MIC (Minimum Inhibitor Concentration) values, since evaluation of antibiotic susceptibility by using an automatic method generates too many mistakes [18, 26, 27].

Progress in medicine makes the lifespan of CF patients longer. However, the constantly growing proportion of adult patients entails the necessity of providing patients with specialist care. The Institute of Tuberculosis and Lung Diseases is one of the few units in Poland dealing with the treatment of adult CF patients.

The aim of the study was to assess the spectrum of microorganisms isolated from clinical materials from cystic fibrosis patients, treated in the Outpatient Clinic and the First Department of Lung Diseases of the Institute of Tuberculosis and Lung Diseases in the period from January 2008 to
December 2011, and to evaluate the drug-resistance of isolated strains.

**Material and methods**

Eighty-nine cystic fibrosis patients, treated in the Institute of Tuberculosis and Lung Diseases in the years 2008–2011, hospitalized in the First Department of Lung Diseases of the Institute of Tuberculosis and Lung Diseases and treated in the Outpatient Clinic, were analysed. A total of 1,422 isolated strains from 1,078 materials (1,062 spu- ta and 16 bronchoscopic materials) were examined.

**Bacterial strains**

Strains were identified by using routine diagnostic methods. Phenotypic properties, biochemical traits and drug susceptibility of the studied strains were analysed. Microbiological diagnosis was made based on cultures grown on solid media Columbia blood agar (bioMérieux, Oxoid), MacConkey agar (bioMérieux, Oxoid), Mannitol-salt agar (bioMérieux, Oxoid),Chocolate agar (bioMérieux, Oxoid) and medium to identify *Burkholderia cepacia* complex (BD Cepacia Medium). Columbia blood agar and Chocolate agar were incubated for 24 and 48 hours at a temperature of 35–37°C in an atmosphere of 5–10% of CO₂; MacConkey agar, BD Cepacia Medium and Mannitol-salt agar were incubated for 24 and 48 hours at a temperature of 35–37°C. The cultured strains were identified using automatic system Phoenix (BD) and Vitek2Compact (bioMérieux). For identification of *P. aeruginosa*, *H. influenzae* and *X* (hemina), and by using the API NH test (bioMérieux). Identification of *Streptococcus* beta-haemolitícus commercial tests were used. *P. aeruginosa* was identified using a positive oxidase test and classic biochemical tests. *H. influenzae* was classified as a specific species based on the demand for growth factors V (NAD) and X (hemina), and by using the API NH test (bioMérieux). Identification of *Streptococcus* beta-haemolitícus in the group was made using a latex agglutination test for group typing of *Streptococcus* from serologic groups A, B, C, D, F and G according to Lancefield Slidex Strepto Plus (bioMérieux).

*S. aureus* strains were identified in an automatic system and by using the following tests: 1) latex agglutination test Slidex-Phast Kit (bioMérieux); 2) test tube — evaluating the ability to produce coagulase, with the use of lyophilized plasma (BectonDickinson).

**Resistance tests**

Drug-resistance of the strains was defined in a Phoenix (BD) automatic system and Vitek-2Compact (bioMérieux). Antibiotic susceptibility of *P. aeruginosa* strains was determined using the Kirby-Bauer disk-diffusion method and/or by determination of MIC with the use of strips with antimicrobial concentration gradient. *In vitro* drug activity towards *H. influenzae* was made on HTM medium (Haemophilus Test Medium) using the disk diffusion susceptibility method. Susceptibility of beta-haemolytic streptococcus was determined in Mueller-Hinton 2 medium with Sheep Blood (bioMérieux). In relation to the recommendations for determination of antimicrobial susceptibility that were introduced in May 2011 by the European Committee for Antimicrobial Susceptibility Testing (EUCAST), drug-resistance of *Haemophilus influenzae* strains and beta-haemolytic streptococcus were determined on Mueller-Hinton medium with Horse Blood + 20 mg/l β-NAD.

The results were interpreted in accordance with recommendations of the National Reference Centre for Antimicrobial Susceptibility and American recommendations of CLSI, and since May 2011, in accordance with the guidelines of the National Consultant for medical microbiology according to EUCAST.

**Results**

Eighty-nine cystic fibrosis patients between 19 to 42 years of age (49 women and 40 men), treated in the Institute of Tuberculosis and Lung Diseases in the years 2008–2011, were analysed.

Among the 89 patients, from 22 (24.7%) only one microorganism was isolated, from 39 (43.8%) there were two (the most frequently *S. aureus* and *P. aeruginosa*), and from 28 (31.5%) there were more than three (Fig. 1).

A total of 1,078 materials taken from the airways of CF patients were analysed. From the material studied, 1,422 strains were cultured. *P. aeruginosa* was isolated from 74 (83.1%) patients treated in the Institute of Tuberculosis and Lung Diseases. Mucoid strains were cultured from 49 (55.1%) patients, and non-mucoid from 55 (61.8%) patients, treated in the Institute of Tuberculosis and Lung Diseases. Among the 89 patients studied, from 68 (76.4%) *S. aureus* strains were isolated, and in 16 patients (18.0%) MRSA were noted (Table 1).

Among all strains studied, 790 (55.6%) were classified as *P. aeruginosa*, and 538 (37.8%) as *S. aureus*. Gram-negative bacteria were represented by *S. marcescens* (0.9%), *K. pneumoniae* (0.7%), *E. coli* (0.6%) and *Enterobacter* spp. (0.2%). Among non-fermenting bacteria other than *P. aeruginosa,*
14 strains of *S. maltophilia* (0.9%) and 4 strains of *A. baumanii* complex (0.3%) were identified. The following bacteria were isolated from the material analysed: *Achromobacter* spp. — 22 strains (1.5%), *H. influenzae* — 11 strains (0.8%) and 9 strains of beta-haemolytic streptococcus (0.7%).

Figure 2 shows the species contribution of microorganisms isolated from clinical materials taken from cystic fibrosis patients in the years 2008–2011.

Among the 790 strains of *P. aeruginosa* studied, 482 (61.0%) were classified as mucoid phenotypes.

Analysis of drug-resistance of mucoid and non-mucoid phenotypes showed greater susceptibility of mucoid phenotypes to the antibiotics studied. The proportion of mucoid *P. aeruginosa* susceptible to aminoglycosides ranged from 42.5% (to gentamycin) to 67.4% (to tobramycin), and for non-mucoid phenotypes — from 25.7% (to gentamycin) to 50.7% (to tobramycin). Susceptibility to beta-lactam antibiotics ranged from 66.0% (to ceftazidime) to 72.2% (to meropenem) for mucoid phenotype, and from 46.1% (to piperacillin with tazobactam) to 56.2% (to meropenem) for non-mucoid phenotype. Susceptibility to ciprofloxacin reached 59.1% for mucoid phenotypes and 41.2% for non-mucoid phenotypes. All studied strains showed susceptibility to colistin (Fig. 3).

All methicillin-sensitive *Staphylococcus aureus* (MSSA) showed susceptibility to linezolid and glycopeptides (vancomycin and teicoplanin). More than 90% of the strains studied were susceptible to trimethoprim/sulfamethoxazole, and 88.9% - to tobramycin. The proportion of strains susceptible to erythromycin was 45.8%, and to clindamycin — 63.9%. 61.1% of strains were susceptible to ciprofloxacin.

Among the 538 *S. aureus* strains, 86 (16.0%) demonstrated resistance to methicillin. The analysed MRSA showed lower susceptibility to the antibiotics studied than MSSA. More than 50% of analysed MRSA proved to be susceptible to trimethoprim/sulfamethoxazole, and 66.3% — to tobramycin. Susceptibility to erythromycin amounted to 24.4%, and to clindamycin — 61.6%. 24.4% of strains were susceptible to ciprofloxacin. All MRSA strains demonstrated susceptibility to linezolid and glycopeptides (vancomycin and teicoplanin) (Fig. 4).

---

Table 1. Microorganisms isolated from the airways of 89 patients with cystic fibrosis

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Number of isolates (%)</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucoid phenotype</td>
<td>308 (21.7)</td>
<td>55 (61.8)</td>
</tr>
<tr>
<td>Non-mucoid phenotype</td>
<td>482 (33.9)</td>
<td>49 (55.1)</td>
</tr>
<tr>
<td>Mucoid and non-mucoid phenotype</td>
<td></td>
<td>64 (71.9)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA</td>
<td>452 (31.8)</td>
<td>68 (76.4)</td>
</tr>
<tr>
<td>MRSA</td>
<td>86 (6.0)</td>
<td>16 (18.0)</td>
</tr>
<tr>
<td><em>Achromobacter</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 (1.5)</td>
<td>8 (9.0)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>14 (0.9)</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>14 (0.9)</td>
<td>7 (7.9)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>11 (0.8)</td>
<td>7 (7.9)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>9 (0.7)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td><em>Streptococcus</em> beta-haemoliticus (gr. C, G)</td>
<td>9 (0.7)</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8 (0.6)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td><em>Acinetobacter baumanii</em> complex</td>
<td>4 (0.3)</td>
<td>2 (2.2)</td>
</tr>
</tbody>
</table>
Discussion

Chronic bronchopulmonary disease changes in the respiratory system dictate the quality and life expectancy of cystic fibrosis patients [4–7, 30]. Data from the literature indicate that the species contribution of pathogens in airway infections in cystic fibrosis patients depends on the age of the patient [12].

The appropriate treatment of CF patients prolongs their lives; therefore, it is necessary to monitor the occurrence of microorganisms in the airways and determine the drug-resistance of isolated strains [5, 7, 28, 29].

Treatment of airway infections of CF patients will be effective if it is based on the results of microbiological examinations that take into consideration the drug-resistance of isolated microorganisms [31]. Thus, it is important to know local bacterial flora and their corresponding drug-resistance, especially in cases when experimental treatment is going to be involved [11].
Microbiological diagnosis of lower respiratory tract infections involves cultures, sputa, bronchial secretion or washings (bronchoalveolar lavage) [27]. In this paper the most frequent material obtained from patients was sputum (98.5%).

In children with cystic fibrosis, in the first years of disease infections caused by Staphylococcus aureus and Haemophilus influenzae lead to epithelium damage, which facilitates colonization by other microorganisms. The most commonly isolated species from adult CF patients is Pseudomonas aeruginosa, which causes chronic infection with subsequent exacerbations [7, 9, 11, 16].

In this paper we analysed the frequency of isolation of microorganisms and the susceptibility of the isolated strains taken from adult CF patients treated in the Institute of Tuberculosis and Lung Diseases in Warsaw.

The study group consisted predominantly of patients from whom two pathogens were isolated — 39 (43.8%) patients. More than three pathogens were isolated from 28 (31.5%) patients, and one pathogen was cultured from 22 (24.7%) patients. In the research conducted by Moore et al. the most numerous group constituted patients from whom barely one microorganism was isolated — 53%, from 38% of patients two microorganisms were isolated, and from the remaining 4% of patients three microorganisms were isolated [28]. A different profile of isolation of microorganisms from cystic fibrosis patients was presented by Paixão et al. They discovered that from 85% of patients only one microorganism was isolated [12].

In our study among airway infections in adult cystic fibrosis patients, infections of P. aeruginosa aetiology predominated, which is in accordance with reports by other authors [27, 28, 32]. P. aeruginosa was isolated from 83.1% of patients; in the majority (61.8%) it was of non-mucoid phenotype. The research results presented by Valenza et al. [30] and Paixão et al. [12] also indicated higher participation of non-mucoid P. aeruginosa in the group of adult patients. However, Paschola et al. noted higher participation of mucoid P. aeruginosa [32]. According to the researchers, there is a close relationship between the presence of mucoid strains of P. aeruginosa and the worsening of functional indices of the respiratory system in cystic fibrosis patients as mucoid bacteria do not undergo eradication and show high resistance to antibiotics [9, 12, 20].

The results presented show higher susceptibility to mucoid P. aeruginosa than to non-mucoid strains. The highest activity to all studied P. aeruginosa strains was shown by colistin, carbapenems and combinations of -lactams and inhibitors (piperacillin/tazobactam). Similar results were obtained by Srifuengfung et al. [33] and Valenza et al. [30]. Paixão et al. [12] noted higher susceptibility of non-mucoid P. aeruginosa to antibiotics. However, irrespective of resistance phenotype, isolation of mucoid strains from patients is an adverse predictor as these strains produce exopolysaccharide.
biofilm, and in this way they become resistant to phagocytosis.

It was shown in this paper that the second, in respect of prevalence, microorganism isolated from patients was *S. aureus*. These data are similar to other reports, in which the frequency of colonization by *S. aureus* strains ranged from 10.1% to 57.0% [17, 27, 28, 30, 32].

The problem concerning the treatment of cystic fibrosis patients are *S. aureus* strains resistant to methicillin. In this paper methicillin resistant *S. aureus* strains (MRSA) were isolated from 18.0% of patients, which corresponds to the research from other centres. In the USA MRSA was isolated from 18.8% of patients [27], in Spain — from 18% [34], in Brasil — from 6% and in Germany — from 5% [30]. The risk of occurrence of MRSA strains among patients increases with the number and length of hospitalizations. Valenza et al. suggest that the prevalence of MRSA strains in CF patients correlates with the prevalence of infections of MRSA aetiology in many other groups of patients in a given country [30].

Among the analysed *S. aureus* strains the majority were susceptible to cefoxitin, which is connected with susceptibility to isoxazolyl penicillin, penicillins with inhibitor and cephalosporins active towards *Staphylococcus*. In the material studied all *S. aureus* strains were susceptible to glycopeptides and linezolid. Similar results were obtained by Valenza et al. and Puxaio et al. [12, 30].

Many researchers report on isolation the pathogens such as *Stenotrophomonas maltophilia* and Achromobacter sp in adult cystic fibrosis patients. We also confirmed the occurrence of these microorganisms in our material. The prevalence of *S. maltophilia* and *Achromobacter* spp. in the group analysed was 0.9% and 1.5%, respectively. Burns et al. noted a higher proportion of occurrence of the above-mentioned microorganisms: 10.3% and 8.7%, respectively [27]. The influence of the above-mentioned pathogens on the course of the disease and the length of patients’ lives is unknown. The observed increase in the frequency of isolation of such strains may result from a facilitated colonization of a changed ecological niche of cystic fibrosis patients [29, 35].

According to the available literature, *H. influenzae* is often isolated from clinical materials of CF patients at an initial stage of the disease, rarely during the third decade of their lives [8, 16, 18]. The results obtained in this paper confirm that in the group of adult patients the proportion of isolated *Haemophilus* was low, in the material taken from 7.9% of patients. According to the literature, the proportion of adult patients from whom this species was isolated varied from 1.5% to 24.0% [17, 30, 32, 36].

Since the beginning of the 1980s *Burkholderia cepacia* complex (Bcc) has been isolated from clinical materials taken from cystic fibrosis patients [6, 9, 18]. Ten genomic variants of this pathogen are currently distinguished. *B. cenocepacia* and *B. multivorans* are usually isolated from cystic fibrosis patients. Although the frequency of colonization in cystic fibrosis is low, the presence of pathogens in the culture is an adverse prognostic factor [13, 14, 31]. The occurrence of Bcc bacteria in the airways is connected with the worsening of lung function and increased frequency of hospitalizations, and disqualifies patients from lung graft. The consequence of Bcc infections is necrotic lung inflammation, often with complicated septicemia [2]. Furthermore, a therapeutic problem is resistance of Bcc to numerous groups of antibiotics and chemotherapeutic agents. The percentage of Bcc isolations differs significantly in individual centres and results probably from the possibility of the pathogen’s identification and patient-to-patient transmission of the strains or infections from unknown sources [6, 13, 15, 16, 18]. In this study *Burkholderia* was not isolated from the group of adult patients. Similar results were presented by Perpati et al. [36]. In the studies of other authors the proportion of Bcc isolation ranged from 1.8% to 22.5% [28, 29, 32].

The results obtained in this paper and data from literature confirm that in order to allow the clinician to apply an appropriate therapy there is the need to conduct systematic microbiological research in cystic fibrosis patients.

**Conclusions**

1. The microbiological analysis of materials taken from adult cystic fibrosis patients treated in the Institute of Tuberculosis and Lung Diseases enabled the prevalence of potentially pathogenic microorganisms to be established:
   — in lower airway infections in cystic fibrosis patients *Pseudomonas aeruginosa* (55.6%) and *Staphylococcus aureus* strains (37.8%) predominate;
   — mixed bacterial infections were discovered in 66 (74.2%) patients.

2. Higher susceptibility to antibiotics was noted in mucoid *Pseudomonas* strains. The greatest activity towards *P. aeruginosa* strains was shown by colistin, piperacillin with tazobactam, imipenem and meropenem.
3. All *S. aureus* strains were susceptible to glycopeptides and linezolid.

4. An appropriate microbiological diagnosis of materials taken from cystic fibrosis patients and expertise in microorganisms colonizing the airways allows a clinician to apply the best available therapy.

References:


