The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original Article

A survey on pulmonary pathogens and their antibiotic susceptibility among cystic fibrosis patients

Ghamartaj Khanbabaee[a*], *Majidreza Akbarizadeh*[b], *Aliakbar Sayyari*[c], *Mitra Ashayeri-Panah*[d], *Fatemeh Abdollahgorji*[e], *Kourosh Sheibani*[f], *Nima Rezaei*[g]

[a]*Department of Pediatric Respiratory Diseases, CF Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[b]*Department of Pediatrics, Zabol University of Medical Sciences, Zabol, Iran*
[c]*Department of Pediatric Gastroenterology Diseases, CF Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[d]*Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran*
[e]*Clinical Research and Development Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[f]*Clinical Research and Development Center, Imam Hossein Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[g]*Research Center for Immunodeficiencies; and Molecular Immunology Research Center, Children's Medical Center, Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 7 August 2011
Accepted 30 August 2011

Keywords:
Cystic fibrosis
Respiratory tract infections
Products with antimicrobial action

A B S T R A C T

Objective: This study was performed to investigate frequency and antimicrobial susceptibility of pulmonary pathogens in cystic fibrosis (CF) patients.
Methods: 129 pediatric patients with CF were enrolled in this cross-sectional study. Microbiological cultures were performed based on sputum or pharyngeal swabs. Antibiotic susceptibilities of the isolated bacteria were determined by the disk diffusion method.
Results: The main infecting pathogens were *Pseudomonas aeruginosa* (38.8%), *Klebsiella pneumoniae* (11.6%) and *Staphyloccus areus* (9.3%), respectively. The most active antibiotics included rifampin (91.7% susceptibility), vancomycin (85%) and imipenem (83.5%). Emerging resistance against aminoglycosides was observed.
Conclusion: Regarding *in vitro* susceptibility results, cyclic treatment of long-term oral azithromycin and inhaled tobramycin could prophylactically be applied, and during exacerbations, imipenem or ceftazidime in combination with an aminoglycoside such as amikacin could be considered the drugs of choice.

*Corresponding author at: Department of Pediatric Respiratory Diseases, Mofid Children Hospital, Shariati St, Tehran, 15468-15514, Iran
E-mail address: khanbabaeegh@yahoo.com (Ghamartaj Khanbabaee)

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal pediatric disease caused by mutation in the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene, which results in multiple organ failure, and 85% of the mortality is due to pulmonary infections.[1,2] According to the most accepted hypothesis that explains the mechanism underlying respiratory infection acquisition in CF patients, defective CFTR results in a disturbance in chloride secretion across the apical membrane of epithelial cells causing airway surface liquid depletion and impaired mucociliary clearance, which leads to bacterial colonization.[3-5] Despite the polymicrobial nature of CF lung infections, *Staphylococcus aureus* or *Haemophilus influenzae* are often pioneers in colonization. Later on *Pseudomonas aeruginosa* (PA) and other gram-negative non-fermenting organisms, such as *Stenotrophomonas maltophilia*, members of the *Burkholderia cepacia*-complex, and *Alcaligenes xylosoxidans* prevail.[3] Biofilm formation due to bacterial conversion to mucoidy and inflammation due to host inflammatory response result in airway obstruction, which is the key cause of morbidity and mortality in CF patients.[4] To postpone the decline of lung function and prevent the spread of antimicrobial resistant pathogens, accurate antibiotic treatment strategies are of great importance.[6-7] Since there are no published data on microbiological surveillance of CF patients from the Middle East, this study aims to investigate the frequency and antimicrobial susceptibility patterns of bacterial pathogens in CF patients admitted to the Mofid Children's Hospital, Tehran, Iran during a six-year-period.

Materials and methods

Patients and samples

129 pediatric patients with CF who had acute exacerbation and were hospitalized in the Mofid Children's Hospital, from 2004 to 2010 were enrolled in this cross-sectional study. Diagnosis of CF was performed according to guidelines recommended by the Cystic Fibrosis Foundation Consensus.[8] Data on gender, age of diagnosis, age of first exacerbation, age at admission, and hospitalization period were gathered based on a questionnaire and on a review of patients' medical records. This study was approved by the Shahid Beheshti University of Medical Sciences' Ethical Committee and an informed consent was obtained from the patients' parents.

Microbiological cultures

During exacerbation, sputum samples or pharyngeal swabs were obtained and cultured on agar medium for 48 hours at 37°C. The dominant bacteria grown for each patient sample were isolated and identified by standard biochemical tests.[9]

Statistical analysis

To compare differences between age, gender and culture based groups of the patients, a non-parametric statistical analysis was performed using the two-tailed Mann Whitney U test and the Kruskal-Wallis test, allowing for continuous variables and non-normal distribution.

Susceptibility tests

Susceptibility of the isolated bacteria to 29 antibiotics (Mast Diagnostics – UK), shown in Fig. 1, was performed by disk diffusion technique according to the CLSI criteria.[10]

Results

Among the 129 CF patients, 51 (39.5%) were female and 78 (60.5%) were male. The mean (± SD) age of the patients was 75.57 (± 6 2.48) months; the median age was 63 months with the age range of 4-215 months. The average age at diagnosis, age of first exacerbation and age at admission were 4.95, 9.3 and 75.57 months, respectively. Demographic data of the patients are shown in Table 1. There was no significant difference between female and male groups in age at admission, age of first exacerbation and age at diagnosis, as well as rate of PA colonization (two-tailed Mann-Whitney U test, $p > 0.05$). Hospitalization period was 12.2 days on average, and significant difference was observed in hospitalization periods between patients who had positive cultures and patients with negative cultures (10.44 vs. 12.62 days, two-tailed Mann-Whitney U test, $p < 0.05$). Among culture positive patients, the hospitalization period was significantly longer in patients infected with PA compared to other microorganisms (13.28 vs. 11.5, two-tailed Mann Whitney U test, $p < 0.05$). 123 bacterial and six fungal strains were isolated and characterized. In 24% of the patients, no microorganisms were isolated and 24% of the patients were infected by more than one species. Patients were sub-divided by age: under or equal to 24 months (32.6%), 24 to 60 months (49.6%), and over 60 months (50.4%). Differences in bacterial colonization by age groups are demonstrated in Fig. 2.

Table 1 – Demographic findings of enrolled CF patients in this study

	Variable (unit)	Range	Mode	SD	Mean
Age at admission (months)	4-215	11	63	62.5	75.6
Age of first exacerbation (months)	1-120	1	4	19.3	9.3
Age at diagnosis (months)	3-6	6	5	1.16	4.95
Hospitalization period (days)	3-21	10	12	4.1	12.2

SD, standard deviation.

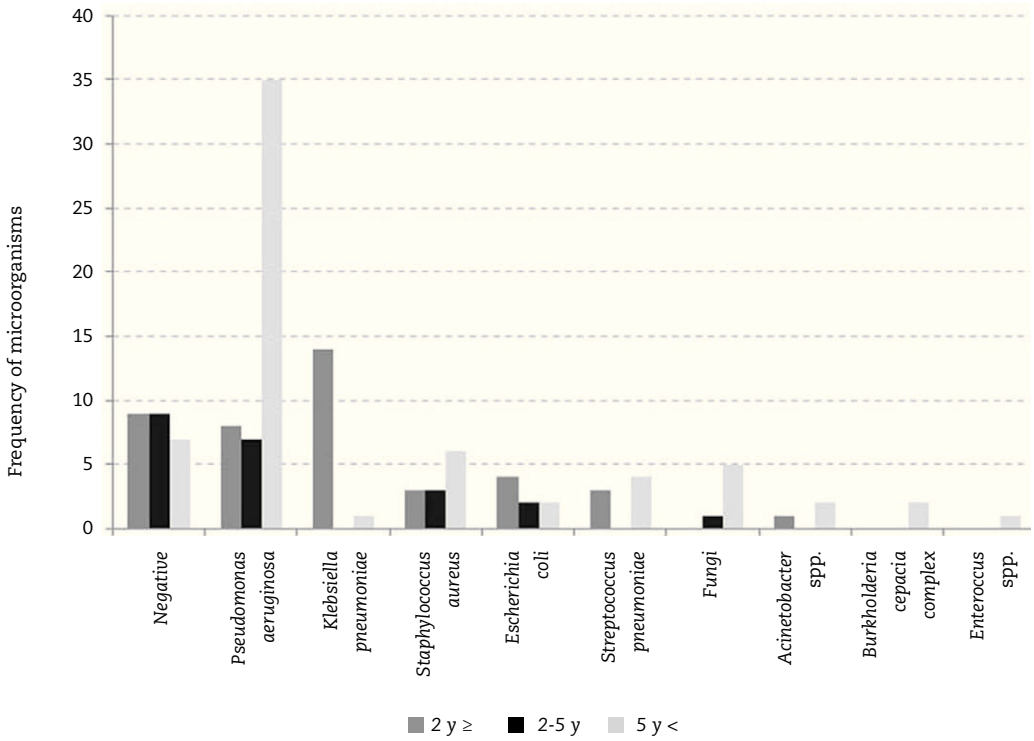

Fig. 1 – Rate of *in vitro* susceptibility (%) of *Pseudomonas aeruginosa* isolates against different antibiotics.

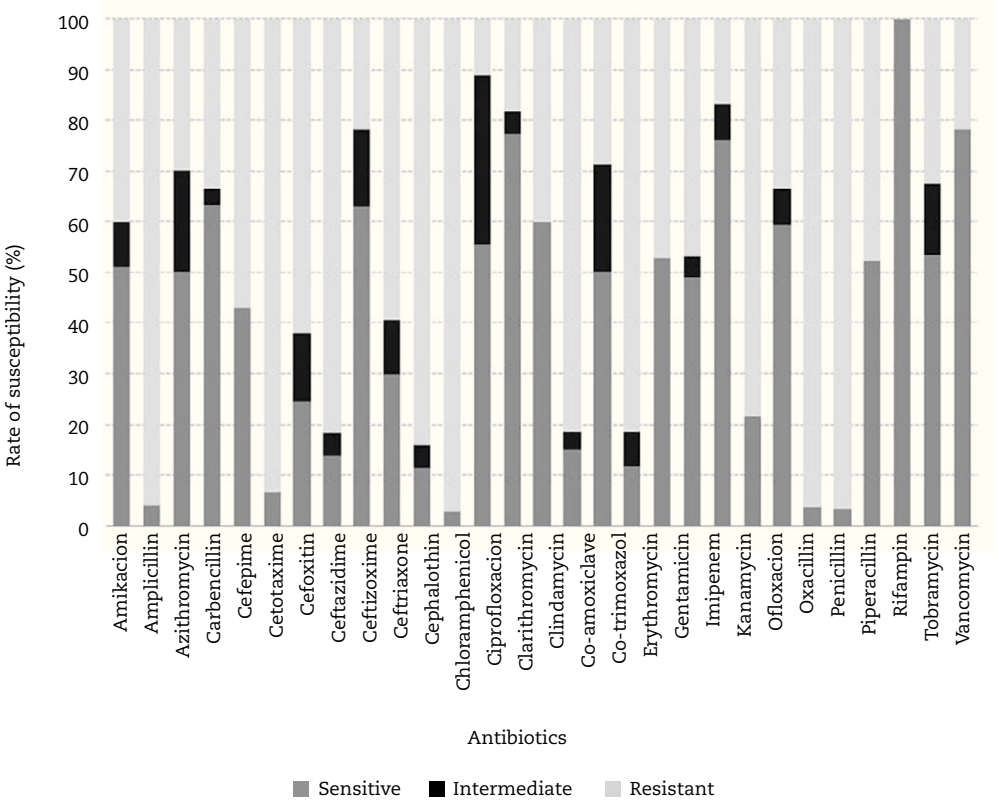

Fig. 2 – Frequencies of negative and positive cultures for each microorganism based on age groups.

Overall, PA was isolated as the most prevalent germ. Among patients under two years of age, *K. pneumoniae* was the most frequently isolated bacteria, and among patients over two years of age, PA. The main infecting pathogens were PA (38.8%), *Klebsiella pneumoniae* (11.6%), *Staphyloccus areus* (9.3%), *Escherichia coli* (6.2%) and *Streptococcus pneumoniae* (5.4%), respectively. In total, colonization rate was significantly age-related: 78.6% in patients under the age of two years, 71.9% in patients under the age of five years, and 89.2% in patients over the age of five years (Kruskal-Wallis test, $p < 0.01$). It was observed that as the age of patients increased, colonization rate of PA, *S. aureus*, *S. pneumoniae* and *Acinetobacter spp*. increased, and the frequency of *K. pneumoniae* decreased. The colonization rate of PA was 19% in patients younger than 2 years of age, 23.4% in patients younger than five years of age and 53.8% in patients older than five years of age. Fungi were isolated only from patients older than two years of age and some microorganisms, including *B. cepacia*-complex and *Enterococcus spp*. were only detected in patients older than five years of age (Fig. 2). Antibiotic susceptibility results of the three most prevalently isolated bacteria are shown in Figs. 1, 3 and 4. Differences in the rate of *in vitro* resistance to antibiotics and the rate of *in vitro*

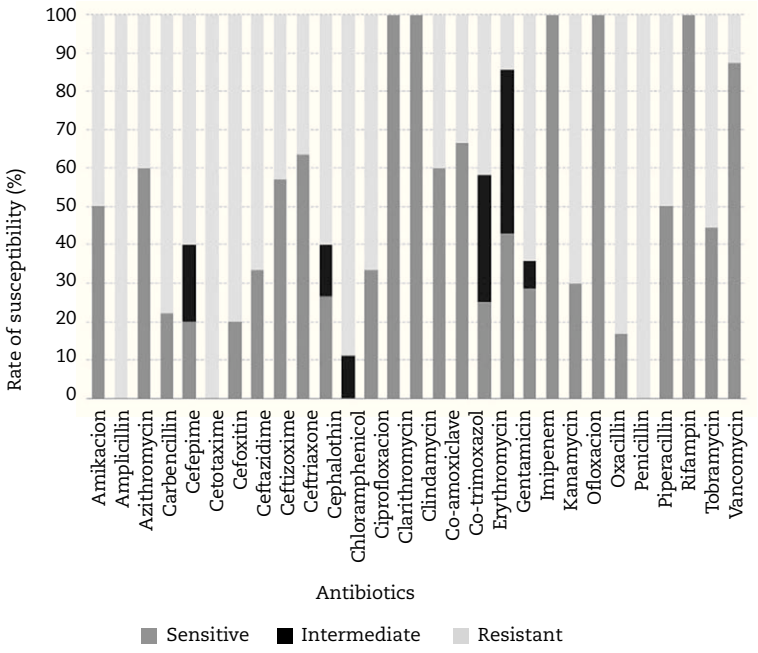

Fig. 3 – Rate of *in vitro* susceptibility (%) of *Klebsiella pneumoniae* isolates against different antibiotics.

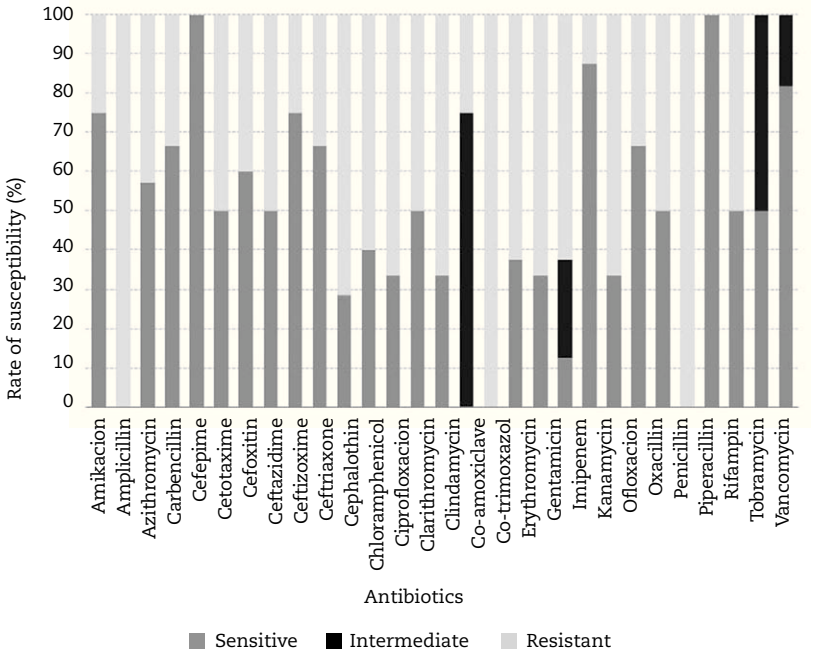

Fig. 4 – Rate of *in vitro* susceptibility (%) of *Staphylococcus aureus* isolates against different antibiotics.

susceptibility to antibiotics by age groups are demonstrated in the Figs. 5 and 6, respectively. The most active antibiotics included rifampin (91.67% susceptibility), vancomycin (85%), imipenem (83.54%), ciprofloxacin (79.49%), ofloxacin (75.86%), ceftazidime (67.82%) and azithromycin (61.22%) and the less active antibiotics were penicillin (97.87% resistance), ampicillin (95.56%), cephalothin (92%), oxacilin (89.13%) and cefixime (86.9%), respectively. Among various identified bacteria, *E. coli,* PA, *K. pneumoniae, S. aureus*, and *Enterococcus spp*. exhibited the highest antimicrobial resistance, respectively.

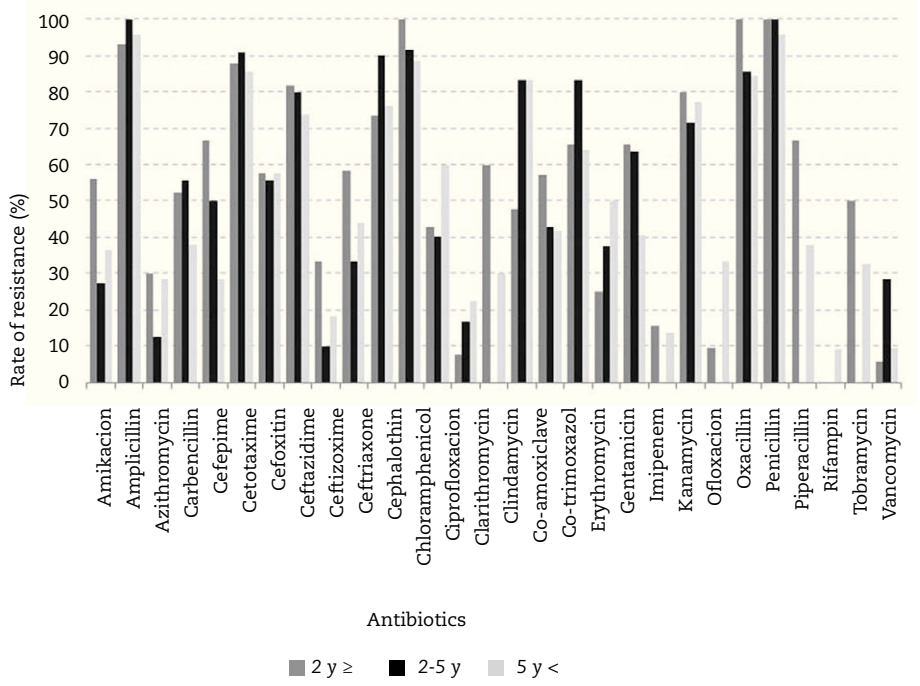

Fig. 5 – Rate of *in vitro* antibiotic resistance based on age groups of the patients.

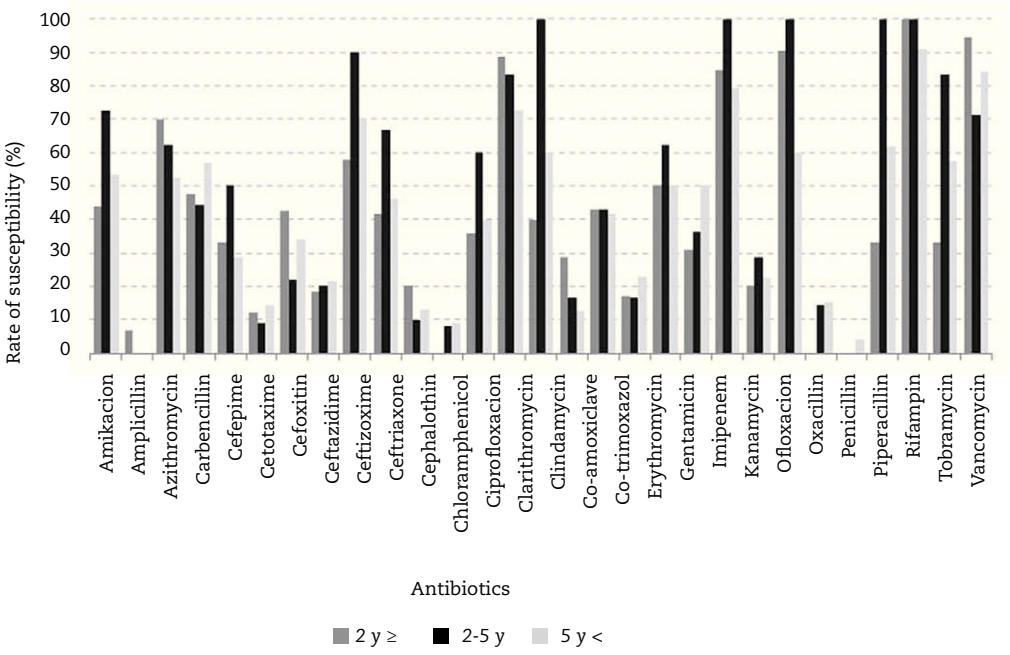

Fig. 6 – Rate of *in vitro* antibiotic susceptibility based on age groups of the patients.

Discussion

Several previous studies have evaluated the microbiology of pulmonary infections in patients with CF, but there have been no studies on this subject from the Middle East.[11-14] In this study, we aimed to survey pulmonary infections of CF patients from the Middle East, specifically from Iran. In contrast to some studies that have reported female gender as a risk factor for colonization of PA in CF patients, we found no significant difference in PA colonization between gender groups in our patients.[15-16] Although the colonization rate of PA among our patients (38.8%) was less than the overall percentage reported by the Cystic Fibrosis Foundation, USA, in 2006 (55.0%), in accordance with other reports, we detected PA as the most prevalent pulmonary pathogen in our CF patients, mainly in older children and caused longer hospitalization periods.[2-3,17] According to data from the Cystic Fibrosis Foundation (USA) in 2006, the initial PA colonization often occurs in patients with CF under the age of 6 years and increasing age is a risk factor for PA infection in CF patients.[2] The same results were obtained in our study – colonization rate of PA was 19% among patients younger than two years of age and 23.4% among patients younger than five years of age, which was increased to 53.8% in patients older than five years of age. There is paucity of reports regarding colonization of *K. pneumoniae* in CF patients which was the second most prevalent microorganism isolated from our patients.[18,19] In agreement with other reports, *S. aureus* was one of the prevailing isolated bacteria in our study. Conflicting with reports that have detected *S. aureus* as one of the first bacteria infecting pediatric patients with CF, in our study *S. aureus* was more common among patients over two years of age.[3,6] Recent studies have reported the advent of *S. pneumoniae* as a resistant pathogen in CF patients.[20,21] Although in our study *S. pneumoniae* showed a higher frequency than its common frequency (3-5%), it was the most susceptible germ among the investigated bacteria (data not shown).[22] Some pathogens such as *H. influenzae*, *S. maltophilia* and *A. xylosoxidans* have been recently detected more frequently in CF patients, which could be considered as a result of modern therapies.[12,13] In our study, these microorganisms were not frequent, perhaps because of the traditional therapies that have been applied to our patients.

Regarding in vitro[23]

Although in our study rifampin was shown to be the most active antibiotic, resistance to rifampin was found among a few *S. aureus* isolates. Recent concerns have been raised about the emergence of heteroresistant vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-resistant *Streptococcus* (VRS).[24,25] In our study, all *S. pneumoniae* isolates were sensitive to vancomycin, but some isolates of *S. aureus* appeared to be intermediate. Except for *K. pneumoniae* and *S. pneumoniae* isolates, all of which were sensitive to imipenem. Resistance against imipenem among three other prevalent bacteria (remarkably so in PA) could be considered as a confirmation for increasing resistance against this antibiotic among CF patients.[26] Ciprofloxacin, ceftazidime and azithromycin have been recommended as therapies for PA eradication in CF patients.[27] Because of relative *in vitro* resistance observed against these antibiotics among our PA isolates, if one of them was not effective on a patient, another could be applied as second-line therapeutic agent. Inhaled tobramycin is a front-line aminoglycoside used successfully for early eradication of PA.[22] Because of the emerging resistance to tobramycin observed in our study, the maintenance of its clinical benefits depends on its chronic and cyclic use, as reported by other studies.[27-28] In total, *in vitro* decrease of the formation of biofilms by quorum sensing disruption, modulation of the host inflammatory response, reduction in sputum viscosity, and enhancement of sputum clearance were discussed in a previous study, and in agreement with another long-term study, oral azithromycin may cause improvement in respiratory function in CF patients.[29,30] Conflicting to a recent study that reported treatment with azithromycin for 24 weeks resulting in no improvement in pulmonary function of CF patients uninfected with PA, in our study azithromycin was one of the most active antibiotics *in vitro*, not only against PA isolates but also against other isolated respiratory pathogens;[31] and activities of macrolides were greater than aminoglycosides. Among macrolides, azithromycin was the most active antibiotic *in vitro*. Due to positive effects of macrolides on susceptibility results, rifampin is a typical treatment of *S. aureus* infections in CF patients.

Because of the high frequency of PA among our patients, a policy of segregating patients to prevent epidemic strain transmission is recommended.[21] Although the clinical relevance of *in vitro* antimicrobial susceptibility of bacterial pathogens involved in CF pulmonary infections has been questioned, as there are no clear alternatives, based on our *in vitro* results we assume that oral azithromycin and nebulized tobramycin could be effective if used in a cyclic fashion as a long-term prophylaxis, and in cases of resistance, an off-period for these antibiotics should be applied.[22] During exacerbations, imipenem or ceftazidime in combination with an aminoglycoside such as amikacin could be drugs of choice that rely on clinical responses of the patients, and in cases of gram positive infections, rifampin could be added.

Acknowledgements

The work was thankfully supported financially by the Mofid Children's Hospital and the Shahid Beheshti University of Medical Sciences.

Conflict of interest

All authors declare to have no conflict of interest.

REFERENCES

1. Boat TF. Cystic Fibrosis. In: Behrman RE, Kliegman RM, Jenson HB, ed. Nelson Textbook of Pediatrics, 17th edition. Philadelphia: Saunders; 2004. p. 1437-49.
2. Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Patient Registry, 2006 Annual data report to the center directors. Bethesda, Maryland: Cystic Fibrosis Foundation; 2007.
3. Davies JC, Bilton D. Bugs, biofilms, and resistance in cystic fibrosis. Respir Care. 2009;54:628-40.
4. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. Respir Care. 2009;54:595-605.
5. Matsui H, Grubb BR, Tarran R, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell. 1998;95:1005-15.
6. Stone A, Saiman L. Update on the epidemiology and management of *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus*, in patients with cystic fibrosis. Curr Opin Pulm Med. 2007;13:515-21.
7. Merlo CA, Boyle MP, Diener-West M, et al. Incidence and Risk Factors for Multiple Antibiotic-Resistant *Pseudomonas aeruginosa* in Cystic Fibrosis. Chest. 2007;132:562-68.
8. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr. 2008;153:S4-S14.
9. Mac Faddin JF. Biochemical tests for identification of medical bacteria. 3rd edition. Baltimore: Williams & Wilkins, 1999.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests, 10th edition. Approved standard M2-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
11. Burns JL, Emerson J, Stapp JR, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin Infect Dis. 1998;27:158-63.
12. Valenza G, Tappe D, Turnwald D, et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cyst Fibros. 2008;7:123-27.
13. Emerson J, McNamara S, Buccat AM, et al. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. Pediatr Pulmonol. 2010;45:363-70.
14. Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. Chest. 2009;136:1554-60.
15. Olesen HV, Pressler T, Hjelte L, et al. Gender differences in the Scandinavian cystic fibrosis population. Pediatr Pulmonol. 2010;45:959-65.
16. Levy H, Kalish LA, Cannon CL, et al. Predictors of mucoid *Pseudomonas* colonization in cystic fibrosis patients. Pediatr Pulmonol. 2008;43:463-471.
17. Champs NS, Gomes DL, Alvin CG, et al. Early colonization by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in newborn screening children. J Cyst Fibros. 2009;8:S64.
18. Leão RS, Pereira RHV, Folescu TW, et al. KPC-2 Carbapenemase-producing *Klebsiella pneumoniae* isolates from patients with cystic fibrosis. J Cyst Fibros. 2011;10:140-42.
19. Bittar F, Richet H, Dubus JC, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. PLoS One. 2008;3:e2908.
20. Tazumi A, Maeda Y, Goldsmith CE, et al. Molecular characterization of macrolide resistance determinants [*erm* (B) and *mef* (A)] in *Streptococcus pneumoniae* and viridans group streptococci (VGS) isolated from adult patients with cystic fibrosis (CF). J Antimicrob Chemother. 2009;64:501-06.
21. Maeda Y, Elborn JS, Parkins MD, et al. Population structure and characterization of viridans group streptococci (VGS) including *Streptococcus pneumoniae* isolated from adult patients with cystic fibrosis (CF). J Cyst Fibros. 2011;10:133-139.
22. Foweraker J. Recent advances in the microbiology of respiratory tract infection in cystic fibrosis. Br Med Bull. 2009;89:93-110.
23. Garske LA, Kidd TJ, Gan R, et al. Rifampicin and sodium fusidate reduce the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation in adults with cystic fibrosis and chronic MRSA infection. J Hosp Infect. 2004;56:208-14.
24. Cafiso V, Bertuccio D, Campanile F, et al. Methicillin resistance and vancomycin heteroresistance in *Staphylococcus aureus* in cystic fibrosis patients. Eur J Clin Microbiol Infect Dis. 2010;29:1277-85.
25. Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae*: trends and management. Expert Rev Anti Infect Ther. 2008;6:619-635.
26. Wolter DJ, Acquazzino D, Goering RV, et al. Emergence of carbapenem resistance in *Pseudomonas aeruginosa* isolates from a patient with cystic fibrosisin the absence of carbapenem therapy. Clin Infect Dis. 2008;46:e137-e141.
27. Stuart B, Lin JH, Mogayzel Jr PJ. Early Eradication of *Pseudomonas aeruginosa* in Patients with Cystic Fibrosis. Paediatr Respir Rev. 2010;11:177-84.
28. Ratjen F, Brockhaus F, Angyalosi G. Aminoglycoside therapy against *Pseudomonas aeruginosa* in cystic fibrosis: A review. J Cyst Fibros. 2009;8:361-69.
29. Nguyen T, Louie SG, Beringer PM, et al. Potential role of macrolide antibiotics in the management of cystic fibrosis lung disease. Curr Opin Pulm Med. 2002;8:521-528.
30. Southern KW, Barker PM, Solis A. Macrolide antibiotics for cystic fibrosis. Cochrane Database of Syst Rev. 2004;2:CD002203.
31. Saiman L, Ansteasd M, Mayer-Hamblett, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*. J Am Med Assoc. 2010;303:1707-15.