Microbiology of Upper Respiratory Tract Pathogens in Cystic Fibrosis Patients

Maryam Node Sharifi[1], Hamid Reza Kianifar[2], Sepideh Bagheri[3], Seyed Javad Sayedi[4]

[1] *Department of Pediatrics, Ghaeam Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

[2] *Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[3] *Department of Pediatrics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[4] *Neonatal Research Center, Akbar Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

Received: 15 Nov. 2017; Accepted: 02 Mar. 2018

Abstract- Cystic fibrosis (CF) is an inherited genetic disorder with chronic respiratory manifestations. The respiratory symptoms may start very early in life. The aim of this study was to evaluate the prevalence and antimicrobial susceptibility of respiratory pathogens in children with CF. In this clinical laboratory study, 100 CF patients were prospectively collected from February 2016 to March 2017. Microbiological cultures and antimicrobial susceptibility tests of the most frequently isolated upper respiratory tract bacteria were performed. According to the results of this study, *Staphylococcus aureus* was the most frequent microorganism 24 (24%) in CF patients followed by *Pseudomonas aeruginosa* 21 (21%). In children younger than one-year-old, Enterococci and Klebsiella pneumonia were the most frequently isolated pathogens. In other age groups, *Staphylococcus aureus* and *Pseudomonas aeroginosa* were most frequent. All pathogens showed more sensitivity to Ceftriaxone, Amikacin, and Ceftazidime. However, *Staphylococcus aureus* was most sensitive to Cefoxitin, Clindamycin, and Linezolid and *Pseudomonas aeroginosa* were most sensitive to Amikacin, Ceftazidime, and Ceftriaxone respectively. In conclusion, *Staphylococcus aureus* and *Pseudomonas aeroginosa* were the most frequent microorganisms in CF patients in our population. In patients younger than one-year-old, the most frequent pathogens were *Enterococci* and *Klebsiella*. All pathogens and *Pseudomonas aeroginosa* were sensitive to Ceftriaxone, Amikacin, and Ceftazidime but *Staphylococcus aureus* was most sensitive to Cefoxitin, Clindamycin, and Linezolid respectively. It seems that Ceftriaxone, Amikacin, and Ceftazidime are the most suitable antibiotics for the treatment of pulmonary infections in CF patients in our population.

© 2018 Tehran University of Medical Sciences. All rights reserved.

Acta Med Iran 2018;56(7):450-456.

Keywords: Antibiotic; Antibiogram; Cystic fibrosis; Microbiology; Respiratory pathogen

Introduction

Cystic fibrosis (CF) is an inherited genetic condition caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (1). It is the most common life-threatening autosomal recessive disease in the white population and affects over 30.000 people in the United States (2).

The main feature of CF is its chronic respiratory infections, which might start very early in the life of these patients. Between 2000 and 2010, the number of CF patients increased from 21 000 to 26 000, median age increased from 14.3 to 16.7 years old, and adjusted mortality decreased by 1.8% per year (95% CI, 0.5% to 2.7%) (3). The history of disease management in CF is overcoming one manifestation of the disease only to be confronted with another. Today more than 90% of the morbidity and mortality of CF is due to the lung failure associated with chronic pulmonary infections (4-6).

Chronic pulmonary infection with opportunistic bacteria is the major cause of morbidity and mortality in CF. Staphylococcus aureus (S. aureus) has been one of the first pathogens infecting CF airways for extended periods (7). Later, up to 80% of adults with CF are chronically colonized with Pseudomonas aeruginosa, (8) indicating progression of the pulmonary destruction (9). So it seems that aggressive antibiotic treatment can improve life expectancy (10).

Previous longitudinal studies have shown that most CF patients had periods of long-term colonization by one predominant S. aureus genotype, but variation in S. aureus genotypes within a single patient has also been

Corresponding Author: S.J. Sayedi
Neonatal Research Center, Akbar Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
Tel: +98 915 5177719, Fax: +98 511 38709201 E-mail address: sayedij@mums.ac.ir

observed (11). When CF patients become colonized with *P. aeruginosa*, the same results were seen, namely long-term colonization by one or two genotypes (12).

In CF patients, methicillin-susceptible S. aureus (MSSA), non-typeable *Haemophilus influenzae*, and *Pseudomonas aeruginosa* are the most common airway pathogens during the first decade of life. Infections with *P. aeruginosa* and *Burkholderia cepacia* complex are associated with a decline in lung function and are predictors of morbidity and mortality in CF patients, (13) and methicillin-resistant S aureus (MRSA), *Stenotrophomonas maltophilia*, and *Alcaligenesxylosoxidans* are increasingly identified as potential pathogens in patients with CF (14-16).

In the last four decades, median predicted survival has risen four-fold with individuals born today expected to survive well into their fifth decade of life. In parallel to the changing epidemiology of patients, recent reports have highlighted the changes that are occurring within the spectrum of organisms causing infection in CF patients (17,18).

As respiratory manifestations continue to be the hallmark of CF and are primarily responsible for the attributable morbidity and mortality, understanding the spectrum and role of organisms involved in CF airways disease is of paramount importance.

Recently, an increasing antibiotic resistance against the most commonly used antibiotics in CF patients is being reported. Above all related to the emergence of hypermutable bacteria, this implies difficulties on therapeutic approach (19,20).

The specific epidemiology of bacteria associated with CF may vary from different centers in different areas of the world and awareness of the local epidemiology may be useful for developing prevention strategies.

Therefore, the aim of this study was to evaluate the prevalence, and antimicrobial susceptibility of respiratory pathogens in children with CF followed in our CF center.

Materials and Methods

Subjects and data collection

In this clinical laboratory study, 100 CF patients referred to Dr. Sheikh, and Ghaem Hospitals between February2016 and March 2017 were enrolled in the study. The diagnosis of CF was confirmed according to the criteria of the CF Foundation (21).

Upper respiratory tract culture was obtained for each patient. All specimens were examined microscopically and cultured in agar blood, agar chocolate, Eosin methylene blue agar (EMB) incubated for a period of 18 to 48 hours at 37° C, followed by room temperature incubation for up to 72 hours. Preparation of suspensions, inoculations, incubation times, temperatures, and interpretation of reactions were performed according to the manufacturer's instructions. Additional biochemical tests for bacterial identification were performed when necessary.

Ethical considerations

The study was approved by the Ethical Committee of Mashhad University of Medical Sciences. Written informed consent and verbal assent were obtained from each patient or children's parent or guardian and from all study participants, respectively.

Data analysis

Statistical analysis was carried out using SPSS, version 17. The Kolmogorov-Smirnov test was performed to assess normal distribution. The normal quantitative and abnormal quantitative data expressed as mean±standard deviation by one sample *t*-test and median±interquartile range by Man-Whitney test respectively. The Chi-square test was performed for qualitative data and expressed as number (percentage). *P* less than 0.05 was considered statistically significant.

Results

Demographic results of all patients are described in table 1.

In our study population, 48% of patients reported respiratory symptoms like cough, wheezing, and pneumonia as their initial presentation.

We also evaluated the use of prophylactic antibiotics in our study population as this could affect the antimicrobial susceptibility tests. 86% of the study population reported the use of prophylactic antibiotics. 86% had received Azithromycin, 8% amoxicillin-clavulanate, 4% ciprofloxacin and 2% levofloxacin. 38% had received amikacin in the form of nebulizers.

Table 2 shows the prevalence of pathogens according to sex. The results show that S. aureus was the most frequent microorganism 24 (24%) in CF patients. After that *Pseudomonas aeruginosa* was isolated 21 (21%). Other frequent pathogens were Streptococcus beta non hemolytic group A 8 (8%), *Enterococci* 7 (7%), Escherichia coli 6 (6%), *Klebsiella pneumonia* 6 (6%), Staphylococcus epidermis 5 (5%), Streptococcus alpha hemolytic 6 (6%), *Streptococcus* beta-hemolytic group A 4 (4%), Candida spp. 2 (2%), Serratia spp. 1 (1%), *Citrobacterfrondii* 1 (1%) and *Streptococcus pneumoniae*

1 (%1).

Table 1. Demographic characteristics of patients according to sex

Variable		Male (n=48)	Female(n=52)	Total (n=100)	*p*
Age (Month)		78.94±50.68	87.31±61.06	83.25±56.140	0.458
Diagnosis time (month)		4.00±(1.25-15.75)	4.00±(2.00-22.50)	4.00±(2.00-18.00)	<0.001**
Hospitalization Frequency (Number)		2.00±(1.00-4.75)	2.00±(1.00-4.00)	2.00±(1.00-4.00)	<0.001**
location	**village**	10(21.3%)	13(25%)	23(23.2%)	0.812
	city	37(78.7%)	39(75%)	76(76.8%)	

All values are mean ± standard deviation for quantitative normal distribution variables; also number (percentage)for Qualitative variables, * *P*<0.05, ** *P*<0.001

Table 2. The prevalence of pathogens according to sex

Pathogens	Male (n=48)	Female(n=52)	Total (n=100)
Pseudomonas aeroginosa	11(22.9%)	10(19.2%)	21(21%)
Staphylococcus aureus	11(22.9%)	13(25%)	24(24%)
Staphylococcus epidermis	1(2.1%)	4(7.7%)	5(5%)
Streptococcus beta non hemolytic group A	5(10.4%)	3(5.8%)	8(8%)
Streptococcus beta hemolytic group A	0(0%)	4(7.7%)	4(4%)
Streptococcus alpha hemolytic	2(4.2%)	4(7.7%)	6(6%)
Enterococci	3(6.2%)	4(7.7%)	7(7%)
Escherichia coli	4(8.3%)	2(3.8%)	6(6%)
Candida spp.	1(2.1%)	1(1.9%)	2(2%)
Klebsiella pneumoniae	2(4.2%)	4(7.7%)	6(6%)
Serratia spp.	0(0%)	1(1.9%)	1(1%)
Citrobacterfrondii	1(2.1%)	0(0%)	1(1%)

All values are number (percentage) for Qualitative variables

Table 3 shows the frequency of airway pathogens in different age groups. As it shows in CF children younger than 12 months, the frequency of isolated respiratory pathogens were *Enterococci* 30%, *Klebsiella pneumonia* 30%, *Pseudomonas aeruginosa* 20% and Escherichia coli 10%. In other age groups, *Pseudomonas aeruginosa* and S. aureus are more prevalent.

Table 3. Pathogens in different age groups

Age (years)	% of patients	PA%	SA%	SE%	SBNHA%	SAH%	E%	EC%	KP%	Others%
<1	6.5	20	0	10	0	0	30	10	30	0
1-3	17.4	20	20	0	6.7	13.3	0	13.3	6.7	6.7
3-5	23.9	14.3	33.3	9.5	9.5	9.5	9.5	4.8	0	9.5
5-7	2.2	35.7	14.3	0	14.3	0	7.1	7.1	0	14.3
7-9	8.7	30	20	0	0	0	10	10	0	10
9-11	0	16.7	41.7	16.7	8.3	8.3	0	0	0	0
11-13	2.2	16.7	16.7	0	16.7	0	0	0	16.7	33.3
13-15	6.5	28.6	14.3	0	14.3	0	0	0	14.3	0
≥15	8.7	0	75	0	0	0	0	0	0	25

PA: Pseudomonas aeruginosa, SA: Staphylococcus aureus, SE: Staphylococcus epidermis, SBNHA: Streptococcus beta nonhemolytic group A, SAH: Streptococcus alpha hemolytic, E: Enterococci, EC: Escherichia coli, KP: Klebsiella pneumonia, others: Citrobacterfrondii, Serratia spp., Candida spp., Streptococcus beta-hemolytic group A

Table 4 shows the rate of susceptibility and resistance (%) of all pathogens to different antimicrobial agents.

As shown in this table, S. aureus is most sensitive to linezolid and is also sensitive to cefoxitin, teicoplanin, and clindamycin. Vancomycin was not promising in this study.

Pseudomonas aeruginosa was most sensitive to amikacin and ceftazidime.

Table 4. The rate of susceptibility and resistance (%) of pathogens against different antimicrobial agents

Antimicrobial agents	All pathogens		Staphylococcus aureus		Pseudomonas aeruginosa	
	Susceptibility (%)	Resistance (%)	Susceptibility (%)	Resistance (%)	Susceptibility (%)	Resistance (%)
Amikacin	28	2	4.2	0	76.2	4.8
Amoxicillin	0	24	0	41.7	0	33.3
Ampicillin	12	30	8.3	58.3	0	33.3
Ampicillin-sulbactam	10	12	0	0	9.5	28.6
Azitromycin	7	12	20.8	20.8	0	4.8
Cefazolin	4	15	0	4.2	0	38.1
Cefixim	15	5	33.3	4.2	0	19
Cefoxitin	14	4	45.8	0	0	0
Ceftazidime	22	6	0	4.2	57.1	9.5
Ceftizoxime	14	7	33.3	4.2	0	28.6
Ceftriaxone	34	6	33.3	4.2	57.1	9.5
Ceftuzid	2	0	0	0	0	0
Cefuroxime	8	12	0	0	4.8	42.9
Cephalexin	0	21	0	33.3	0	33.3
Ciprofloxacin	11	6	29.2	0	4.8	28.6
Claritomycin	0	0	0	0	0	0
Clindamycin	14	13	41.7	33.3	0	0
Cloxacillin	0	13	0	29.2	0	9.5
Colistin	10	3	0	0	38.1	0
Co-amoxiclave	0	1	0	4.2	0	0
Co-trimoxazole	2	21	0	33.3	4.8	33.3
Doxycycline	2	18	4.2	29.2	0	33.3
erythromycin	5	27	12.5	58.3	0	9.5
Gentamycin	4	20	4.2	33.3	0	19
Imipenem	7	0	0	0	0	33.3
Kanamycin	1	1	0	4.2	0	0
Linezolid	19	0	41.7	0	0	0
Levofloxacin	1	0	0	0	4.8	0
Meropenem	17	1	0	4.2	33.3	0
Nalidixic acid	2	0	0	0	0	0
Nitrofurantonin	3	0	0	0	0	0
norfloxacin	2	0	8.3	0	0	0
Ofloxacin	2	0	0	0	0	0
Oxacillin	0	1	0	4.2	0	0
Oxytetracyclin	2	0	4.2	0	0	0
Penicillin	13	25	8.3	83.3	0	9.5
Piperacillin-tazobactam	19	0	4.2	0	38.1	0
Teicoplanin	13	5	37.5	4.2	0	0
Tetracycline	2	21	4.2	33.3	0	33.3
Vancomycin	10	2	12.5	4.2	0	0

Discussion

According to the results of this study, S. aureus was the most frequent microorganism 24 (24%) in CF patients. The second most prevalent microorganism was *Pseudomonas aeruginosa* 21 (21%). Other microorganisms found in the upper airway culture of our study population were as follows: Streptococcus beta non hemolytic group A 8(8%), *Enterococci* 7 (7%), *Escherichia coli* 6 (6%), *Klebsiella pneumonia* 6 (6%), *Staphylococcus epidermis* 5 (5%), Streptococcus alpha hemolytic 6 (6%), *Streptococcus* beta-hemolytic group A 4 (4%), *Candida spp*. 2 (2%), *Serratia spp*. 1 (1%), *Citrobacterfrondii* 1 (1%) and *Streptococcus pneumoniae* 1 (%1). In patients, younger than 12 months, the most frequent pathogens were *Enterococci* and *Klebsiella pneumonia*. In other age groups, *S. aureus* and *Pseudomonas spp.* were most frequent. All pathogens were sensitive to Ceftriaxone, Amikacin, and Ceftazidime. Furthermore, S. aureus was sensitive to Cefoxitin, Clindamycin and Linezolid and *Pseudomonas aeruginosa* were sensitive to Amikacin, Ceftazidime, and Ceftriaxone.

In a study by JE Hoppe *et al*., in 1995 in Germany on

50 pediatric CF patients, the most frequent airway pathogens were *Candida albicans*, *Pseudomonas aeruginosa*, *S. aureus* , *Enterobacteriaceae* family, *Aspergillus fumigates*, *Haemophilus influenza* type b, Candida parapsilosis (22). Their microbiologic pattern seems different from our population.

Razvi *et al*., studied the respiratory microbiology of patients with CF in the United States from 1995 to 2005. The number of patients with CF in the patient registry increased from 19,735 in 1995 to 23,347 in 2005. During the study period, the reported annual prevalence of *Pseudomonas aeruginosa* significantly declined from 60.4% in 1995 to 56.1% in 2005 (P<0.001). The decline was most marked in children 6 to 10-year-old (48.2 to 36.1%) and adolescents 11 to 17-year-old (68.9 to 55.5%). Both the incidence (21.7% in 1995 and 33.2% in 2005) and prevalence (37.0% in 1995 and 52.4% in 2005) of MSSA significantly increased, and the age-specific prevalence was highest in patients 6 to 17-year-old. The prevalence of MR SA increased from 0.1% in 1995 to 17.2% in 2005 and from 2002 to 2005 was highest in adolescents 11 to 17-year-old. Both the prevalence and incidence of *Burkholderia cepacia* complex declined, while the prevalence of Haemophilus influenza, *Stenotrophomonas maltophilia*, and Alcaligenesxylosoxidans increased (23). The results of our study showed that S. aureus is the most frequent pathogen that is different from findings of this study.

A Lambiase *et al*., investigated the microbiology of airway disease in a cohort of 300 patients with C in 2006 in Italy. During their study period, 40% of patients were infected by *Pseudomonas aeruginosa*, 7% by *Burkholderia cepacia* complex, 11% by *Stenotrophomonas maltophilia* and 7% by Alcaligenesxylosoxidans. Of the strains isolated, 460 were multidrug-resistant. Multi-resistant organisms were *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (24). The result of our study showed that *Pseudomonas aeruginosa* is resistant to Ampicillin and Trimethoprim-sulfamethoxazole and less resistant to Colistin, Cefepime, and Ceftazidime.

In a study of MC Berkhout *et al*., bacteriology of upper airways of 100 patients with CF at The Netherlands in 2013 were investigated. Their most frequent pathogens were *Pseudomonas aeruginosa, S. aureus, penicillium spices, Escherichia coli, Stenotrophomonas maltophilia, Aspergillus fumigates* and *Proteus mirabilis*, respectively (25).

G Valenza *et al*., investigated the prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of 60 patients with CF in 2008 in Germany. 464 bacterial and 414 fungal strains were isolated and characterized. 63.3% of the patients harbored S. aureus, 50% P. aeruginosa, 16.6% *Haemophilus influenzae*, 15% Stenotrophomonas maltophilia and 13.3% nontuberculous Mycobacteria (NTM). Methicillin-resistant S. aureus (MRSA) and MBL-producing P. aeruginosa were detected in 3 (5%) and 5 (8.3%) of patients, respectively. Among the fungi, Aspergillus fumigatus and Candida albicans showed the highest prevalence (26). The result of this study is almost similar to our results. Also in this study, MSSA was most sensitive to Amoxicillin/clavulanate, Cefuroxime, Vancomycin, Teicoplanin, Rifampicin, Linezolid and Fusidic acid and for MRSA. Vancomycin, Teicoplanin, Rifampicin, Linezolid, Fosfomycin and Fusidic acid were the most sensitive antibiotics.

In a study by VA Paixão *et al*., 279 respiratory specimens of 146 patients were prospectively collected from July to December 2006. Microbiological cultures and antimicrobial susceptibility tests of the most frequently isolated bacteria were performed. Sputum and oropharyngeal swabs were processed for culture. During the study period, 50% of the patients harbored *S. aureus*, 35% *Pseudomonas aeruginosa*, 4.7% *Haemophilus influenzae*. MRSA was detected in 8 (6%) patients; ESBL and MBL-producing *P. aeruginosa* were not identified in these patients (27). The result of this study is almost similar to our results. Also in this study, S. aureus was most sensitive to Vancomycin and Linezolid.

B Coburn *et al*. studied 269 CF patients spanning a 60 year age range, including 76 pediatric samples from patients 4-17-year-old. The core microbiota consisted of five genera-*Streptococcus, Prevotella, Rothia, Veillonella,* and *Actinomyces*. CF-associated pathogens such as *Pseudomonas, Burkholderia, Stenotrophomonas*, and Achromobacter were less prevalent than core genera, but have a strong tendency to dominate the bacterial community when present (28).

As mentioned above, studies show different results. It seems that ecological and geographical conditions and the type of human race may play an important role in the frequency of pathogens and antimicrobial susceptibility of pathogens in different populations.

In conclusion, S. aureus was the most frequent airway pathogen 24 (24%) of our CF patients. After that *Pseudomonas aeruginosa* was recognized 21(21%). In patients, younger than 12 months, the most frequent pathogens were *Enterococci* and *Klebsiella pneumoniae*. All pathogens and Pseudomonas aeruginosa were sensitive to Ceftriaxone, Amikacin, and Ceftazidime but S. aureus was most sensitive to Cefoxitin, Clindamycin,

and Linezolid, respectively. These findings help us in choosing suitable antibiotics for the treatment of respiratory tract infections in patients with CF. It seems that Ceftriaxone, Amikacin, and Ceftazidime are suitable antibiotic choices to administer in patients with CF presenting due to respiratory tract infections.

Acknowledgments

The MUMS (Mashhad University of Medical Science) has provided the financial supports for this study. We are particularly grateful to the patients and their family members who volunteered to participate in this study. The results presented in this work have been taken from student's thesis in MUMS, with the following ID number: 931667.

References

1. Welsh M, Ramsey B, Accurso F, Cutting G, Scriver C, Beaudet A, et al. Cystic fibrosis. Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic and molecular basis of inherited disease. 8th ed. New York: McGraw-Hill, 2001:5121-89.
2. Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. N Engl J Med 1996;335:179-88.
3. MacKenzie T, Gifford AH, Sabadosa KA, Quinton HB, Knapp EA, Goss CH, et al. Longevity of Patients With Cystic Fibrosis in 2000 to 2010 and Beyond: Survival Analysis of the Cystic Fibrosis Foundation Patient RegistryLifetime of Patients With Cystic Fibrosis in 2000 to 2010 and Beyond. Ann Int Med 2014;161:233-41.
4. O'Sullivan B, Freedman S. Carrier screening for cystic fibrosis–Authors' reply. Lancet 2009;374:978.
5. LiPuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 2010;23:299-323.
6. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev 2002;15:194-222.
7. Kahl BC, Duebbers A, Lubritz G, Haeberle J, Koch HG, Ritzerfeld B, et al. Population dynamics of persistent Staphylococcus aureus isolated from the airways of cystic fibrosis patients during a 6-year prospective study. J Clin Microbiol 2003;41:4424-7.
8. Marshall B, Elbert A, Petren K, Rizvi S, Fink A, Ostrenga J, et al. Cystic fibrosis foundation patient registry 2014 annual data report. Cyst Fibros Found 2014:1-92.
9. Henry RL, Mellis CM, Petrovic L. Mucoid Pseudomonas aeruginosa is a marker of poor survival in cystic fibrosis. Pediatr Pulmonol 1992;12:158-61.
10. Frederiksen B, Lanng S, Koch C, Hølby N. Improved survival in the Danish center-treated cystic fibrosis patients: Results of aggressive treatment. Pediatr Pulmonol 1996;21:153-8.
11. Renders NH, Belkum Av, Overbeek SE, Mouton JW, Verbrugh HA. Molecular epidemiology of Staphylococcus aureus strains colonizing the lungs of related and unrelated cystic fibrosis patients. Clin Microbiol Infect 1997;3:216-21.
12. Renders N, Sijmons M, van Belkum A, Overbeek S, Mouton J, Verbrugh H. Exchange of Pseudomonas aeruginosa strains among cystic fibrosis siblings. Res Microbiol 1997;148:447-54.
13. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 2002;34:91-100.
14. Goss CH, Rosenfeld M. Update on cystic fibrosis epidemiology. Curr Opin Pulm Med 2004;10:510-4.
15. LiPuma JJ. Expanding microbiology of pulmonary infection in cystic fibrosis. Pediatr Infect Dis J 2000;19:473-4.
16. Li Puma JJ. Burkholderia and emerging pathogens in cystic fibrosis. Semin Respir Crit Care Med 2003;24:681-92.
17. Millar F, Simmonds N, Hodson M. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985-2005. J Cyst Fibros 2009;8:386-91.
18. Emerson J, McNamara S, Buccat AM, Worrell K, Burns JL. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. Pediatr Pulmonol 2010;45:363-70.
19. Conway SP, Brownlee KG, Denton M, Peckham DG. Antibiotic treatment of multidrug-resistant organisms in cystic fibrosis. Am J Respir Med 2003;2:321-32.
20. Banerjee D, Stableforth D. The treatment of respiratory Pseudomonas infection in cystic fibrosis. Drugs 2000;60:1053-64.
21. Dakin CJ, Numa AH, Wang H, Morton JR, Vertzyas CC, Henry RL. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. Am J Respir Criti Care Med 2002;165:904-10.
22. Hoppe JE, Theurer-Mainka U, Stern M. Comparison of three methods for culturing throat swabs from cystic fibrosis patients. J Clin Microbiol 1995;33:1896-8.
23. Razvi S, Quittell L, Sewall A, Quinton H, Marshall B, Saiman L. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. Chest 2009;136:1554-60.
24. Lambiase A, Raia V, Del Pezzo M, Sepe A, Carnovale V, Rossano F. Microbiology of airway disease in a cohort of

patients with cystic fibrosis. BMC Infect Dis 2006;6:4.
25. Berkhout M, Rijntjes E, El Bouazzaoui L, Fokkens W, Brimicombe R, Heijerman H. Importance of bacteriology in upper airways of patients with cystic fibrosis. J Cystic Fibros 2013;12:525-9.
26. Valenza G, Tappe D, Turnwald D, Frosch M, König C, Hebestreit H, et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cystic Fibros 2008;7:123-7.
27. Paixão VA, Barros TF, Mota CMC, Moreira TF, Santana MA, Reis JN. Prevalence and antimicrobial susceptibility of respiratory pathogens in patients with cystic fibrosis. Braz J Infect Dis 2010;14:406-9.
28. Coburn B, Wang PW, Caballero JD, Clark ST, Brahma V, Donaldson S, et al. Lung microbiota across age and disease stage in cystic fibrosis. Sci Rep 2015;5:10241.