JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Antimicrobial susceptibility of *Pseudomonas aeruginosa* before and after initiation of inhaled tobramycin in Bulgaria

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Abstract

Introduction: In aim to achieve better infection control and possible eradication of the pathogens involved in chronic infections of patients with cystic fibrosis (CF) scientists have developed a new way to administer antimicrobials – inhalation. The first and so far the only available inhalable antimicrobial in Bulgaria is inhaled tobramycin (TOBI), introduced in 2009.We aimed to evaluate the antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) patients before and after initiation of TOBI in the regular treatment regimen.

Methodology: We have determined the minimal inhibitory concentration (MIC) of 17 antimicrobials by the E-test (LIOFILCHEM) in sputa samples of 118 CF patients for the period of 2005–2014. The results were interpreted according to the annual Clinical and Laboratory Standards Institute guidelines.

Results: In the sputa of 70 patients a total of 102 *P. aeruginosa* isolates were found. Sixty-eight out of 102 (66.7%) were susceptible to all studied antimicrobials. We divided the isolates in two chronological groups: those collected before the introduction of TOBI as a regular treatment in 2009 and those collected after 2009. A significant reduction ($p < 0,001–0,02$) in susceptibility for the strains after 2009 was noted towards piperacillin (100% vs 50%), ceftazidime (100% / 77.3%), cefepime (97.9% / 68.2%), amikacin (100% / 63.6%), gentamicin (95.7% / 40.9%), tobramycin (93.6% / 59.1%) and ciprofloxacin (93.6% / 45.5%).

Conclusion: The introduction of inhaled tobramycin as a regular therapy for CF patients in Bulgaria lead to a significant change in antimicrobial susceptibility of CF *P. aeruginosa*.

Key words: cystic fibrosis; *Pseudomonas aeruginosa*; antimicrobial sensitivity; inhaled tobramycin; regular treatment.

J Infect Dev Ctries 2016; 10(11):1265-1267. doi:10.3855/jidc.7658

(Received 08 September 2015 – Accepted 29 October 2015)

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal hereditary disorder in Caucasians [1]. For CF patients, lung damage secondary to chronic infection is the main cause of death. Antibiotic therapy aims at eradicating *Pseudomonas aeruginosa*, the major bacterial pathogen in CF, after early lung infection. During the last decades, improved regimens for treating chronic *P. aeruginosa* infection have played a major role in increasing median survival of CF patients up to 40 years [2], a goal yet to be achieved in our country.

In Bulgaria the only approved and available inhaled antibiotic for chronic *P. aeruginosa* infection TOBI was introduced in 2007 and became part of the regular treatment of all patients in 2009. Therefore, we aimed at evaluating the antimicrobial susceptibility of *Pseudomonas aeruginosa*, isolated from CF patients before and after initiation of regular therapy with inhaled tobramycin (TOBI).

Methodology

For the period of 2005–2014 we evaluated sputa from 118 patients (50 female and 68 male) aged 5 to 34 years of age, with genetically confirmed CF disease. The samples were obtained during pulmonary exacerbation of the disease, one sample per patient. Sputum samples were plated on appropriate nutrient media and incubated at 35◦C for 24–48 hours. Bronchopulmonary infection pathogens were identified by a BBL Crystal identification system (Becton Dickinson, USA). The susceptibilities of the investigated *P. aeruginosa* isolates to 17 antimicrobial agents were determined by a gradient MIC method with E-test strips (LIOFILCHEM) on Mueller–Hinton II agar plates (Becton Dickinson, USA) according to the annual Clinical and Laboratory Standards Institute guidelines [3]. The samples were divided into 2 chronological groups - the first group included sputa collected before 1st January 2009, and the second group included sputa obtained afterwards.

All statistical analyses were performed with Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, United States). The differences between the measured parameters in the two groups were tested by two-tailed unpaired t-test.

Results

In the sputa of 70 patients (32 males and 38 females) a total of 102 isolates of *P. aeruginosa* were found. Age distribution was as follows: 42.1% of all children under 9 years of age, 57.9% for children aged 10 to 16 years and 86.9% for patients over 16 years of age. A total of 68 out of 102 (66.7%) isolates were susceptible to all 17 studied antimicrobials ('wild-type' phenotype). Low resistance levels were found towards: imipenem (5.9%), meropenem (3.9%), amikacin (3.9%), gentamicin (7.8%), tobramycin (5.9%), netilmicin (13.7%) and ciprofloxacin (13.7%).

In Bulgaria the first inhaled tobramycin regimens were introduced in 2007 and this treatment has become regular for chronically infected CF patients since 2009. For this reason, we have divided the isolates of our study into two chronological groups: group I included all isolates before 2009 (64 *P. aeruginosa* isolates) and group II included all isolates after 2009 (38 isolates). Significant reduction ($p < 0.001–0.02$) in susceptibility of the strains after 2009 relatively to the strains before 2009 was noted towards piperacillin (100%/50%), ceftazidime (100%/77.3%), cefepime (97.9%/68.2%), amikacin (100%/63.6%), gentamicin (95.7% /40.9%), tobramycin (93.6%/ 59.1%) and ciprofloxacin (93.6%. /45.5%) respectively. All strains (in both groups) were susceptible to colistin. The comparative table of susceptibility of both groups is presented in Figure 1.

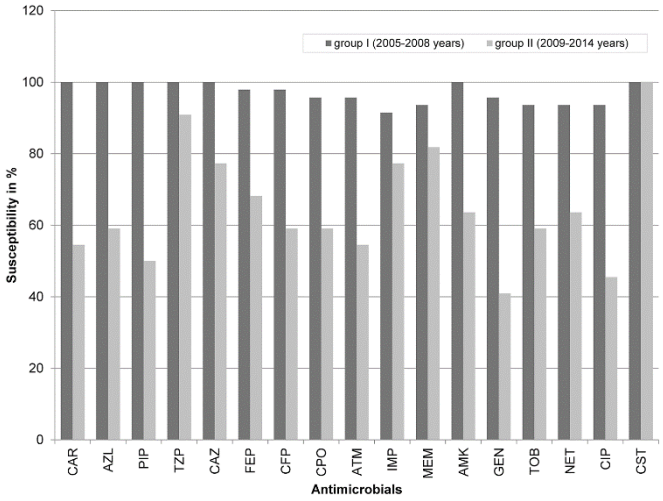
Discussion and conclusion

The increased resistance of *P. aeruginosa* to antibiotics in CF patients with advanced lung disease has been attributed to genetic mechanisms, including the emergence of hypermutable *P. aeruginosa*, the generation of biofilms, and exposure of the microorganisms to an environment which is anaerobic, acidic, and nutrient depleted [4].

In one recent multicenter study on *P. aeruginosa* isolates susceptibility, it was found that isolates from USA were more susceptible than those from Europe [5]. Maybe due to the lack of a strict standard policy of care for CF patients in Bulgaria, the isolates of this study, before TOBI introduction, were much more susceptible compared to those reported by other countries until 2009. For instance, the antibiotic resistance rates in Spain (2003–2004), UK (2000) and USA (2000) were, respectively: piperacillin – 20.9, 29.7 and 48.2 %; ceftazidime – 14.6, 39.6 and 49.9 %; tobramycin – 13.1, 10.1 and 22.2 %; gentamicin – 33.0, 47.0 and 52.4 %; ciprofloxacin – 28.6, 29.7 and 37.4% [6-8]. After the initiation of inhalation regimens with TOBI the susceptibility of *P. aeruginosa* became much lower compared to the susceptibility reported from Spain and Germany regarding aminoglycosides, cephalosporins and fluoroquinolones (at less than 60% susceptibility for all of the three classes) and much higher regarding carbapenems (over 75% susceptibility) as in USA [5]. Additionally, excluding the data for carbapenem, the susceptibility of our strains, from 2009 onwards, is similar to that reported by Kuti *et al*. for multidrug-resistant (MDR) *P. aeruginosa* isolates from CF children with susceptibility to meropenem, ceftazidime, and piperacillin/tazobactam of 46%, 58%, and 50%, respectively [9]. Maybe this could be explained by the indiscriminate use of anti-microbials for home therapy, because in Bulgaria carbapenems, can be used only during hospital stay. Additionally, it is possible that anaerobic cultures and biofilms generated *in vitro* do not accurately reflect the hostile microenvironment of lungs with CF and/or that the concentrations of antibiotics used according to the consensus guidelines may not reflect the concentrations found in CF airway mucus and thus may not be effective [13].

The increasing prevalence of MDR *P. aeruginosa* in recent years is not only a local but also a global problem [10]. The difference in susceptibility before

Figure 1. Antimicrobial susceptibility of the two groups of 102 isolates of *P. aeruginosa* from Bulgarian CF patients.



CAR – carbenicillin; AZL – azlocillin; PIP – piperacillin; TZP – piperacillin/ tazobactam; CAZ – ceftazidime; FEP – cefepime; CFP – cefoperazone; CPO – cefpirome; ATM – aztreonam; IMP – imipenem; MEM – meropenem; AMK – amikacin; GEN – gentamicin; TOB – tobramycin; NET – netilmicin; CIP- ciprofloxacin; CST- colistin.

and after TOBI introduction in Bulgaria is a worrisome problem, not only because there is no other available approved inhalable antimicrobial therapy for CF patients right now, but also because recent studies in the literature report an increasing colistin-resistance [6,7,12,14]. At the moment, due to the 100% susceptibility to colistin, we use this antimicrobial as the last line of defence. The combined use of colistin–tobramycin inhalation in animal and *in vitro* studies has been shown to be superior to monotherapy since its use significantly decreased bacterial burden [11].

Unfortunately, some colistin-resistant CF isolates of *P. aeruginosa* have been found lately in Spain [7], UK [6] and Denmark [12]. However, as colistin is not covered by NHI and its high price is making it almost unaffordable for over 90% of CF patients in Bulgaria, it is possible that such colistin-resistant strains are not to be seen in the near future in Bulgaria.

In the face of the selection pressure posed by the exposure to daily inhaled doses of tobramycin, the susceptibility of *P. aeruginosa* is changing, thus making the goal of eradication from the chronically infected CF patients difficult to achieve, while using current antimicrobials.

Acknowledgements

The authors would like to express their gratitude for the technical help and support to Miss Svetlana Atanasova, from Department of Medical Microbiology, Faculty of Medicine, Medical University of Sofia, Bulgaria.

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Conflict of interests: No conflict of interests is declared.