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CROSS-INFECTIONS WITH *PSEUDOMONAS AERUGINOSA* IN PATIENTS WITH CYSTIC FIBROSIS ATTENDING THE WARSAW CENTRE

ZAKAŻENIA KRZYŻOWE *PSEUDOMONAS AERUGINOSA* U PACJENTÓW Z MUKOWISCYDOZĄ POZOSTAJĄCYCH POD OPIEKĄ INSTYTUTU MATKI I DZIECKA

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Abstract

Aim: 1. To assess the prevalence of cross-infections with *P. aeruginosa* in order to evaluate the epidemiological situation of this infection in patients with cystic fibrosis attending our centre; 2. To correlate the clinical features of the patients carrying a potentially transmissible strain with the entire study group in order to determine the risk factors and possible effects of its acquisition.

Material and methods: 170 *Pseudomonas aeruginosa* strains obtained from the respiratory tract of 75 cystic fibrosis patients attending the Warsaw Centre in 2011 and 2012 were typed using restriction enzyme analysis-pulsed field gel electrophoresis (*Spe I* restriction enzyme was used). Simultaneously, the information concerning contacts between patients, as well as several clinical data regarding the course of the disease were collected.

Results: Twenty four clusters of strains were detected. The main cluster included 49 isolates derived from 21 patients. The other detected clusters included 2 to 12 isolates derived from 1 to 7 patients. Three clusters comprised the isolates derived from three pairs of siblings. There were 15 clusters containing 2 to 7 strains belonging to the same patient. The remaining 24 patients were infected with their own strains, not fitting any clonal group. Several clinical parameters showed that the 21 patients whose strains constituted the main cluster, were in worse clinical condition than the other patients in the study group. Moreover, the total duration of their hospitalizations in order to perform intravenous antibiotic treatment was longer.

Conclusions: 1. Frequent hospitalizations of CF patients with a more severe course of the disease seem to be a risk factor of cross-infections with *P. aeruginosa*. 2. Intensification of measures to prevent cross-infection, such as hygienic precautions, patient segregation, introduction of home intravenous antibiotic therapy programme, as well as further education of patients and their parents should lead to the improvement of the epidemiological situation in our centre.

Key words: cystic fibrosis, *Pseudomonas aeruginosa*, cross-infections, PFGE

Streszczenie

Cel: 1. Ocena częstości występowania zakażeń krzyżowych *Pseudomonas aeruginosa* i ich możliwych konsekwencji klinicznych u pacjentów chorujących na mukowiscydozę leczonych w naszym ośrodku; 2. Porównanie cech klinicznych grupy pacjentów, u których doszło do zakażenia krzyżowego z cechami klinicznymi całej grupy badanej, w celu określenia czynników ryzyka i możliwych skutków takiego zakażenia.

Materiał i metody: 170 szczepów *Pseudomonas aeruginosa* wyizolowanych w latach 2011-2012 z dróg oddechowych 75 pacjentów z mukowiscydozą pozostających pod opieką Instytutu Matki i Dziecka

typowano metodą makrogenomowej analizy restrykcyjnej z elektroforezą w zmiennym polu elektrycznym (z wykorzystaniem endonukleazy *Spe I*). Zbierano wywiady dotyczące najważniejszych parametrów klinicznych, a także kontaktów pomiędzy pacjentami.

Wyniki: W toku analizy wyodrębniono 24 grupy klonalne. Największa grupa zawierała 49 izolatów pochodzących od 21 chorych. Inne grupy obejmowały od 2 do 12 izolatów pochodzących od 1 do 7 pacjentów. Trzy grupy zawierały szczepy pochodzące od trzech rodzeństw. Piętnaście grup klonalnych obejmowało od 2 do 7 szczepów należących do tego samego pacjenta. Pozostałych 24 pacjentów było zakażonych szczepami, nie należącymi do żadnej grupy klonalnej. Ocena parametrów klinicznych wykazała, że przebieg choroby u 21 pacjentów, których szczepy stanowiły największą grupę klonalną, był cięższy niż u pozostałych pacjentów w grupie badanej. Także łączny czas trwania ich hospitalizacji (antybiotykoterapii dożylnych) był dłuższy.

Wnioski: 1. Częste hospitalizacje pacjentów z mukowiscydozą w gorszym stanie klinicznym wydają się być istotnym czynnikiem ryzyka zakażeń krzyżowych *Pseudomonas aeruginosa*. 2. Intensyfikacja działań zapobiegających zakażeniom krzyżowym, takich jak środki higieny, izolacja chorych, wprowadzenie w Polsce programu domowej antybiotykoterapii dożylnych, a także dalsza edukacja pacjentów i ich rodziców odnośnie dróg transmisji zakażeń bakteryjnych, prawdopodobnie przyczynią się do poprawy sytuacji epidemiologicznej w naszym ośrodku.

Słowa kluczowe: mukowiscydoza, pałeczka ropy błękitnej, zakażenia krzyżowe, PFGE

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INTRODUCTION

The factor determining the duration and quality of life of patients with cystic fibrosis (CF) in more than 90% of the cases is the degree of severity of chronic bronchopulmonary disease. Accumulation of thick mucus in the bronchi leads to their chronic obstruction. It promotes bacterial infection overlapping the chronic inflammation present in the airways. In consequence, bronchiectases, mucous plugs, atelectasis and lung fibrosis develop. This leads to irreversible destructive changes of the respiratory system, to chronic respiratory failure and finally death. The bacterial infection plays the essential role in the described process. *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most common organisms isolated from the respiratory tract of CF patients. It affects about 60% of adolescents and about 80% of adults [1]. It is a gram-negative rod which is reported to be ubiquitous in the natural environment. The presence and persistence of the organism in the respiratory tract of CF patients correlates with the deterioration of lung function and the clinical decline of the patient [2].

The bacterial infection, inflammation of the mucous membrane and bronchial obstruction form a vicious circle of events leading to the destruction of the lungs. The most characteristic feature of persistent *P. aeruginosa* infection is the production of mucoid alginate and the formation of biofilm which is especially correlated with poor prognosis. At this stage, the infection is virtually impossible to eradicate [3]. Therefore, there is no doubt that the initial *P. aeruginosa* infection by the strain carried by another patient with cystic fibrosis (cross-infection) is potentially a greater risk than infection with an environmental strain.

During the last 25 years, numerous publications described patient-to-patient transmission and acquisition of the pathogen from CF clinics, rehabilitation centres and summer camps (e.g. the highly transmissible Liverpool epidemic strain) [4, 5, 6, 7]. Nowadays, due to hygienic precautions, the epidemiological situation has improved in Western Europe, the United States of America and Australia [8]. However, siblings with CF are typically colonized with identical or clonally related strains [5, 9].

AIM

The aims of this study were:

- to assess the prevalence of cross-infections with *P. aeruginosa* in order to evaluate the epidemiological situation of this infection in patients with cystic fibrosis attending our centre;
- to correlate the clinical features of the patients carrying a potentially transmissible strain with the entire study group in order to determine risk factors and possible effects of its acquisition.

MATERIAL AND METHODS

From January 2011 until December 2012 *P. aeruginosa* isolates from the respiratory tract were collected from the pediatric patients with the diagnosis of CF attending the Cystic Fibrosis Centre in the Institute of Mother and Child (outpatient and inpatient care). According to the Polish Cystic Fibrosis Patient Registry by the end of 2012 there were 376 patients. Most of them inhabited central and eastern regions of Poland. Patients with both the chronic as well as the intermittent infection with this

pathogen were included. The materials for the culture were: sputum, throat swab (when sputum was not possible to obtain) or bronchoalveolar lavage (BAL) received during bronchoscopy. The cultures were taken every 3 months in the outpatient clinic and in every case of admission to hospital. In total, 170 isolates from 75 patients were collected (44 isolates obtained from throat swab, 122 from sputum, 4 from BAL). The study was approved by the Bioethics Committee.

The research material was sown on a solid medium, the identification of species was based on the analysis of phenotypic characteristics (type of growth and colony morphology – differentiating strains of mucous/non-mucous). The identification of cultured strains was performed using mini API and ID 32 GN stripes. Susceptibility to antibiotics was tested by disc-diffusion tests.

The detailed information concerning social contacts between patients and their simultaneous hospitalizations was collected, as well as several clinical data regarding the course of the disease. The parameters taken into consideration were: the age of the first *P. aeruginosa* positive culture during the study period, sex, mutations in the *CFTR* gene, other CF manifestations, body mass index (BMI), the Shwachman-Kulczycki (SK) scoring, forced expiratory volume in 1 second (FEV1), type of *P. aeruginosa* infection (chronic, intermittent, first-time), number of pulmonary exacerbations, total duration of oral (at home) and intravenous (at the hospital) antibiotic therapy treatment during the study period. The infection was defined as chronic if more than 50% of respiratory

tract cultures during the study period were *P. aeruginosa* positive.

The mean age of the 75 patients who took part in the study was 11.6 years (range 1.0-19.3). Two patients died during or shortly after the study period. There were 37 (49%) boys. Forty four (55%) patients were F508del homozygotes, 25 (33%) – F508del heterozygotes and in 9 (12%) patients two other mutations in the *CFTR* gene were found. Sixty five (87%) patients were able to perform pulmonary function tests (PFTs) and the mean FEV1 in the study group was 78.12% predicted. Concerning the other CF manifestations – 71 (95%) patients suffered from pancreatic insufficiency, 4 (5%) were treated for diabetes, 49 (65%) had chronic sinusitis and 37 (49%) had sinus polyposis. The mean BMI standard deviation amounted to -0.65 and the mean S-K score – 75.5. The *P. aeruginosa* infection was considered chronic in 37 patients (49%), and intermittent in 38 (51%). The mean duration of infection was 5.60 years (range 0-19). The first-time infection with this pathogen was observed in 13 (17%) patients. In the study group the frequency of pulmonary exacerbations was 2.69 per patient per year. The average oral antibiotic therapy time was 26.36 days and the intravenous one – 16.71 per patient per year (the data collected during 2011 and 2012) (Table I).

Subsequently, the strains were sent to the National Institute of Public Health – National Institute of Hygiene, to determine the genetic relatedness by the pulsed field gel electrophoresis method (PFGE). PFGE analysis, with *Spe I* restriction enzyme, was conducted as described previously [10] using the CHEF-DR II system (Bio-Rad,

Table I. Clinical characteristics of the study group.

Tabela I. Charakterystyka kliniczna grupy badanej.

	Study group Grupa badana (n=75)	Group A Grupa A (n=21)	Group A1 Grupa A1 (n=16)
Boys (patients) <i>Chłopcy (pacjenci)</i>	37 (49%)	14 (67%)	11 (69%)
Mean age (years) <i>Średni wiek (lata)</i>	11.6 (1.0-19.3)	14.2 (9.5-17.8)	13.8 (9.5-17.0)
F508del homozygotes (patients) <i>Homozygoty F508del (pacjenci)</i>	44 (55%)	13 (62%)	10 (63%)
Mean FEV1 (% predicted) <i>Średnia FEV1 (% wartości należnej)</i>	78.12	73.52	70.44
Mean BMI standard deviation <i>Średnia wartość BMI – odchylenie standardowe</i>	-0.65	-0.91	-0.99
Mean S-K score <i>Średnia punktacja w skali S-K</i>	75.5	69.5	67.5
Chronic <i>P. aeruginosa</i> infection (patients) <i>Przewlekłe zakażenie P. aeruginosa (pacjenci)</i>	37 (49%)	16 (76%)	15 (94%)
Mean duration of infection (years) <i>Średni czas trwania zakażenia (lata)</i>	5.60 (0-19)	6.14 (0-11)	17.19 (3-11)
Average intravenous antibiotic therapy time (days per patient per year) <i>Średni czas trwania antybiotykoterapii dożylniej (dni na pacjenta rocznie)</i>	16.71	25.93	31.63

USA). PFGE profiles were analysed using the BioNumerics v 6.6 software (Applied Maths, Belgium) with the following parameters: Dice coefficient, tolerance and optimization equals 1.5, clustering algorithm UPGMA. Isolates were grouped based on the similarity with a coefficient higher than 90% to show clonal relationships.

RESULTS

Twenty four clusters of *P. aeruginosa* strains were detected. The main cluster (cluster 19) included 49 isolates derived from 21 patients (28%). The other 5 clusters included 2 to 12 isolates derived from 1 to 7 patients. There were 15 clusters containing 2 to 7 strains belonging to the same patient. Three clusters comprised the isolates derived from three pairs of siblings. The remaining 24 patients were infected with their own strains, not shared by other patients (Figure 1).

As mentioned before, the widest cluster was cluster 19 which consisted of 21 subjects (carrying 49 isolates). The interviews with the patients and their parents, together with the analysis of the dates of admissions to our ward, revealed the possible sources of cross-infection. In this group, 16 (76%) persons stated they had personal contacts with each other (social contacts, using the same items, eg. computers, playstations). Furthermore, shared periods of hospitalizations were very common in this population.

In the group of 21 children, whose strains constituted cluster 19 (group A), the majority – 14 (67%) were boys. The mean age was 14.2 years (range 9.5-17.8). In 16 (76%) subjects the *P. aeruginosa* infection was defined as chronic, in 4 (19%) – intermittent and in 1 (5%) patient a first-time infection was diagnosed. Thirteen (62%) patients were F508del homozygotes. All of them performed PFTs and the mean FEV1 in this group was 73.52% predicted. The mean BMI standard deviation amounted to -0.91 and the mean S-K score was 69.50. The average intravenous antibiotic therapy time due to pulmonary exacerbations (equals the hospitalization time) was 25.93 per patient per year (Table I).

Among the 16 patients who stated having had personal contacts and shared periods of hospitalizations (group A1), there were 11 (69%) boys. The mean age was 13.8 years (range 9.5-17.0). In 15 (94%) subjects the *P. aeruginosa* infection was chronic and in 1 (6%) – intermittent. The mean duration of infection was 7.19 years (range 3-11). The average FEV1 in this group was 70.44% predicted. The mean BMI standard deviation amounted to -0.99 and the mean S-K score – to 67.50. The average intravenous antibiotic therapy time due to pulmonary exacerbations (equals the hospitalization time) was 31.63 per patient per year (Table I).

DISCUSSION

Until the 1990 the environmental strains of *P. aeruginosa* were believed to constitute the main cause of infection of CF patients. The occurrence of cross-infections by highly transmissible clones in patients participating in summer camps and training courses in several Western Europe and

American countries induced intensive research on this problem. Hygienic precautions, segregation of patients according to their bacteriological status, as well as the abandonment of summer camps considerably improved the epidemiological situation in those countries [11]. For example, in the Danish CF centre in Copenhagen, after having taken such preventive measures, the incidence of new cases of chronic *P. aeruginosa* infection has dropped significantly to 1-2% per year [12].

Typing of *P. aeruginosa* strains obtained from cystic fibrosis patients using the PFGE method disclosed one largest cluster including 21 (28%) out of 75 patients in the study group. The other clusters were much smaller and most of them included isolates belonging to one patient per cluster. This means that the diversity among the strains in an individual patient was very low and also shows that during two years of observation the incidence of superinfections by another *P. aeruginosa* strain was rare. The results of this typing also showed that each of the three pairs of siblings included in the study carried the strains belonging to the same, i. e. their own cluster. Furthermore, the relatedness of the isolates within each of those clusters was very high. This reflects the results of several previous studies [5, 9].

Although it is thought that the most common source of *P. aeruginosa* infection for CF patients are environmental strains, the results of our study strongly suggest that cross-infection has taken place in some patients. Cluster 19 included 49 isolates derived from 21 patients (group A) – 28% of our study group. The interviews obtained from the patients and their parents revealed that most of the patients in this group (16 children) maintained social contacts with each other, despite the fact that they were advised to avoid them. Several parameters showed that the patients from group A were in worse clinical condition than the other patients in the study group (Table II). In the majority of cases the *P. aeruginosa* infection was defined as chronic and there was only 1 first-time infection. The average number of days of hospitalizations in order to perform intravenous antibiotic treatment was higher than in the entire study group. In group A, there were two individuals in whom the infection occurred either after a long time free of *Pseudomonas*, or as a first-time infection, soon after one single hospitalization shared with some other patients from this group. The second case was a boy whose diagnosis of CF was established very late (at the age of 12 years) and the infection occurred 4 weeks after his first hospitalization in our department. The 16 subjects from group A1 had an even worse course of the disease than group A. Most of them had chronic infection and they were hospitalized for a longer time.

In addition, we observed that in the mentioned group and subgroup the boys constituted the majority. It is evident that items of everyday use may constitute vectors for *P. aeruginosa* infection [13]. As the boys often share electronic equipment because they play computer games together, this might be one of the causes why those cross-infections occur.

The total duration of hospitalization throughout the study period was longer in patients from group A than in the entire study group. Although in our ward several

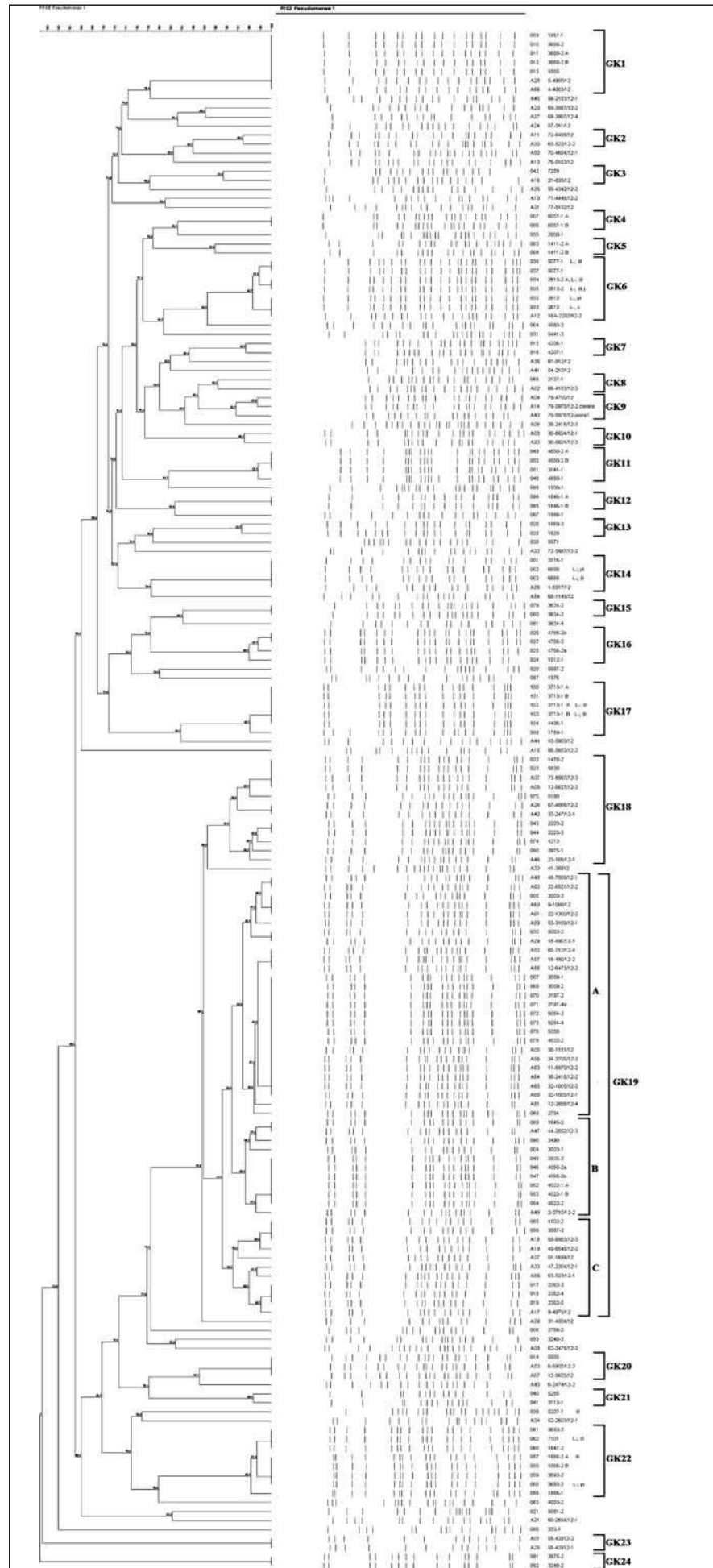


Fig. 1. Dendrogram of PFGE results for 170 *P. aeruginosa* isolates.

Ryc. 1. Dendrogram przedstawiający wyniki analizy PFGE 170 szczepów *P. aeruginosa*.

preventive measures were taken, we were not able to isolate all the patients due to unsatisfactory accommodation conditions. Furthermore, the education of patients regarding the consequences of cross-infections and the ways to prevent them has been implemented for only several years. Then, it was much easier to prevent social contacts between small children and their parents than between teenagers. In the early childhood of the latter group, the hygienic conditions in hospitals and the awareness regarding prevention of infections among patients was much worse than nowadays. Therefore, unfortunately many of them still do not accept the "new rules" of patient segregation. They have private meetings with each other out of the hospital and during hospitalizations they try to maintain contact despite bans. Both, worse clinical parameters and longer total periods of hospitalizations were observed in group A. There was no evidence that the isolates carried by the patients in that group were associated with a worse clinical course or if more frequent hospitalizations (due to more severe form of CF in an individual) led to the occurrence of cross-infections. The question about cause and consequence was practically impossible to answer due to a relatively short time of observation in this study.

Finally, we would like to emphasize that the introduction of a national program of home intravenous antibiotic therapy in several countries in Western Europe has contributed to a significant improvement of the epidemiological situation among patients suffering from CF [14]. In Poland, the project has already been approved by the authorities of the National Health Fund and we are waiting for its implementation. We anticipate that this will be an important step in reducing the incidence of cross-infection in our centre and in our country.

CONCLUSIONS

1. Frequent hospitalizations of CF patients with a more severe course of the disease seem to be a risk factor of cross-infections with *P. aeruginosa*.
2. Intensification of measures to prevent cross-infection such as hygienic precautions, patient segregation, introduction of a home intravenous antibiotic therapy programme, as well as further education of patients and their parents should lead to the improvement of epidemiological situation in our centre.

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Conflicts of interest/Konflikt interesu

The Authors declare no conflict of interest.

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