PRESENCE OF MULTIPLE ANTIMICROBIAL RESISTANT STRAINS AND FREQUENCY OF PSEUDOMONAS AERUGINOSA IN BRONCHIECTASIS PATIENTS IN BALOCHISTAN

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ABSTRACT

OBJECTIVE: To discover the frequency and presence of multiple antimicrobial resistant strains of Pseudomonas aeruginosa (P.aeruginosa) in patients with Post tuberculosis bronchiectasis in Baluchistan.

DESIGN: A prospective descriptive study.

PLACE AND DURATION: this study was done in Pulmonology and tuberculosis outpatient department (OPD) of Fatimah Jinnah Chest Hospital and Rehan Hospital Quetta Pakistan from 1st October 2011 to 1st April 2012.

METHODOLOGY: The samples were obtained from post tuberculosis patients (n=60, Age = 40+ years) suspected of bronchiectasis who were subjected to spirometry test, Mueller Hinton sensitivity testing, oxidase test and disk-diffusion method to find out frequency and multiple drug resistance of P. aeruginosa..

RESULTS: Our results confirmed the association of chronic P. aeruginosa infection with poor lung function. It is not clear whether P. aeruginosa caused the accelerated decline in lung function or it was just a marker of those whose lung function was already declining rapidly. The antibiotic susceptibility testing confirmed the presence of resistant strains of the P. aeruginosa most of which were mucoid strains. It proved that a single antibiotic therapy is not a good treatment for patients with P. aeruginosa infection in airways.

CONCLUSION: Bronchiectasis is more frequently encountered in middle-aged and elderly persons and resistant strains of P. aeruginosa are highly prevalent in these patients.

KEYWORDS: Bronchiestasis, Pseudomonas aeruginosa, multiple antimicrobial resistance.

INTRODUCTION

Bronchiectasis, the abnormal and permanent dilation of the bronchi, is a chronic lung disease that can be difficult to manage. In most patients with bronchiectasis, airway damage is related to a combination of infection and the associated release of inflammatory mediators.[1]

Bronchiectasis is diagnosed in patients with chest radiographs and more recently High Resolution Computed Tomography (HRCT) scans.[2,3] Recognition of the presence of bronchiectasis prompts an investigation of possible causes and associated conditions some of which are treatable.[4]

In United States, the most common causes are the presence of the respiratory tract infection by microorganisms particularly Pseudomonas aeruginosa, Heamophilusinfluenzae, Streptococcus pneumonae, Staphylococcus aureus, and Moraxella catarrhalis. Of these, P aeruginosa infection is one of the most frequently observed.[5]

Emerging evidence suggests that P. aeruginosa stimulates a neutrophilic and inflammatory mediator response in the airway.[6]The presence of P. aeruginosa is associated with increased sputum production, more extensive bronchiectasis visible on CT scans more hospitalizations, and reduced quality of life .[7]

The resistance offered by P. aeruginosa is usually due to the formation of biofilm micro colonies that is believed to provide resistance to various antibiotics.[8, 9] hypermutable strains of p. aeruginosa exhibiting increase mutation rates are common in chronic infections such as those that occur in the lings of cystic fibrosis patients10. Increase in the frequency of multi drug resistant strains of p. aeruginosa has severely limited the availability of therapeutic options ongoing studies on current antimicrobial resistance profiles of P. aeruginosa are essential to find out the susceptibilities of this pathogen against commonly prescribed antibiotics in any health care facility. This would help the physicians to optimize the current therapeutic treatment options. Data on antimicrobial susceptibility of p. aeruginosa is limited in Nepal.[10]

METHODOLOGY

This prospective/descriptive study was conducted in

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Pulmonology and tuberculosis outpatient department (OPD) of Fatimah Jinnah chest hospital and Rehan hospital Quetta Pakistan. From 1st October 2011 to 1st April 2012 we interviewed and included all those patients who were diagnosed with post tuberculous bronchiectasis, and were exposed to different smoking conditions. patients with cystic fibrosis, other congenital diseases and those with no history of tuberculosis in the past were excluded to evaluate the frequency of P. aeruginosain patients with Post tuberculosis bronchiectasis in Baluchistan. Investigations were carried out on 60 samples from the patients included (male =38, female=22) that were already diagnosed with the underlying post-tuberculous bronchiectasis having strong clinical and radiological evidence in a randomized, multicenter study in Fatima Jinnah Chest Hospital and Rehan Hospital Quetta Baluchistan to evaluate the antimicrobial susceptibility and influence of P. aeruginosa. As per direction of the physicians the patients were prescribed to undergo CT scans and chest x-rays to ascertain the degree of lung damage caused by P. aeruginosa.

Allthe necessary clinical details were obtained from the patientsusing predesigned questionnaire . The fresh specimen swere collected in sterile containers directly from the patients. All the samples were processed at pathology laboratory of Fatimah Jinnah Chest Hospital Quetta for culture and smear examination.

All patients were subjected to Spirometry test. Soft nose clips were used to prevent air escaping through the nose. Filtered mouthpieces were used to prevent the spread of microorganisms.

Sputum and Throat swabs were then taken from patients for the microbiological evaluation.

All the samples were streaked on three different media viz; McConkey, KIA (*Kligler's Iron Agar*) and Cetrimide agar. After gram staining the bacterial growth was confirmed through a series of different biochemical tests.

All the positive 60 plates of P. aeruginosa samples were then grown on blood agar to get an approximate number of mucoid P. aeruginosa strains which is responsible for biofilm formation in the airways in turn causing antimicrobial resistance.

Mueller Hinton sensitivity testing agar was used to evaluate the antimicrobial sensitivity of P. aeruginosa isolated from the patients.

The disk-diffusion method was done to test the susceptibility of the P. aeruginosa for all the 60 plates to various antibiotics using discs that of amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin and meropenem. We selected these limited number of antibiotic due to the fact of no availability of other two groups of antibiotics for which we have requested the higher authorities that would be taken into consideration. After taking all the calculations the final result was described.

RESULTS

A total of 60 patients with broncheictasis with all apparent symptoms were selected in the study in Fatimah Jinnah chest hospital and Rehan hospital Quetta Pakistan. The patients recruited were 22(36%) females, out of which some (n=16 ,73%) were above 40 year and some (n=6, 27%)were below 40 years; and 38(64%) males, out of which some (n=30 ,79%) were above 40 year and some (n=8, 21%)were below 40 years.(Table - I)

All of the patients were post tuberculous and were under treatment for broncheictasis. Out of 60 patients 24(40%) were active smokers, 14 (23%) ex-smokers, 15 (25%) were subjected to smoke from other sources and rest of the 7 (12%) had no smoking history. (Table - III)

The lung function results of the 60 patients from both the hospitals showed clear decline in the volume of the lung and in its function.

Sputum volume, sputum purulence and sputum viscosity was also checked according to the modified score systems. The results of the sputum analysis indicated the damaged lung function. Majority of the patients had gray or opaque color sputum with about 30 (50%) patients out of the total had opaque / milky white color of the sputum while 26 (40%) patients had gray sputum appearance. About 78% patients had mild to severe sputum viscosity indicating the bacterial colonization. Increased sputum volume was seen in more than 50% patients mostly in the concentrations of 0 to 30mL (Table - V). The sputum samples (n=60) were mucopurulent indicating the presence of chronic infection

Out of the 60 culture plates 58 (98 %) were positive for the presence of gram-negative organisms.

Out of the 60 plates 30 (50 %) plates were separated and cultured on KIA medium test tubes. These tubes confirmed the presence of the P. aeruginosa in the 29 clinical isolates.

The remaining 30 plates were further cultured on the cetrimide agar medium which only allowed the growth of P. aeruginosa. Yellowish green color colonies of P. aeruginosa were seen in all the 30 respective plates that confirmed that bacilli are present in all the plates. Furthermore the plates showed fluorescent colonies and a grapy odor was coming from all the plates.

The oxidase test was performed for all the 60 plates P. aeruginosa is cytochrome oxidase positive so when the artificial electron donors were oxidized by enzyme, dark purple color was produced on the paper. All the plates showed positive oxidase test that further confirmed the presence of Pseudomonas in the isolates from the patients.

The growths from the 58 plates were separately tested by Kirby-Bauer disk diffusion antibiotic susceptibility tests. The antimicrobial discs prepared were of ceftazidime, amoxicillin-clavulanic acid, ciprofloxacin and meropenem.Meropenem was the most effective agent that was bactericidal against 94% (n=57)of the isolates. Among other antibiotics Ciprofloxacin, Ceftazidime, amoxicillin-clavulanate shown bactericidal activity only against 31% (n=18) isolates.

The results showed that 32 isolates were resistant to Ceftazidime, and 24 isolates were resistant to Amoxicillin-calvulanic acid, while 19 isolates were resistant to Ciprofloxacin. Meropenem was resisted by only 5 isolates. (Table IV).

TABLE- I: AGE CRITERIA (n=60)

Age of Patients	Males		Females	
	Age >40	Age >40	Age >40	Age >40
	N=30	N=8	N=16	N=6
TOTAL =60	N=38		N=22	

(Legend) FEV= Forced External Volume,
FVC= Forced Vital Capacity

TABLE-II: SPIROMETERY TEST (n=60)

Spirometery	Spirometery Results		
	FEV1 %	FVC %	FEV1/FVC ratio
	50-75%	65-82 %	51-67 %

TABLE-III: PATIENTS DEMOGRAPHY FOR SMOKING, (n=60)

	Gender		Smoking Habit				Etiology
	F	M	Active Smokers	Ex-Smokers	Smokers Inhaled From Other Source	Non Smokers	Post TB etiology
No. of Patients	N=23	N=39	N=24 (40%)	N=14 (23%)	N=15 (60%)	N=9 (15%)	N=60

TABLE-IV: SPUTUM ANALYSIS (n=60)

Sputum Analysis	Sputum Appearance		Sputum Purulence	Sputum Volume
	Gray sputum	Opaque/milky white sputum	Mucopurulent sputum	0-30mL volume
No. of Patients	N=26 (40%)	N=30 (50%)	60	N=30 +

TABLE-V: ANTIBIOTIC SUSCEPTIBILITY TEST (n=60)

Sr. No.	Antibiotic Names	Number and Percentage of Susceptible Isolates	Number and Percentage of Resistant Isolates
I	Meropenem	57 (94%)	3 (6%)
II	Ciprofloxacin	43 (72%)	17 (28%)
III	Amoxicillin-calvulanic acid	38 (63%)	22 (37%)
IV	Ceftizidime	28(47%)	32(53%)

DISCUSSION

Our study was prospective and therefore a thorough evaluation for underlying genetic or acquired disease was not always performed. However, our findings are in agreement with a recent very meticulous and thorough prospective study of adults with broncheictasis.[12]

Patients between 35-80 years were included in our study because bronchiectasis is more common in this age group. Our study confirms the study carried out by Weycker et al (2005) that reported that bronchiectasis is more frequently encountered in middle-aged and elderly persons.[13]

In our study we found a percentage of FEV1 of the patients in the range of 50-75% which lower than the normal range. The FVC percentage (65-82%) and FEV1/FVC percentage (51-67%) that clearly indicated a decrease in lung function. The findings support the studies of Martinez GMA et al 2007, demonstrating that the accelerated rate of decline of lung function is associated with the chronic colonization by P. aeruginosa.[14]

According to the report by the American Thoracic Society in 1986 proposed that during infective exacerbations P. aeruginosa was an important pathogen in Chinese patients with bronchiectasis.[15]Our findings support it because during this study we found patients colonized with P. aeruginosa having severe exacerbations.

The parameters for evaluating the lung damage in patients with P. aeruginosa colonization clearly indicated decreased lung function and severe exacerbations. The study carried out by Guerreiro et al in 1993 supports our findings in which it was reported that P. aeruginosa itself is the cause of the rapid decline in the lung function.[16]

The relative frequencies of P. aeruginosa isolated in this study in patients with bronchiectasis were different from those reported in sputum studies from the West.[17] Bronchiectasis is the disease most prevalent in the underdeveloped countries of the East then in the West. It is much more controlled in the West. This statement supports our study findings that the P. aeruginosa colonization is prevalent in the bronchiectasis patients.

We believe our study wouldbe the first reported series of bronchiectasis patients in Quetta where in the findings would prove that P. aeruginosa colonization or infection is found to be associated with moderately severe airflow obstruction and high sputum output.

In the current study Colistin and Meropenem were the most effective single agents. Interestingly, there was no clear correlation between the class of drug or mode of growth and bactericidal activity. Colistin, Meropenem and Tobramycin were bactericidal against more isolates than ciprofloxacin, amoxicillin calvulanic acid and ceftazidime. Resistance to Colistin has been seen in few patients but otherwise such cases have been rarely reported in other studies. [18]

In this study the frequency of resistance to all antibiotics was 94.1% by P. aeruginosa while a study conducted in Turkey, multidrug resistance was reported to be 45.4% in case of P. aeruginosa.We found in our study that the patients developed resistance to the treatment within 3 months which supported the findings of Rayner et al 1994 that reported that flouroquinolones often develop resistance after one or two of the treatment cycles.

CONCLUSION

Our study showed that the resistant strains of P. aeruginosaare highly prevalent in patients with post TB bronchiactatsis. In addition, these post TB patients have poorer lung function giving an opportunity to the microbes most frequently to the P. aeruginosa to be colonized.

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