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Evaluation of Antibiotic Resistance Trend among Cystic Fibrosis Patients: A Single Center Study from 2014-2019

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Abstract

Background: Antibiotic resistance in Cystic Fibrosis (CF) patients has been a serious issue in their survival. The objective of this study was to investigate the antibiogram trend in serial admissions among CF patients.

Materials and Methods: In this retrospective descriptive-analytical study, from 2014 to 2019, 95 known cases of CF patients with repeated admissions at Masih Daneshvari Hospital, Tehran, Iran, were evaluated. Demographic data and para-clinical parameters were evaluated. Fourteen antibiotic disks were used to determine antibiotic resistance. Resistance trend in 2014 and 2018 was compared.

Results: Out of 95 patients, 48 patients (52.2%) were female. The mean age of patients was 19.43 ± 6.7 years. Pseudomonas positive culture in the first admission was 68.4%; while it was 45.3% in the second admission and 28.4% in the third admission ($p> 0.05$). Staph positive culture was 20%; 18.9%, and 16.8%, respectively ($p> 0.05$). Pseudomonas aeruginosa was the most sensitive to vancomycin (93.8%), followed by colistin (93.3%) and ceftazidime (65.2%). There was no correlation between patients' age and multidrug resistance (MDR). FEV1 was significantly lower in both the patient with positive pseudomonas (p-value: 0.01), and culture and MDR ($p= 0.023$). Furthermore, in terms of antibiotic resistance over time, resistance to colistin statistically decreased from 25% in 2014 to 2% in 2018 ($p= 0.02$).

Conclusion: Vancomycin, Colistin, Ceftazidim, Imipenem, Amikacin, and Gentamycin had the highest drug sensitivity; while Cefotaxime, Clindamycin, and Chloramphenicol antibiotics had a low sensitivity. From 2014 to 2019, resistance to Colistin dramatically decreased.

Key Words: Antibiotic Resistance, Cystic fibrosis, Drug resistance, Microbial culture.

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1- INTRODUCTION

Cystic fibrosis (CF) as a multisystem disorder is the most common autosomal recessive inherited disorder (1). However, there is no precise information on the exact prevalence of CF in Iran. Genetic impairment of CFTR protein causes imbalance of ions on both sides of the membrane, and ultimately the fluids levels of the respiratory tract are reduced, leading to the colonization of opportunistic bacteria, especially Pseudomonas aeruginosa, staphylococcus aureus, and B. cepacia in patients' lungs (2) which causes impairment in the lung function. Chronic respiratory infections are among the most common causes of mortality and morbidity in children and result in premature death in 90% of patients (3). They also cause many cases of pansinusitis, nasal polyps, rectal prolapse, pancreatitis, cholelithiasis, and insulin-induced hyperglycemia. Recently, the incidence and prevalence of resistant infections have increased. The quality of life and the duration of the patient's survival are thoroughly dependent on the success of antibiotic therapy and the elimination of initial infection (4).

Over the past decade, CF therapy has been changed from periodic venous treatments in cases of disease exacerbation to the strategy of periodic venous treatments in addition to chronic oral or inhaled suppressive antibiotics. Antibiotics are one of the most essential therapeutic components in CF and have a significant responsibility to increase the life expectancy to 40 years. Antibiotic resistance and changes in treatment regimens, as well as the results of antibiograms based on the emergence of new pathogens, have a very important role in the prognosis and survival of CF patients (5–7). CF patients need to be exposed to a wide range of antibiotics by various regimes including oral, inhaled and intravenous antibiotics.

As antibiotic resistance is induced by adaptive and mutational mechanisms, it is affected by antibiotic consumption patterns over time (8). Indeed antibiotic resistance to Pseudomonas aeruginosa as one of the most common organisms in the culture of respiratory secretion in patients has been a serious issue in CF compared to non-CF patients (9). Furthermore, other antibiotic resistance to staph (MRSA), and gram-negative organisms (B. cepacia and S. maltophilia) has been regarded as a new concern in CF care (10–12). Overall, information about antibiotic resistance evolution and its trend during repeated hospitalizations are critical for infection-control policymakers while literature lacks data corresponded to the prevalence of antibiotic resistance in this regard. The objective of this study was to determine the antibiogram and antibiotic resistance among hospitalized CF patients over time and repeated hospitalizations.

2- MATERIALS AND METHODS

2-1. Study design and population

In this descriptive retrospective study, we reviewed records of 95 known cases of respiratory CF patients referred to Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, from June 2014 to June 2019. The study was approved by the Institute's Ethics Committee of National Research Institute of Tuberculosis and Lung Diseases, with the reference number of IR.SBMU.NRITLD.REC. 1397.080.

The research population consisted of all CF patients older than 6 years admitted to the respiratory pediatric ward. Lung transplantation candidates, immunodeficient patients, and patients with comorbidity of collagen vascular diseases or malignancy were excluded from the study. Moreover, patients undergoing corticosteroid treatment were excluded from the study, too.

2-2. Methods

All data were gathered from the patient's medical records by a research assistant. Antibiogram pattern was defined as the frequency of microbial resistances according to data obtained from microbial culture and antibiogram of hospital lab reports. Multidrug resistance was defined as "resistance to multiple antimicrobial agents, classes or subclasses of antimicrobial agents" (13). In addition, a higher than 5% decrease in oxygen saturation during the 6-min walk test or the walking distance less than 200 meters was reported as functional impairment. Disk antibiogram of colistin, piperacillin, vancomycin, co-trimoxazole, ciprofloxacin, cefepime, ceftazidime, clindamycin, amikacin, imipenem, chloramphenicol, erythromycin, and gentamicin was performed for respiratory secretion samples. The results were reported as minimum inhibitory concentration (MIC) according to antibiotic potency of disks which was standardized by staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853.

2-3. Laboratory measurements

The research tools consisted of microbial cultures and antibiograms. Other variables were investigated as follows: demographic information including (age, gender), familial history, comorbidity of Diabetes Mellitus (DM), chief complaint at admission such as failure to thrive (FTT), gastrointestinal (GI), and respiratory complications, duration of admission, Pulmonary Function Test pattern, Forced Expiratory Value in one score (FEV1) as well the results of 6-min walk, microbial species (as recorded in respiratory secretion culture reports for staph aureus and Pseudomonas aeruginosa), and drug resistance (or sensitivity) as recorded in antibiograms forms.

2-4. Comparisons

Antibiotic resistance was compared in different admissions to assess the pattern of changes in repeated hospitalizations. Also, in order to investigate the effect of time, in two time points including 2014 and 2019, we compared the prevalence of drug resistance too.

2-5. Data Analysis

Data were collected and analyzed using SPSS software (version 22.0). Quantitative variables such as age, admission duration, walked distance, O2 saturation and FEV1 were presented along with the mean and standard deviation. Kolmogorov-Smirnov test was used to assess normal distribution. Nominal variables including antibiotic resistance were reported in percentages. In order to compare the bivariate value, chi-square and Fisher's exact test or Student's t-test were performed. For comparison of the same within-subjects in the groups, repeated measures ANOVA, Cochran test or Friedman were conducted. Correlation between age, the amount of walking distance decrease and FEV1 change percentage and the number of drugs with resistance, was measured using scatter plot and R^2 value. A 95% confidence interval (CI), and 0.05% significance level were used for the analysis.

3- RESULTS

3-1. Demographic data

Ninety-five patients enrolled in the study with the mean age of 19.43 ± 6.7 years, 58.3% of whom were over 18 years old and 52.2% of the patients were female. Frequency of various parameters in both genders is summarized in **Table.1**. In both genders, there was no considerable difference in terms of spirometry pattern, FEV1 actual value, the prevalence of functional impairment based on the walking test, the prevalence of Diabetes Mellitus and MDR (**Table.1**).

Table-1: Baseline characteristics and comparison of spirometry pattern.

Parameter	Male	Female	P-value
Age (year), mean± SD	12.5± 0.69	33.3+ 0.58	0.29
Family Relative, %	61.9	63.6	0.86
Positive family history, %	19.14±7.01	19.84±6.8	0.45
Obstructive pattern in PFT, %	12.8	39.5	0.056
Restrictive pattern in PFT, %	25.6	20.9	0.06
Normal PFT, %	35.9	20.9	0.47
FEV1,%	48.4±20.7	21.5± 48	0.51
6-minute test functional impairment, %	73.3	57.1	0.051
DM, %	10.5	2.6	0.1
MDR, %	82.2	77.1	0.6

FEV1: Forced expiratory volume in 1 second; DM: Diabetes Mellitus; MDR: Multidrug Resistance.

3-2. Frequent admission

Twenty-seven patients (28.4%) were hospitalized once; while 17 patients (17.9%) were admitted twice and 51 patients (53.7%) were admitted three times. The mean duration of admission was 11.4 ± 7.3; 9.9 ± 4.6 and 11.3 ± 4.8 days in the serial admissions.

3-3. Multidrug resistance and sputum culture

The rate of MDR in the first admission was estimated to be 78.9% that fell to 61.1% in the second admission, followed by to 43.2% in the third admission. Mean age in MDR patients was 19.5 ± 6.5 years and in non-MDR patients was 19.07 ± 9.4 years, with no considerable difference. 88.2% of male and 77.1% of female patients were MDR (p= 0.6). Microbial colonization of sputum culture was positive in 68.4% for Pseudomonas in the first admission, while it was 45.3% in the second admission, followed by 28.4% in the third admission (p=0.00). On the other hand, staphylococcus aureus was found to be 20% in the first admission, 18.9% in the second and 16.8% in the third admission. Cochran test showed no significant difference even though there was a decreasing trend. Also, there was no statistically significant difference in the mean age of patients with either a positive culture of staph or pseudomonas compared to negative groups.

3-4. Chief complaints at the time of admission

As it is depicted in **Figure.1** the prevalence of failure to thrive (FTT) was 20.2% in the first admission; while it was 26.9% in the second and 40.8% in the third admission. FTT rate had an increasing trend. However, Cochran test showed no significant difference in the rate of FTT in repeated admissions. Also, the frequency of gastrointestinal (GI) complaints was 36.4% in the first admission; followed by 32.3% in the second admission and 36.4% in the third admission with no significant difference. Moreover, the prevalence of productive cough was 95.9%, 97%, and 6%, respectively.

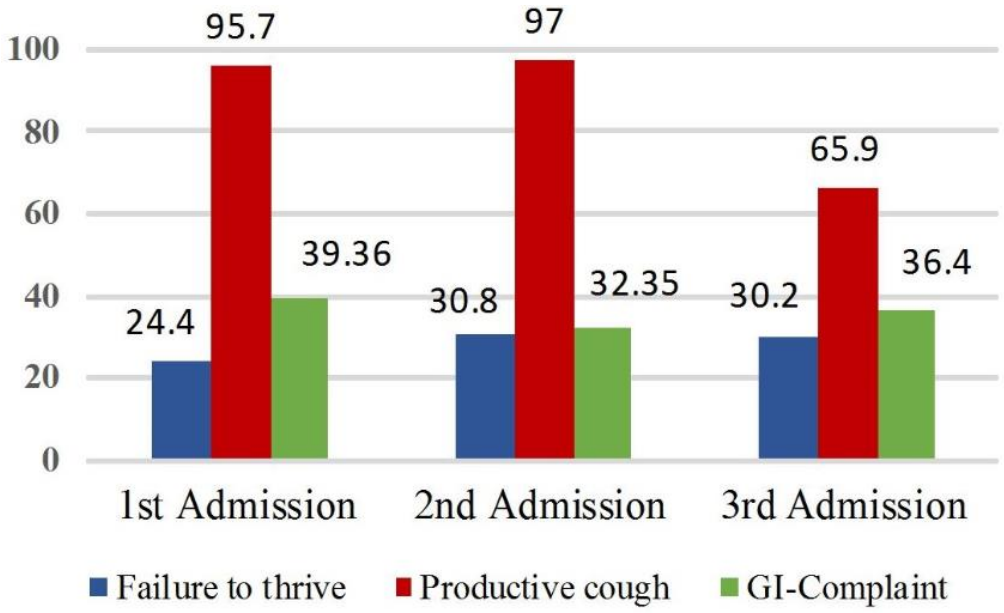

Fig.1: Prevalence of chief complaints at admission time of recurrent admissions.

(In all admissions, productive cough is the most prevalent complaint. GI complaint as well as FTT are in a steady state situation and are in the second and third place. GI: Gastrointestinal; FTT: Failure to thrive).

3-5. Spirometry findings

Regarding the spirometry findings, ANOVA revealed a significant drop of FEV1 in repeated admissions, (p=0.01). Frequency of normal spirometry also decreased from 28.6% (first admission) to 18.2% (third admission). Moreover, in the first admission, 26.2% of patients had obstructive and 22.6% had a restrictive pattern; during the second hospitalization, these patterns were 22.8 and 36.8%, respectively and in the third admission, these rates changed to 13.6 and 40.9%. Subsequently, the rate of restrictive pattern increased gradually while the obstructive pattern was limited over time. The mean prediction of FEV1 in patients with a positive culture of Pseudomonas was significantly lower than that of patients with a negative result, (43.4 ± 20.3 vs. 62.7 ± 17.2, respectively; p=0.034). Besides, FEV1 was significantly higher in the group of non-MDR compared to the MDR group. In MDR vs. non-MDR patients, the predicted FEV1 was 46.8 ± 20.8% and 50± 22.9%, respectively, the t-student test declared no significant difference between groups.

3-6. Walking test

Concerning the results of the six minute walking test, 63.3% of the patients in the first admission, 66.7% in the second admission and 48% in the third admission had a functional impairment and ANOVA demonstrated no significant difference (**Figure.2**). The relationship between MDR and walking impairment indicated that 31.8% of MDR patients had normal walking tests and 68.2% of MDR patients had a functional impairment (p = 0.361).

3-7. Diabetes Mellitus

Data obtained from 95 CF patients revealed that 6.6% of all patients had diabetes, which did not have any significant association with the functional impairment of walking test, MDR and colonization of staph or Pseudomonas.

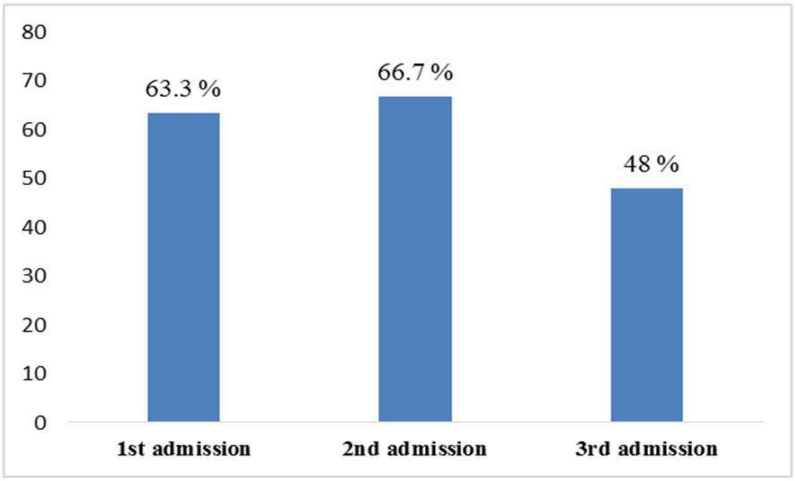

Fig.2: Prevalence of functional impairment based on 6-minute walking test.

(Functional impairment according to walking test involves a majority of patients and its prevalence has a fluctuated pattern).

3-8. Antibiogram

In **Figure.3** drug resistance of various antibiotics in first, second and third admission is summarized. The results of antibiogram represented that in the first admission, vancomycin, colistin, and ciprofloxacin had the maximum drug sensitivity; while cefotaxime, clindamycin, and chloramphenicol had the minimum drug sensitivity. Also, antibiogram trend over time with regard to drug resistance is presented via **Table.2**. Indeed, in addition to the admission time, in this table, we compare drug resistance in two different time intervals including 2014 and 2019.

Resistance to Colistin dramatically decreased from 25% in 2014 to 2% in 2019. However, resistance to Gentamycin increased in 5-year follow up and reached 55.6%. The correlation between the number of drugs with resistance out of 14 antibiotics, and patients' age is presented in **Figure.4 A**. Indeed there was no correlation in this regard (R^2=0.02), and also there was no correlation between the number of resistance-drugs and age, predicted FEV1 percentage change, and decrease in walking distance (**Figure 4-B, 4-C**).

Table-2: Antibiotic resistant evolution from 2014 to 2019 in a CF referral center.

Antibiotic	Resistance Percentage in 2014	Resistance Percentage in 2019	P-value
Vancomycin	5	12.5	0.43
Colistin	25	2	**0.02**
Ceftazidime	41.7	55	0.37
Amikacin	54.5	40.9	0.36
Imipenem	31.8	47.1	0.33
Co-Trimoxazole	16.7	57.1	0.13
Ciprofloxacin	25	35.3	0.4
Piperacillin	28	43.5	0.26
Cefepime	56.3	43.5	0.43
Gentamycin	20.8	55.6	**0.01**
Clindamycin	66.7	87.5	0.34
Cefotaxime	81	83.3	0.84

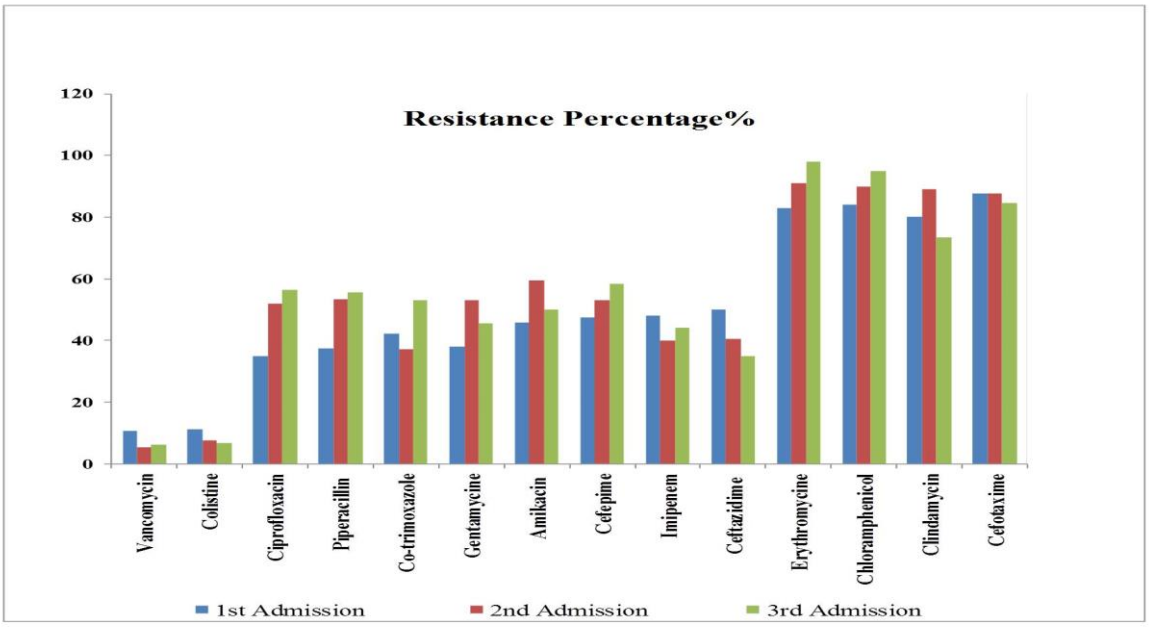

Fig.3: Drug resistance of various antibiotics over time.
As the diagram shows, sensitivity to Vancomycin, Colistin, Ceftazidime, Imipenem, Amikacin, and Gentamycin is higher than other agents including Erythromycin, Cefotaxime and Chloramphenicol.

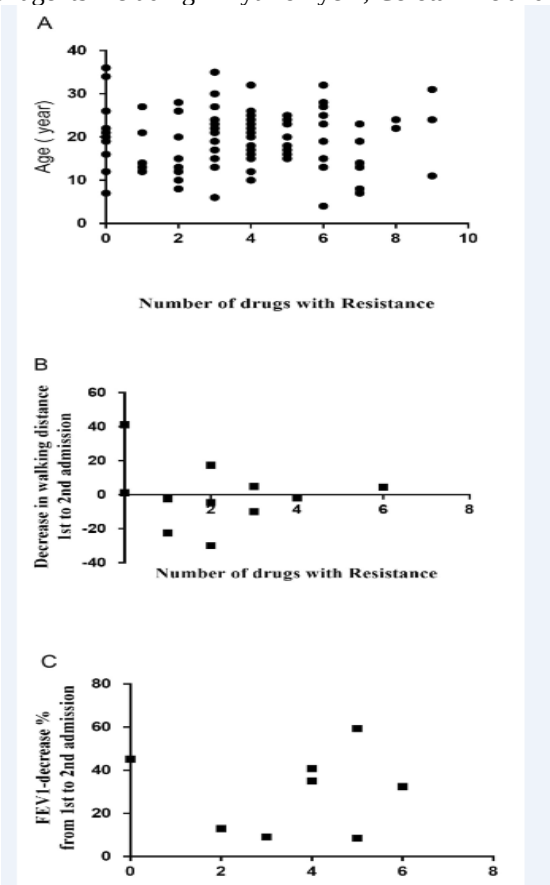

Fig.4: The correlation between the number of drugs with resistance and (A): Age, (B) walking distance change % and (C) decrease in FEV 1. There is no correlation between age and the number of drugs with resistance. Also, there was no relationship between change in ability to walk and the number of drugs with resistance. However, in case of FEV1 decrease, there was a weak correlation ($R^2 = 0.2$).

4- DISCUSSION

In the present study, the antibiotic results of respiratory secretion in 95 CF patients, whose disease was proven by a sweat-test and clinical examinations, showed that drug resistance was different in successive admissions for different antibiotics; whereas in some cases there was a decreasing trend and in some cases, there was an increasing trend. Generally, vancomycin, colistin, and ciprofloxacin had the highest drug sensitivity; while cefotaxime, clindamycin, and chloramphenicol antibiotics had the least drug sensitivity. In three repeated admissions in five years, 68.4% of patients in the first admission were infected with Pseudomonas; while in the second admission the trend reached 45.3%, and in the third admission it was reported to be 28.4%. It seems that in the first admission, contamination rate of pseudomonas was much higher in comparison to most CF clinics worldwide (14,15), and the positive sputum culture for Pseudomonas significantly decreased from the first admission to the third admission, which could be due to effective medical care during hospitalization, informing the patients, their families and their caregivers about the nature of the disease, following-up the patients in the intervals between disease flares and hospitalization, the consumption of antibiotics and anti-inflammatory drugs, as well as the methods of respiratory physiotherapy and concentrated respiratory secretions exit at home. In a study conducted in Qatar in 2018, 60.9% positive culture for Pseudomonas was reported at the first admission, which was relatively similar to our study (16). The results of studies suggested that the primary treatments for Pseudomonas infection were only 80% effective. This clarified the importance of preventing early infection by bacteria. Positive cultures of staphylococcus aureus were reported in 20% of cases in the first admission, which decreased to 18.9% in the second and to 16.8% in the third admission. Despite the decreasing trend of staphylococcal cultures that could be due to proper eradication by beneficial antibiotics, this difference was not significant in recurrence admissions. In a study conducted on microbiological changes in the sputum of CF patients in the United States, the prevalence of staphylococcus aureus as the second most common organism isolated from respiratory samples in CF patients was reported to be 43.2 - 54.3%, which was higher than in our study (10). Also, none of the patients were infected with Burkholderia cepacia, and no contamination with Candida albicans was detected, which was justified due to the antifungal activity in Pseudomonas. From spirometry point of view, restrictive type increased over time and predicted FEV1 in patients with positive Pseudomonas culture was significantly lower than other patients, unlike staph positive cases. Furthermore, in the group of patients with multidrug resistance, FEV1 was significantly lower than other patients. In this regard, MDR Pseudomonas was correlated to pulmonary function parameters, FEV1 deterioration lung transplantation candidate and high mortality (7). The present study indicated high sensitivity of colistin (93.3%), and vancomycin (93.8%) for Pseudomonas and staphylococcus aureus strains despite routine consumption. Sensitivity to amikacin was in a somewhat steady state in different hospitalizations and over time. It was reported to be 44 to 55% as Khalilzad et al. stated in 2012 (17). Unlike our results, there are other reports based on 97.8%, 91.4%, and 67.2% in Iran, Turkey and Qatar, respectively (4, 18, 19). Pseudomonas antibiotic sensitivity to colistin as the most effective aminoglycoside increased gradually and the sensitivity to gentamicin was also 55.5% in our study with the compatible result of 59% in Qatar (19); while in

Yageia's study amikacin was the most effective aminoglycoside, followed by colistin and gentamicin (18). Resistance to aminoglycosides is associated with poor clinical outcome and is usually due to overexpression of the efflux pump MexXY as one of the most commonly mutated genes in Peudomonas aeruginosa related to CF (20). Ciprofloxacin has only been used for children above 10 years and the susceptibility was 50 - 56% during the study, though in Italy it decreased from 55.6 to 25.8% during the 3 year follow-up (21). In our study, susceptibility to ciprofloxacin in repeated admissions decreased from 65.2% in the first admission to 48.1% in the second admission and eventually reached 43.5% in the third admission, which was a significant decrease and may be contributed to the widespread use of medication. It worthy to mention that in 2012, sensitivity to ciprofloxacin was 71.4% in our center. Microbial sensitivity to imipenem was 56% in 2018, while in 2003 a 100% sensitivity to Pseudomonas was mentioned (22); but, in Lithuania, the sensitivity of 76% was reported for imipenem among cystic fibrosis patients. Likewise, sensitivity to ceftazidime as well as piperacillin was 65.2% and 44.4% in the present study, however it was 77% and 97.8%, respectively (21) in other centers.

In our study DM affected 10.5% of male and 2.6% of female patients. Cystic fibrosis-related diabetes is an extra-pulmonary complication in CF patients which affects 9% of CF patients in the age of 5 to 9 years, and 26% of patients aged 10 to 20 years. Accelerated decline in pulmonary function, FTT and high mortality are correlated with CF-diabetes mellitus (23, 24). Diabetes, and frequent acute pulmonary exacerbations requiring hospitalization or IV antibiotics increase the risk for MDR. In terms of MDR trend, during 5 year follow up, we obtained a high rate of MDR in CF sputum cultures in comparison with the published literature but its decreasing pattern is promising. Indeed, proper usage of antibiotics, the establishment of CF foundation in our center as well as data registry, improved patients' management.

4-1. Study Limitations

This study has some limitations including retrospective data gathering and poor information as regards inhaled antibiotics used by patients. In addition, we are unaware of intervals between rehospitalization. Also, the effect of chest physiotherapy at home and diseases severity are not rolled out.

5- CONCLUSION

An investigation in antibiotic resistance in a five year period and repeated admissions showed a dynamic change over time. Considerable antibiotic resistance was identified in all categories. Therefore, it is suggested to perform microbial culture test in order to choose the appropriate medication. Vancomycin, Colistin, Ceftazidime, Imipenem, Amikacin, and Gentamycin had the highest drug sensitivity; while Cefotaxime, Clindamycin, and Chloramphenicol antibiotics had a drug sensitivity. From 2014 to 2019, resistance to Colistin dramatically decreased, but resistance to Gentamycin increased.

6 -ACKNOWLEDGMENT

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7- CONFLICT OF INTEREST: None.

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