**Introduction**

*Pseudomonas aeruginosa* is one of the important causes of hospital-acquired infections in Intensive Care Unit (ICU) and considered as a major determinant of morbidity and mortality in patients affected by cystic fibrosis (CF). The aim of this study was to investigate clonal diversity among randomly picked *P. aeruginosa* isolates of CF and the other hospitalized patients in ICU.

**Methods.** Cultivation, identification, and antimicrobial susceptibility testing of *P. aeruginosa* isolates were performed using standard techniques. The genetic similarity of the strains was investigated by amplification of the Enterobacterial Repetitive Intergenic Consensus-polymerase chain reaction (ERIC-PCR) sequence.

**Results and discussion.** Among 49 isolates, sixteen were isolated from 11 patients affected by CF and 33 came from an epidemiological investigation of 25 *P. aeruginosa* infected patients of ICU. Five clusters were generated for all isolates analyzed through ERIC-PCR genotyping. Two major clusters (B and C) were discovered in *P. aeruginosa* isolates of ICU and CF patients during the whole period of this study. Fifteen unique antibiogram patterns obtained from all isolates and multi-resistant *P. aeruginosa* (MRPA) were identified in 23 isolates (47%). MRPA isolates were detected in all clusters (except A) while pan-resistant isolates were recovered only in cluster C. The high prevalence of related or identical isolates in CF and non-CF patients can be due to transmission of particular dominant clones in ICU ward. Therefore, enhanced infection-control may become necessary to prevent further spread of clonal strains.

**Key words**

*Pseudomonas aeruginosa* • Cystic fibrosis • Cross-infection
### Tab. I. Patient details, origin and date of isolation, outcome and microbiological data of all the P. aeruginosa isolates.

| Patient* | Isolates | Age (month) | Gender | Ward        | Type of specimen | Length of hospital stay (Day) | Time of isolation after hospitalization (Day) | Outcome | ERIC-PCR pattern | Antibiogram pattern |
|----------|----------|-------------|--------|-------------|------------------|-------------------------------|---------------------------------------------|---------|-----------------|---------------------|
| 1        | a        | 11          | M      | PICU        | Trachea tube     | 21                            | 4                                           | Death   | D               | 12                  |
| 2        | a        | 12          | M      | PICU        | Wound            | 30                            | 6                                           | Discharge | B               | 3                   |
| 3        | a        | 12          | F      | PICU        | Ear secretion    | 58                            | 5                                           | Discharge | C               | 15                  |
| 4        | a        | 7           | M      | PICU        | Trachea tube     | 34                            | 20                                          | Discharge | E               | 11                  |
| 5        | a        | 2.5         | F      | PICU        | Urine            | 29                            | 2                                           | Discharge | C               | 15                  |
| 6        | a        | 10          | F      | PICU        | Urine            | 30                            | 21                                          | Death    | C               | 15                  |
| 7        | a        | 6           | F      | PICU        | Wound            | 11                            | 10                                          | Death    | A               | 6                   |
| 8        | a        | 3           | M      | PICU        | Urine            | 21                            | 5                                           | Death    | B               | 7                   |
| 9        | a        | 11          | F      | PICU        | Trachea tube     | 5                             | 2                                           | Discharge | B               | 8                   |
| 10       | a        | 3           | F      | PICU        | Wound            | 22                            | 12                                          | Death    | B               | 7                   |
| b        |          |             |        |             | Eye              |                                |                                             |          |                 |                     |
| 11       | a        | 7           | F      | PICU        | Trachea tube     | 15                            | 5                                           | Discharge | B               | 4                   |
| 12       | a        | 120         | M      | PICU        | Urine            | 13                            | 2                                           | Death    | B               | 3                   |
| 13       | a        | 2           | M      | PICU        | Trachea tube     | 5                             | 3                                           | Death    | B               | 11                  |
| 14       | a        | 9           | M      | PICU        | Trachea tube     | 59                            | 4                                           | Discharge | B               | 4                   |
| b        |          |             |        |             | Wound            |                                |                                             |          |                 |                     |
| 15       | a        | 108         | F      | PICU        | Trachea tube     | 54                            | 2                                           | Discharge | B               | 2                   |
| 16       | a        | 24          | M      | PICU        | Trachea tube     | 4                             | 3                                           | Discharge | A               | 7                   |
| a        |          |             |        |             | Blood            |                                |                                             |          |                 |                     |
| b        |          |             |        |             | Alveolar aspirate| 28                            | 14                                          | Discharge | A               | 3                   |
| c        |          |             |        |             | Sputum           |                                |                                             |          |                 |                     |
| d        |          |             |        |             | Alveolar aspirate|                                |                                             |          |                 |                     |
| 17       | a        | 8           | M      | PICU        | Trachea tube     | 18                            | 2                                           | Discharge | C               | 12                  |
| b        |          |             |        |             | Urine            |                                |                                             |          |                 |                     |
| a        |          |             |        |             |                     |                                |                                             |          |                 |                     |
| b        |          |             |        |             | Blood            | 20                            | 20                                          | Discharge | C               | 12                  |
| c        |          |             |        |             | Alveolar aspirate|                                |                                             |          |                 |                     |
| 18       | a        | 36          | F      | PICU        | Wound            | 40                            | 5                                           | Discharge | C               | 14                  |
| b        |          |             |        |             | Eye              |                                |                                             |          |                 |                     |
| a        |          |             |        |             | Alveolar aspirate|                                |                                             |          |                 |                     |
| 19       | b        | 36          | F      | PICU        | Wound            | 40                            | 5                                           | Discharge | C               | 14                  |
| c        |          |             |        |             | Eye              |                                |                                             |          |                 |                     |
| 20       | a        | 4           | M      | PICU        | Urine            | 25                            | 1                                           | Discharge | C               | 15                  |
| 21       | a        | 31          | F      | PICU        | Urine            | 26                            | 5                                           | Discharge | B               | 3                   |
| 22       | a        | 36          | M      | PICU        | Blood            | 58                            | 1                                           | Discharge | C               | 15                  |
| 23       | a        | 120         | M      | PICU        | Trachea tube     | 19                            | 4                                           | Discharge | B               | 3                   |
| 24       | a        | 24          | M      | PICU        | Pharynx          | 15                            | 6                                           | Discharge | C               | 14                  |
| 25       | a        | 3           | F      | PICU        | Wound            | 51                            | 3                                           | Death    | C               | 15                  |
| 26       | a        | 12          | F      | PICU        | Wound            | 14                            | 14                                          | Death    | C               | 15                  |
| a        |          |             |        |             | Blood            |                                |                                             |          |                 |                     |
| b        |          |             |        |             | Alveolar aspirate|                                |                                             |          |                 |                     |
| 27       | b        | 192         | F      | Gastroenterology | Alveolar aspirate| 56                            | 40                                          | Death    | B               | 7                   |
| c        |          |             |        |             | Sputum           |                                |                                             |          |                 |                     |
| 28       | a        | 72          | M      | PICU        | Trachea tube     | 12                            | 7                                           | Death    | B               | 13                  |
| b        |          |             |        |             | Trachea tube     |                                |                                             |          |                 |                     |
| c        |          |             |        |             | Trachea tube     |                                |                                             |          |                 |                     |
| 29       | a        | 5           | F      | PICU        | Sputum           | 10                            | 1                                           | Discharge | C               | 15                  |
| 30       | a        | 45          | M      | Gastroenterology | Sputum           | 14                            | 2                                           | Discharge | B               | 8                   |
| 31       | a        | 120         | M      | Gastroenterology | Sputum           | 12                            | 2                                           | Death    | B               | 7                   |
| 32       | a        | 10          | M      | PICU        | Wound            | 10                            | 9                                           | Death    | C               | 15                  |
| 33       | a        | 7           | F      | Gastroenterology | Urine            | 11                            | 1                                           | Discharge | B               | 9                   |
| 34       | a        | 7           | M      | Gastroenterology | Urine            | 36                            | 2                                           | Death    | C               | 15                  |
| 35       | a        | 72          | M      | PICU        | Trachea tube     | 7                             | 7                                           | Death    | C               | 15                  |
| b        |          |             |        |             | Sputum           |                                |                                             |          |                 |                     |
| 36       | a        | 48          | F      | Gastroenterology | Sputum           | 7                             | 1                                           | Discharge | B               | 10                  |

*Isolates number of 26 to 36 isolated from CF patients. PICU: Pediatric Intensive Care Unit
Cultivation, identification, and antimicrobial susceptibility testing of \textit{P. aeruginosa} isolates were performed using standard techniques \cite{9}. Multi-resistant \textit{P. aeruginosa} (MRPA) was defined as strains resistant to $\geq 3$ of the following classes of antibiotics: antipseudomonal penicillins, antipseudomonal oximino-b-lactams, fluoroquinolones, aminoglycosides, and carbapenems \cite{10}.

The genetic similarity of the strains was investigated by amplification of the Enterobacterial Repetitive Intergenic Consensus-polymerase chain reaction (ERIC-PCR). sequence \cite{11}. Comparison of banding patterns was performed using Gelcompar II, version 6.5 (Applied Maths, Sint-Matens-latem, Belgium). Cluster analysis was accomplished with the unweighted pair group method using average linkages (UPGMA). ERIC-PCR was performed for \textit{P. aeruginosa} isolates and relatedness among genetic clones was defined as 80\% similarity as belonging to one clone.

**Results**

During one year, 36 patients aged 3.5 to 192 month (average 33.4) were entered to this study. Table I illustrated the patient details, origin and date of isolation, outcome, ERIC-PCR and antibiogram pattern of all isolates. Among 49 isolates, sixteen were isolated from 11 patients affected by CF and 33 came from an epidemiological investigation of 25 \textit{P. aeruginosa} strains isolated from infected patients of ICU. \textit{P. aeruginosa} isolates were recovered from trachea tube (26\%), urine (18\%), wound (16\%), sputum (18\%), alveolar aspirate (8\%), blood (4\%), tracheal aspirate (2\%), ear secretion (2\%), and eye (2\%).

Average length of hospital stay in all patients was 24 days. Of the 36 patients, 14 died during the study (case fatality rate, 39\%); whereas half of them were from CF patients (case fatality rate, 64\%). MRPA were identified in 23 isolates (47\%) and consistently detected during the study period. Among all isolates, 11 considered as pan-resistant isolates that 5 of them belonged to CF patients.

Five clusters were generated for all isolates analyzed through ERIC-PCR genotyping, which were designated alphabetically from A to D (Fig. 1). Cluster B and C comprised both ICU and CF isolates while cluster A, D and E had only isolates of ICU ward. Eleven isolates of cluster B were identical whereas cluster C comprised of only 3 identical isolates.

MRPA isolates were detected in all clusters (except A) while pan-resistant isolates were recovered only in cluster C. Cluster B and C was seen during the study period (4 seasons) (Fig. 2). Cluster A was introduced during summer and remained present during the rest
of the study. During the autumn, one patient appeared in cluster D and E. However, cluster D emerged in the third season, detection of this cluster during the winter did not occur.

In our study, 15 unique antibiogram patterns obtained from all isolates (Tab. II). Most of the isolates were resistant to antipseudomonal oxyimino-b-lactams. The highest percentage of susceptibility was seen in aminoglycosides (71%) followed by piperacillin-tazobactam (67%), imipenem (63%), ciprofloxacin (59%), and meropenem (55%). Different antibiogram patterns were displayed in a number of isolates even with ≥ 95% similarity.

Discussion

Our hospital is a tertiary referral center in which the ICU setting includes mixed patients such as CF patients and transmission of *P. aeruginosa* strains between CF and the other hospitalized patients in ICU is probable. Therefore genotyping of *P. aeruginosa* isolates seems to be essential to clarify our hospital epidemiology. The spread of infection from patients with CF is a definite risk in children’s wards especially in ICU [12, 13]. In this study, molecular typing suggests cross-transmission between CF and non-CF patients. According to our results, infections were predominantly caused by strains with cluster B and C. Presence of these patterns during the whole period of this study suggests that these clones are adapted to our hospital. Cluster B was identified in 25 strains (51% of all typed isolates) and highly appeared in the first 3 months of 2010 and circulated until end of the study.

Our analysis demonstrated that CF isolates are genotypically closely related to non-CF isolates. The two major clusters (B and C) in our study were discovered in both patient populations that suggest probable occurrence of cross-infection between these patients. Studies about *P. aeruginosa* cross-infection demonstrated controversy evidence of clonal spread in CF centers. Some studies have revealed convincing evidence of clonal spread at CF holiday camps or centers [14-18]. Another study at the Vancouver CF Centre did not represent any evidence of significant cross-infection with *P. aeruginosa* [19].

Emergence of MDR expect to become more prevalent in many hospitals [20, 21]. There is no doubt that cross-transmission plays an important epidemiological role in MRPA isolates [22, 23], so prevention of the acquisition of these isolates is essential due to limited therapeutic options and increased mortality [24]. On the other hand, prevalence of strains with resistance to all antimicrobial agents is a major risk for hospitalized patients especially in CF patients because of rare *P. aeruginosa* eradication and subsequently occurrence of chronic infection [25, 26]. *P. aeruginosa* has capacity to develop resistance to essentially antimicrobial agents [27] and may require treatment with less commonly used antibacterial agents such as colistin [4].

Our results support other studies [28] that mentioned aminoglycosides are clinically effective against *P. aeruginosa* isolates when administrated intravenously or by nebulization.

Finally, in our study the high prevalence of related or identical isolates in CF and non–CF patients can be due to transmission of particular dominant clones in ICU ward. This suggests cross-infection can occur between CF and non-CF patients. Therefore, enhanced infection-control including strict segregation policies, basic hygiene measures and continued microbiological surveillance may become necessary to prevent further spread of clonal strains.

| Pattern number | Isolates* | CF | FEP | CP | MEM | CAZ | PTZ | IMP | GM | AM |
|----------------|-----------|----|-----|----|-----|-----|-----|-----|-----|-----|
| 1              | 18b       | S  | S   | S  | S   | S   | S   | S   | S   | S   |
| 2              | 15,18a    | S  | S   | S  | S   | R   | S   | S   | S   | S   |
| 3              | 2a,12,14b,17b,17c,21,23,35a | R  | S   | S  | S   | S   | S   | S   | S   | S   |
| 4              | 10b,11,14a | R  | S   | S  | S   | R   | S   | S   | S   | S   |
| 5              | 28a       | S  | S   | S  | R   | S   | S   | S   | S   | R   |
| 6              | 7,27a,27c | R  | R   | S  | S   | S   | S   | S   | S   | S   |
| 7              | 8,10a,16,19a,27b,31 | R  | R   | S  | S   | R   | S   | S   | S   | S   |
| 8              | 9,30      | R  | R   | S  | R   | R   | S   | S   | S   | S   |
| 9              | 3         | R  | R   | R  | R   | R   | S   | S   | S   | S   |
| 10             | 17a,36    | R  | R   | R  | R   | R   | S   | S   | S   | S   |
| 11             | 2c,10b,28c | R  | R   | S  | R   | S   | R   | S   | S   | S   |
| 12             | 1,17d,19c | R  | R   | R  | R   | R   | S   | S   | S   | S   |
| 13             | 28b       | R  | R   | S  | R   | S   | R   | R   | R   | R   |
| 14             | 19b,24    | R  | R   | R  | R   | R   | S   | R   | R   | R   |
| 15             | 2b,5,6,20,22,25,26,29,32,34,35b | R  | R   | R  | R   | R   | R   | R   | R   | R   |

* CF, Cephalothin; FEP, Cefepime; CP, Ciprofloxacin; MEM, Meropenem; CAZ, Ceftazidime; PTZ, Piperacillin-tazobactam; IMP, Imipenem; GM, Gentamycin; AM, Amikacin

* Isolate number of 26 to 36 belongs to CF patients

Tab. II. Antibiogram patterns of 49 *P. aeruginosa* isolates.

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PSEUDOMONAS AERUGINOSA INFECTION AMONG CYSTIC FIBROSIS PATIENTS
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