The Prevalence of *Pseudomonas Aeruginosa* Strains in Infants With Cystic Fibrosis in a Hospital from North Easter Romania

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Abstract

Cystic fibrosis (CF) is the most common autosomal recessive genetic disease caused by mutation of the CFTR gene. In Romania, CF disease has an estimated a prevalence of 1 case per 2500 live births. Approximately 50% of children die in early life, so the exact number of CF patients in our country remains unknown as no national patient registry exists. This situation has limited our knowledge about the disease progression. Complicated with *P. aeruginosa* leads to long progressive diseases and often aggravates morbidity and mortality.

The aim of the study was to phenotypically identify the *P. aeruginosa* strains, according to their antibiotic susceptibility profile in order to administer more precisely the antibiotics and to avoid development of multidrug-resistant strains.

After the diagnosis of CF, the patients return to a medical control at every 3 months. The sputum samples are collected from these patients, in order to perform the bacteriological examination and the DST test. This study was performed during 1 year (01.01-31.12.2015), and all the sputum samples were cultivated on usual and on special culture media to establish more precisely the type of isolated bacteria. The confirmation was performed using the API 20 NE biochemical test and classified in resistance phenotypes according to CLSI 2015 standards.

The study population was represented by 85 infants with CF, from which we isolated 364 bacterial strains. 10.16% of the total bacterial strains were represented by *P. aeruginosa*.

A major problem represents the repeated antibiotic treatments, this being favourable for the emergence of resistant strains. Antibiotic resistance is currently one of the most important problems faced by clinicians (Moskowitz *et al.*, 2004). Multidrug-resistant (MDR) strains of *P. aeruginosa* were identified in this study and we highlighted that this strains limits the antimicrobial treatment and is shortening the patient's life (9).

Keywords: cystic fibrosis, prevalence, *Pseudomonas aeruginosa*

INTRODUCTION

Cystic fibrosis (CF) – the most frequent autosomal recessive disorder of the Caucasian population, is a complex disease. Often, the respiratory tract is the most affected and the lung disease is characterized by chronic infections (positive cultures after 6 months of germ

isolation) or recurrent infections, inflammation and development of bronchiectasis (Kirkby *et al.*, 2009)

The lungs of the patients with CF are the home of numerous bacterial infections, *Pseudomonas aeruginosa* being dangerous bacteria detected in

the respiratory tract of these children (Paduraru *et al.*, 2014).

The objective of this study was to identify children with CF and *P. aeruginosa* infection and to quantify the impact of this infection on their clinical status, the MDR prevalence in these children, as well as the chronic *P. aeruginosa* MDR infection impact on the lung.

MATERIAL AND METHODS

The prospective study between 1 January 2015 and 31 December 2015 was carried out on a group of 85 children suffering from CF, registered in the records of the hospital. All patients with CF were submitted to the micro-bacteriological test of the aspirate or the sputum for *P. aeruginosa*. All the sputum samples were cultivated on usual culture media (blood agar, chocolate agar, MacConkey) and on special culture media (selective *Pseudomonas* Agar CN-Oxoid), PAID media, for establish more

precisely the *Pseudomonas aeruginosa*. (Fig. 1.) (Ciocan *et al.*, 2014)

The confirmation was performed using the API 20 NE biochemical test and finally classified in resistance phenotypes according to CLSI 2015 standards. (Fig. 2.)

The resistance of strains was determined by testing sensitiveness to major classes of antibiotics with micro-caplets on the Mueller-Hinton medium at 37°C for 24 hours. (Fig. 3.) (Ciocan *et al.*, 2015)

Criteria for defining MDR, XDR and PDR in *Pseudomonas aeruginosa* MDR: non-susceptible to 1 agent in 3 antimicrobial categories, XDR: non-susceptible to 1 agent in all but less 2 categories, PDR: non-susceptible to all antimicrobial agents listed (Magiorakos *et al.*, 2011).

RESULTS AND DISCUSSIONS

The study population was represented by 85 infants with CF, from which we isolated 364

Fig.1. Strains of *Pseudomonas aeruginosa* on diverse cultural media

Fig. 2. Biochemical confirmation of *Pseudomonas aeruginosa* strains

Fig. 3. Disc diffusion method for testing antimicrobial activity

Tab.1. Antimicrobial categories and agents used to define MDR, XDR and PDR (Magiorakos *et al.*, 2011)

<i>Antimicrobial category</i>	<i>Antimicrobial agent</i>
Aminoglycosides	Gentamicin
	Tobramycin
	Amikacin
	Netilmicin
Antipseudomonal carbapenems	Imipenem
	Meropenem
	Doripenem
Antipseudomonal cephalosporins	Ceftazidime
	Cefepime
Antipseudomonal fluoroquinolones	Ciprofloxacin
	Levofloxacin
Antipseudomonal penicillins + b-lactamase inhibitors	Ticarcillin-clavulanic acid
	Piperacillin-tazobactam
Monobactams	Aztreonam
Phosphonic acids	Fosfomycin
Polymyxins	Colistin
	Polymyxin B

Fig. 4. Total of bacterial strains isolated from infants with CF

Fig. 5. Graphical representation of age groups

bacterial strains. 10% of the total of bacterial strains were represented by *P. aeruginosa*. (Fig. 4)

Colonization of lung with *Pseudomonas aeruginosa* strains depends on dissimilar number of factors, children from group with 6 years

to 10 years are the most likely infected with *Pseudomonas aeruginosa* in this study. (Fig. 5)

MDR microorganisms are an important issue in the care of patients with CF. Each patient infected with such strains should be assessed individually

Fig. 6. Graphical representation depending on the sex of the child with CF

Fig. 7. Results of antibiotic susceptibility of *Pseudomonas aeruginosa* strains

Fig. 8. Phenotypic identification of *Pseudomonas aeruginosa* strains

and antibiotic treatment administrated according to the results of antibiotic susceptibility. (Fig. 7)

CONCLUSIONS

For a more accurate and rapid isolation we recommend using *Pseudomonas* culture medium CN Oxoid and for sputum PAID chromogen medium, even if it is more expensive;

In our study, 10% of the total bacterial strains were represented by *P. aeruginosa*;

Following the results of antibiotic susceptibility in the current study prevailed MDR strains;

Resistance to colistin is low and this antibiotic should be considered as a valuable second-line drug to be used for multidrug-resistant *P. aeruginosa*;

Treatment of MDR strains is difficult and sometimes without therapeutic success. MDR strains become PDR if antibiotics are not used according to the antimicrobial test. Combined antibiotic therapy is recommended, but the choice of treatment regimen should always be guided by the clinical response of patient's.

According to the schedule in which age groups are represented, group 6-10 years is the most affected, and boys are more infected than girls;

To check if these parameters are relevant must be checked a much larger number of children and over a longer time interval.

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