ORIGINAL ARTICLE

# *Pseudomonas aeruginosa* infection among cystic fibrosis and ICU patients in the referral Children Medical Hospital in Tehran, Iran

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Key words

*Pseudomonas aeruginosa* • Cystic fibrosis • Cross-infection

Summary

**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.*

## 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) [1-3].

Surveillance of nosocomial *P. aeruginosa* infections has revealed trends of increasing antimicrobial resistance [4]. The emergence of transmissible *P. aeruginosa* strains at hospital and its spread to other *P. aeruginosa*-negative patients raise concerns especially in ICU settings, where the number of antibiotic agents with good activity is limited [5, 6]. Several studies describe the patient-to-patient transmission of this bacteria from centers related with CF patients [7, 8].

At our hospital, CF patients are cared in gastroenterology ward and *P. aeruginosa*-positive and *P. aeruginosa*-negative patients are not separated from each other in this ward. In addition, these patients move to other wards especially ICU during their hospitalization. The aim of this study was to investigate epidemiology of antimicrobial resistance and clonal diversity among randomly picked *P. aeruginosa* isolates of CF and the other hospitalized patients in ICU.

## Material and methods

Between January and December 2010, randomly picked *P. aeruginosa* isolates of infected patient in ICU and patients affected by CF were collected from the tertiary referral Children Medical Hospital in Tehran, Iran. The patients investigated in this study were all prone *to P. aeruginosa* infection. An infected patient was defined as a patient with clinical symptoms of infection, and from whom a clinical culture yielded a dominant growth of *P.aeruginosa*. Clinical information on *P. aeruginosa* patient isolates was collected from medical records. Information included age, sex, length of hospital stay, history of transfer from another wards, clinical outcome and microbiological data.

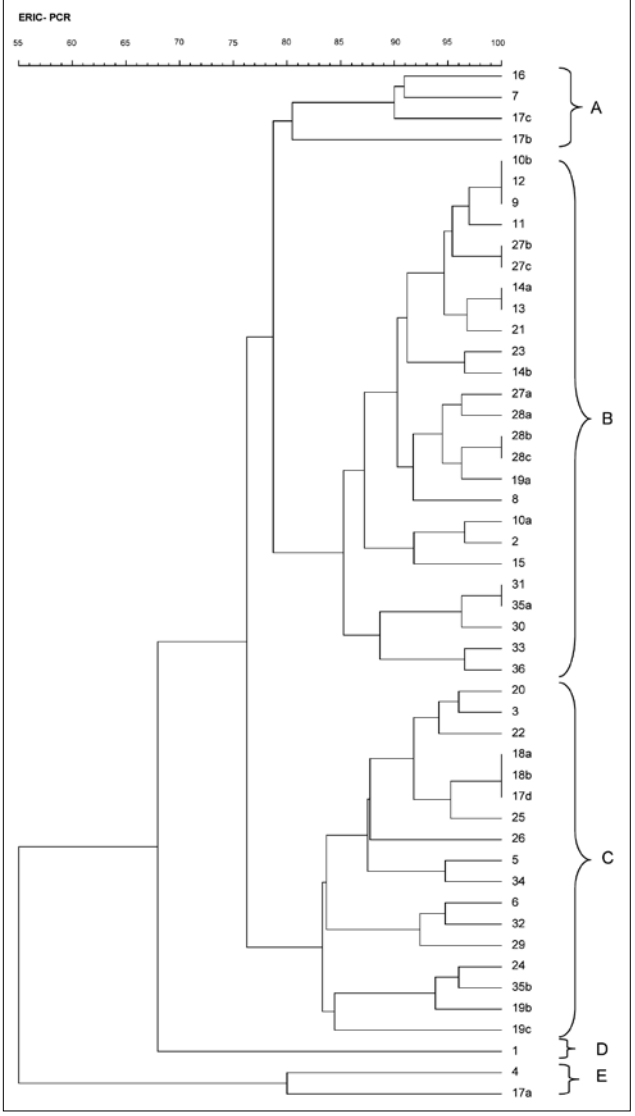
A number of CF patients had more than one isolates and some of them were isolated while they were transferred to the ICU. In addition several cultures were obtained

**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 | lentgh 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 | | 22 | | B | 4 |
| 11 | a | 7 | F | PICU | Trachea tube | 15 | 5 | Discharge | B | 4 |
| 12 | a | 120 | M | PICU | Urine | 13 | 2 | Discharge | B | 3 |
| 13 | a | 2 | M | PICU | Trachea tube | 5 | 3 | Death | B | 11 |
| 14 | a | 9 | M | PICU | Trachea tube | 39 | 4 | Discharge | B | 4 |
| | b | | | | Wound | | 17 | | B | 3 |
| 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 | | 4 | | E | 10 |
| 17 | b | 4 | F | PICU | Alveolar aspirate | 28 | 14 | Discharge | A | 3 |
| | c | | | | Sputum | | 8 | | A | 3 |
| | d | | | | Alveolar aspirate | | 8 | | C | 12 |
| 18 | a | 8 | M | PICU | Trachea tube | 18 | 2 | Discharge | C | 2 |
| | b | | | | Urine | | 14 | | C | 1 |
| 19 | a | 36 | F | PICU | Wound | 40 | 3 | Discharge | B | 7 |
| | b | | F | | | | 5 | | C | 14 |
| | c | | F | | | | 20 | | C | 12 |
| 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 | Dischrge | 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 |
| 27 | a | 192 | F | Gastroenterology | Alveolar aspirate | 56 | 4 | Death | B | 6 |
| | b | | | Gastroenterology | Sputum | | 40 | | B | 7 |
| | c | | | Gastroenterology | Sputum | | 46 | | B | 6 |
| 28 | a | 72 | M | PICU | Trachea tube | 12 | 2 | Death | B | 5 |
| | b | | | PICU | Tracheal aspirate | | 7 | | B | 13 |
| | c | | | PICU | Trachea tube | | 11 | | B | 11 |
| 29 | a | 5 | F | PICU | Sputum | 10 | 1 | Dischage | C | 15 |
| 30 | a | 45 | M | Gastroenterology | Sputum | 14 | 2 | Dischage | 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 | 5 | F | Gastroenterology | Urine | 11 | 1 | Dischage | B | 9 |
| 34 | a | 7 | M | Gastroenterology | Urine | 36 | 2 | Death | C | 15 |
| 35 | a | 72 | M | PICU | Trachea tube | 7 | 2 | Death | B | 3 |
| | b | | | Gastroenterology | Sputum | | 7 | | C | 15 |
| 36 | a | 48 | F | Gastroenterology | Sputum | 7 | 1 | Dischage | B | 10 |

*Isolates number of 26 to 36 isolated from CF patients. PICU: Pediatric Intensive Care Unit

**Fig. 1.** Dendrogram of genotype analysis derived from 49 *P. aeruginosa* isolates. The scale at the top represents the genetic distance between the isolates..




from some patients as long as they stayed in the ICU. Cultivation, identification, and antimicrobial susceptibility testing of *P. aeruginosa* isolates were performed using standard techniques [9].

Multi-resistant *P. aeruginosa* (MRPA) was defined as strains resistant to ≥ 3 of the following classes of antibiotics: antipseudomonal penicillins, antipseudomonal oxyimino-b-lactams, fluoroquinolones, aminoglycosides, and carbapenems [10].

The genetic similarity of the strains was investigated by amplification of the Enterobacterial Repetitive Intergenic Consensus-polymerase chain reaction (ERIC-PCR). sequence [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 *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 *P. aeruginosa* strains isolated from infected patients of ICU.

*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%).

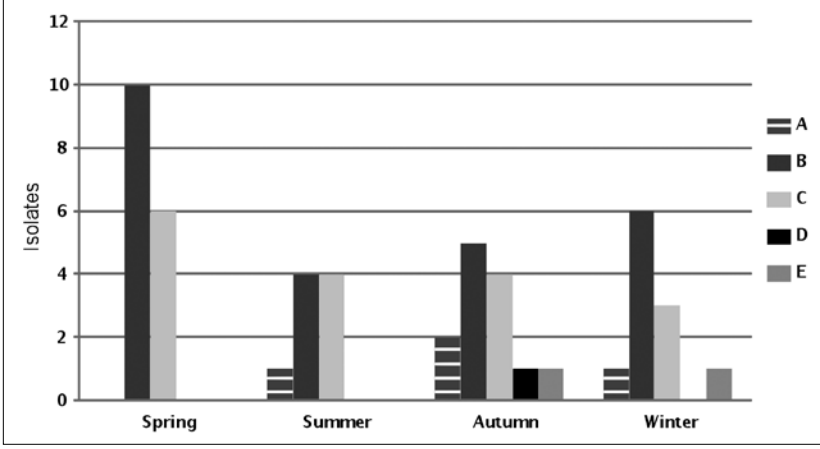
Average length of hospital stay in all patients was 24 days. Of the 36 patients, 14 died during the study (case fatality rate, 39%); whereas half of them were from CF patients (case fatality rate, 64%). MRPA were identified in 23 isolates (47%) and consistently detected during the study period. Among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

**Fig. 2.** Distribution of different clusters during 2010.



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

| 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 | R | S |
| 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 | 33 | R | R | R | R | R | S | S | S | S |
| 10 | 17a,36 | R | R | S | R | R | R | S | S | S |
| 11 | 2c,10b,28c | R | R | R | S | R | S | R | S | S |
| 12 | 1,17d,19c | R | R | R | R | R | R | S | S | S |
| 13 | 28b | R | R | S | S | R | S | 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

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 suggesting 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 are 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.

## Reference


[1] Emerson J, Rosenfeld M, McNamara S, et al. *Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis.* Pediatr Pulmonol 2002;34:91-100.

[2] Gibson RL, Burns JL, Ramsey BW. *Pathophysiology and management of pulmonary infections in cystic fibrosis.* Am J Respir Crit Care Med 2003;168:918-51.

[3] Nixon GM, Armstrong DS, Carzino R, et al. *Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis*. J Pediatr 2001;138:699-704.

[4] Driscoll JA, Brody SL, Kollef MH. *The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections.* Drugs 2007;67:351-68.

[5] Tsukayama DT, van Loon HJ, Cartwright C, et al. *The evolution of Pseudomonas aeruginosa during antibiotic rotation in a medical intensive care unit: the RADAR-trial.* Int J Antimicrob Agents 2004;24:339-45.

[6] Jones AM, Webb AK. *Recent advances in cross-infection in cystic fibrosis: Burkholderia cepacia complex, Pseudomonas aeruginosa, MRSA and Pandoraea spp.* J R Soc Med 2003;96(Suppl 43):66-72.

[7] Festini F, Buzzetti R, Bassi C, et al. *Isolation measures for prevention of infection with respiratory pathogens in cystic fibrosis: a systematic review*. J Hosp Infect 2006;64:1-6.

[8] O'Malley CA. *Infection control in cystic fibrosis: cohorting, cross-contamination, and the respiratory therapist.* Respir Care 2009;54:641-57.

[9] Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing; 18th informational supplement, document M100-S18. Wayne, PA 2008.

[10] Paterson DL. *The epidemiological profile of infections with multidrug-resistant Pseudomonas aeruginosa and Acinetobacter species.* Clin Infect Dis 2006;43(Suppl 2):S43-S48.

[11] Syrmis MW, O'Carroll MR, Sloots TP, et al. *Rapid genotyping of Pseudomonas aeruginosa isolates harboured by adult and paediatric patients with cystic fibrosis using repetitive-element-based PCR assays.* J Med Microbiol 2004;53:1089-96.

[12] Robinson P, Carzino R, Armstrong D, et al. *Pseudomonas cross-infection from cystic fibrosis patients to non-cystic fibrosis patients: implications for inpatient care of respiratory patients.* J Clin Microbiol 2003;41:5741.

[13] Moolenaar RL, Crutcher JM, San J, V, et al. *A prolonged outbreak of Pseudomonas aeruginosa in a neonatal intensive care unit: did staff fingernails play a role in disease transmission?* Infect Control Hosp Epidemiol 2000;21:80-5.

[14] Armstrong DS, Nixon GM, Carzino R, et al. *Detection of a widespread clone of Pseudomonas aeruginosa in a pediatric cystic fibrosis clinic.* Am J Respir Crit Care Med 2002;166:983-7.

[15] Cheng K, Smyth RL, Govan JR, et al. *Spread of beta-lactam-resistant Pseudomonas aeruginosa in a cystic fibrosis clinic.* Lancet 1996;348:639-42.

[16] Hunfeld KP, Schmidt C, Krackhardt B, et al. *Risk of Pseudomonas aeruginosa cross-colonisation in patients with cystic fibrosis within a holiday camp--a molecular-epidemiological study.* Wien Klin Wochenschr 2000;112:329-33.

[17] McCallum SJ, Corkill J, Gallagher M, et al. *Superinfection with a transmissible strain of Pseudomonas aeruginosa in adults with cystic fibrosis chronically colonised by P aeruginosa.* Lancet 2001;358:558-60.

[18] Ojeniyi B, Frederiksen B, Hoiby N. *Pseudomonas aeruginosa cross-infection among patients with cystic fibrosis during a winter camp*. Pediatr Pulmonol 2000;29:177-81.

[19] Speert DP, Campbell ME, Henry DA, et al. *Epidemiology of Pseudomonas aeruginosa in cystic fibrosis in British Columbia, Canada*. Am J Respir Crit Care Med 2002;166:988-93.

[20] Brusselaers N, Vogelaers D, Blot S. *The rising problem of antimicrobial resistance in the intensive care unit.* Ann Intensive Care 2011;1:47.

[21] Aloush V, Navon-Venezia S, Seigman-Igra Y, et al. *Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact.* Antimicrob Agents Chemother 2006;50:43-8.

[22] Suarez C, Pena C, Arch O, et al. *A large sustained endemic outbreak of multiresistant Pseudomonas aeruginosa: a new epidemiological scenario for nosocomial acquisition.* BMC Infect Dis 2011;11:272.

[23] Widmer AF, Wenzel RP, Trilla A, et al. *Outbreak of Pseudomonas aeruginosa infections in a surgical intensive care unit: probable transmission via hands of a health care worker*. Clin Infect Dis 1993;16:372-6.

[24] Giske CG, Monnet DL, Cars O, et al. *Clinical and economic impact of common multidrug-resistant gram-negative bacilli.* Antimicrob Agents Chemother 2008;52:813-21.

[25] Matar GM, Chaar MH, Araj GF, et al. *Detection of a highly prevalent and potentially virulent strain of Pseudomonas aeruginosa from nosocomial infections in a medical center.* BMC Microbiol 2005;5:29.

[26] Tramper-Stranders GA, van der Ent CK, Wolfs TF, et al. *Pseudomonas aeruginosa diversity in distinct paediatric patient groups.* Clin Microbiol Infect 2008;14:935-41.

[27] Chopra I, O'Neill AJ, Miller K. *The role of mutators in the emergence of antibiotic-resistant bacteria.* Drug Resist Updat 2003;6:137-45.

[28] Ratjen F, Brockhaus F, Angyalosi G. *Aminoglycoside therapy against Pseudomonas aeruginosa in cystic fibrosis: a review*. J Cyst Fibros 2009;8:361-9.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