Original Article

A study of prevalence of aerobic bacteria and fungi in sputum specimens of patients with post tubercular bronchiectasis

Kumar GS[1], Lal SB[2], Laxmikanth M[3]

[1]Dr Gattu Suresh Kumar
Assistant Professor
[2]DrSabinkarBabuLal
Professor & HOD
drsblal@yahoo.co.in.
[3]Dr M Laxmikanth
MD, Junior Resident
mudragadalaxmikanth@gmail.com
[1,2,3]Department of Pulmonary Medicine
SMC, Vijayawada
Affiliated to Dr NTR University of Health sciences
Vijayawada, India

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Correspondence to:
Dr Gattu Suresh Kumar
8374358304
saiprabhakarf4u@gmail.com

ABSTRACT

Background: *Patients with Post TB bronchiectasis colonize many aerobic bacteria and fungi and lead to an increase in exacerbations and decrease in quality of life.*

Objective: *To study the prevalence of aerobic bacteria and fungi in sputum specimens of patients with post tubercular bronchiectasis and to find out the local antibiotic sensitivity and resistance patterns.*

Materials and Methods: *This prospective observational study was carried out over a period of one year comprising of 50 patients with post Tubercular Bronchiectasis who were above 18 yrs, completed ATT smear negative and HRCT showing bronchiectactic changes. Identification of organisms from sputum samples were done by classical aerobic microbial staining and culture methods. Descriptive and inferential statistical analysis was carried out in this study.*

Results: *This study comprises of the 50 patients with a larger group of individuals under the age group of 51-60 (30%), along with bronchial colonization of aerobic bacteria being 78% and growth of fungi being 2%.Among the 39 patients,16 patients (32%) had grown Pseudomonas aeruginosa, 12 patients(24%) had grown Klebsiella pneumoniae, 8 patients(16%) had grown Streptococcus species and rest 3 patients(6%) had grown staphylococcus species. The Antibiotic resistance noted highest being Amikacin (56.4%), piperacillin-tazobactam showing the least (2.6%) and highest sensitivity with imipenem(100%).*

Conclusion: *This study signifies that prevalence of aerobic bacteria, especially Pseudomonas aeruginosa and Klebsiella pneumonia was common in patients with post tubercular bronchiectasis. The Antibiotic resistance noted highest being Amikacin (56.4%) and sensitivity with imipenem(100%). In patients colonizing Pseudomonas aeruginosa, there was significant decline in lung function.*

Key Words: *Bronchiectasis, pulmonary tuberculosis, antibiotic sensitivity, antibiotic resistance*

Introduction

Bronchiectasis is defined as abnormal and irreversible dilatation of one or more medium sized bronchi mainly from 4th to 9th generation. It is clinically characterized by persistent cough with the production of sputum which in untreated patients is often copious, purulent and associated with recurrent infective exacerbations. Causes of bronchiectasis include both infectious and noninfectious conditions like bacterial infections, tuberculosis, bronchial obstruction due to foreign bodies or tumours, congenital due to kartageners syndrome and cystic fibrosis, immunodeficiency, autoimmune diseases, recurrent aspirations and idiopathic causes. Several studies found the incidence of post TB bronchiectasis to range between 19% and 65%. [1] Both tuberculosis and bronchiectasis carry an enormous burden worldwide in terms of morbidity and mortality and also financially to the affected population**.** Burden of the disease in terms of respiratory morbidity, effect on patient's quality of life and the economic cost of long term management is important and has become obvious that more research into its causes and management is needed. The most prevalent pathogenic bacteria in post TB bronchiectasis are Pseudomonas aeruginosa, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, anaerobic organisms like prevotella and veillonella and most common fungus is Aspergillus fumigatus. [2] Understanding the pathogenic properties of these microorganisms may allow the development of novel therapies for the management of respiratory exacerbations. This study will give an insight whether bronchial colonization by aerobic bacteria and fungi is common in patients with post tubercular

bronchiectasis with sputum culture being an adequate tool for evaluation.

Materials and Methods

This prospective observational study was carried out over a period of one year from February 2015 to January 2016 in the Department of Pulmonary Medicine, Siddhartha medical college, Vijayawada. This study comprised of 50 patients with post TB bronchiectasis. A detailed history was taken from all the patients with respect to presenting complaints, age at onset of chronic mucopurulent sputum production and past history of pulmonary tuberculosis. A thorough clinical examination was carried out. The following investigations were done: TLC, DLC, Hb%, ESR, Blood sugar, Blood urea, serum creatinine, Chest X ray, High resolution computed tomography, Pulmonary function test, Sputum for AFB, Sputum samples for gram's stain, culture and sensitivity and Sputum for fungal hyphae and culture.

Early morning sputum specimens were obtained on the patient's first visit and transported immediately to Microbiology Department in a sterile wide mouthed jar with a tightly fitted screw cap lid. All sputum samples were subjected to gram staining, staining for AFB, KOH mounting, and cultured on blood agar, chocolate agar, Haemophilus selective agar, cetrimide agar and Sabouraud agar. Identification of organisms was done using classical aerobic-based microbial culture techniques. Antibiotic sensitivity testing (AST) for aerobic bacteria was done. The patients who were above 18 years of age, have completed ATT and smear negative at the time of admission with HRCT showing bronchiectactic changes were included in this study. Patients with age below 18 years or Bronchiectasis due to other aetiologies or having sputum smear positive Pulmonary Tuberculosis and who were unwilling and uncooperative were excluded from the study. Patients with contraindications for spirometry like history of myocardial infarction or congestive heart failure or coronary heart disease were also excluded from this study. Age and sex of the patients, pulmonary function test, high resolution computed tomography and Sputum cultures were the outcome measures monitored during study. Descriptive and inferential statistical analysis was carried out in the present study.

Results on categorical measurements were presented in Number (%). The following assumptions were made on data: Chi-square/ Fischer Exact test was used to find the significance of study parameters on categorical scale between two or more groups. In this study Chi-square / Fisher exact test were used to compare isolated and non-isolated groups according to FEV1. Significance was assessed at 5 % level of significance. Statistical software namely SPSS V19 was used for analysis of the data.

Results

Out of 50 patients, there were 8 patients (16%) in <30 age group, 10 patients (20%) in 31-40 age group, 9 patients (18%) in 41-50 age group, 15 patients (30%) in 51-60 age group and 8 patients (16%) in 61-70 age group and the highest incidence was seen in 51-60 age group accounting for 30%. (Table: 1) Among the 50 patients, 27 patients (54%) were females and 23 patients (46%) were male. (Table: 2) Out of the 50 patients studied 39 patients had growth of aerobic bacteria in their sputum and the rest 11 had no growth. Among the 39 patients, 16 patients (32%) had grown Pseudomonas aeruginosa, 12 patients (24%) had grown Klebsiella pneumoniae, 8 patients (16%) had grown Streptococcus species and 3 patients (6%) had grown staphylococcus species. (Table: 3) Out of the 50 patients 1 patient had grown Aspergillus fumigatus, 1 had grown Candida albicans and 48 had no fungal growth. (Fig.1,3)

As shown in figure (2), percentages of resistance noted among these drugs was as follows: Amikacin (56.4%), Ampicillin (53.8%), Amoxicillin (51.3%), Gentamicin (48.7%) Amoxicillin and Clavulinic acid (41%), Cotrimaxazole (35.9%), ceftriaxone (25.6%), cefuroxime (25.6%), ciprofloxacin (5.1%) and imipenem (0%) and piperacillin and tazobactam (2.6%). The most sensitive drug being imepenim (100%), pipercillin and tazobactam (97.4%), ciprofloxacin (94.9%), cefuroxime (74.4%) and

ceftriaxone (74.4%). Out of 50 patients studied, (Fig.4) 14 patients (28%) had bilateral bronchiectasis, 11 patients (22%) had left lower lobe bronchiectasis, 10 patients (20%) had right upper lobe bronchiectasis, 5 patients (10%) had right middle lobe bronchiectasis, 4 patients (8%) had right middle lobe and right lower lobe bronchiectasis, 3 patients (6%) had right lower lobe bronchiectasis, 2 patients (4%) had left upper lobe bronchiectasis and 1 patient (2%) had left upper lobe and left lower lobe bronchiectasis on HRCT. Out of 50 patients, 25 patients (50%) had cystic bronchiectasis, 12 patients (24%) had cylindrical bronchiectasis, 13 patients (26%) had tractionbronchiectasis and none had varicose bronchiectasis. Infection with Pseudomonas when compared with other aerobic bacteria led to worsening of lung function and increased morbidity and mortality. In Pseudomonas Aeruginosa, the comparison of the FEV1 severity between isolated and non isolated groups showed statistically significance. In case of klebsiella pneumonia and streptococcus species, no statistical significance was observed. (Table: 4)

Table 1: Age distribution

Age	No of patients	Percent
<30	8	16.0
31-40	10	20.0
41-50	9	18.0
51-60	15	30.0
61-70	8	16.0
Total	50	100.0

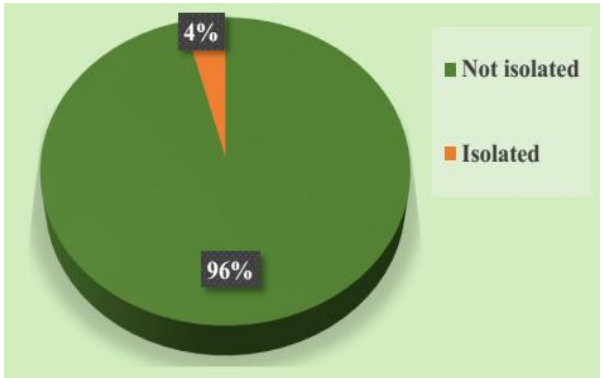

Fig. 1 Fungal Growth

Table 2: Gender distribution

Sex	No of patients	Percent
Female	27	54.0
Male	23	46.0
Total	50	100.0

Table 3: Aerobic bacterial growth distribution

Aerobic bacteria	n	Percent
Streptococcus pneumoniae	8	16.0
Pseudomonas aeruginosa	16	32.0
Staphylococcus aureus	3	6.0
Klebsiella pneumonia	12	24.0
Total	39	78

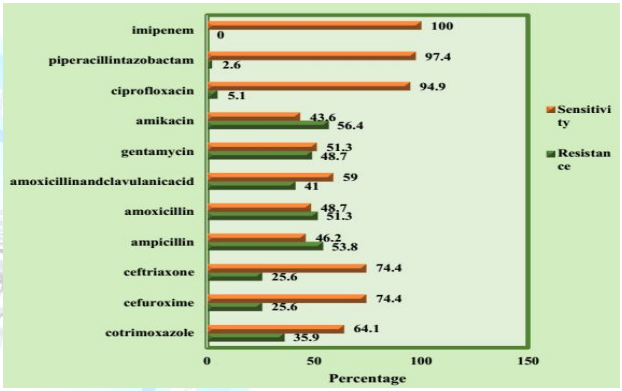

Fig. 2 Antibiotic Susceptibility Patterns

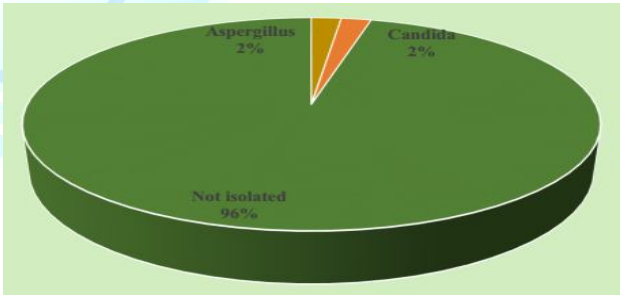

Fig. 3 Distribution of Fungi

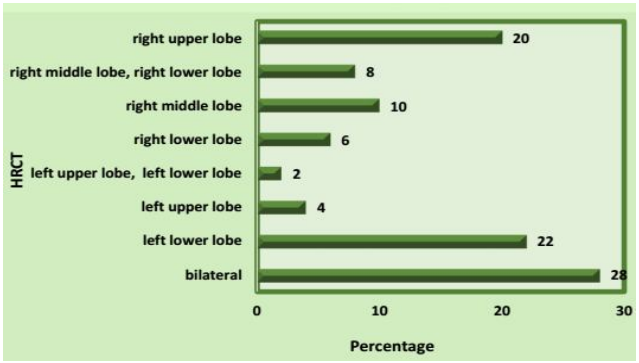

Fig. 4 HRCT Patterns

Table 4: Comparison of FEV1 with Aerobic bacteria

Aerobic bacteria	FEV1 %					Total n=50	P-value
	<35 n=0	35-49 n=10	50-59 n=10	60-69 n=24	≥70 n=6		
Streptococcus pneumonia							
Not isolated	0	9	9	19	5	42	0.34 NS
	0.00%	90.0%	90.0%	79.1%	83.3%	84.00%	
Isolated	0	1	1	5	1	8	
	0.00%	10.0%	10.0%	20.9%	16.7%	16.00%	
Staphylococcus aureus							
Not isolated	0	10	10	21	6	47	0.34 NS
	0.00%	100.0%	100.0%	87.5%	100.0%	94.00%	
Isolated	0	0	0	3	0	3	
	0.00%	0.0%	0.0%	12.5%	0.0%	6.0%	
Pseudomonas aeroginosa							
Not isolated	0	2	7	20	5	34	<0.01 HS
	0.00%	20.00%	70.0%	83.3%	83.3%	68.00%	
Isolated	0	8	3	4	1	16	
	0.00%	80.00%	30.0%	16.7%	16.7%	32.00%	
Klebsiella pneumonia							
Not isolated	0	9	6	18	5	38	0.50 NS
	0.00%	90.00%	60.0%	75.0%	83.3%	76.00%	
Isolated	0	1	4	6	1	12	
	0.00%	10.00%	40.0%	25.0%	16.7%	24.00%	

Discussion

This study showed highest incidence in elderly population especially between 51 and 60 years. In a retrospective study of 144 patients during 1987-1994, Scala R et al have concluded that 40% of study population were in the age group of 51-60 years. [3] Another retrospective cohort study of health claims between 1999–2001 in the US suggested the prevalence of bronchiectasis to be 4.2 per 1,00,000 population aged 18–34 years and 271.8 per 100,000 population aged >75 years. MB Nicotra et al had shown that mean age of their study group was 57.2(+/-16.7) years.[2] In a study by Amorim A et al, the mean age of the patients was 54+/-15 years.[4] Our findings were similar to Scala R et al and MB Nicora et al, that Post TB Bronchiectasis is common among elderly population. The prevalence of Bronchiectasis in patients treated for tuberculosis was estimated to be 46% during the period of study. As there is no follow-up data and the same data to be taken in different intervals, incidence cannot be calculated.

This study showed female preponderance in Post TB Bronchiectasis. Various studies have also observed 63% to 68% female preponderance, perhaps due to both biological and sociological factors. In a study by MA habesogluet al, where 304 patients with bronchiectasis were enrolled into the study, women were more predominant (54.3%).[5] In a study by Amorim A et al, there was a predominance of female patients (63.9%).[4] The most commonly identified cause was post infectious (30.3%), mostly tuberculosis (27.2%).[4] Regarding the sex distribution, this study is in concurrence with above mentioned studies done by MA Habesoglu et al [5] and Amorium A et al [6] where women are affected more than men. The

women affected were more than their male counterparts due to post infectious bronchial destruction caused by childhood TB, severe pneumonia and also due to the habit of voluntary suppression of cough among women which causes retention of secretions and development of bronchiectasis.

Bronchial colonisation by aerobic bacteria especially Pseudomonas (32%) and klebsiella (24%) were common. In a study conducted by S. Rajasekharanet al, 50 patients with bronchiectasis were admitted, the bacteriological profile of the patients showed that the commonest organism producing secondary infection was H. influenza (46%) followed by S. Pneumoniae (16%), Beta- haemolytic streptococci (14%) coliform group (12%), Klebsiella Pneumoniae (8%) and Pseudomonas aeruginosa (4%).[6] A study by Ho et al found pseudomonas aeruginosa (33%), haemophilus influenza (10%), streptococcus pneumonia (6%), and staphylococcus aureus (5%) and yeast (1%) as main respiratory pathogens isolated from the sputum samples.[7] Dimakou et al had shown that pseudomonas aeruginosa was the most common pathogen yielded in sputum cultures (43%) followed by haemophilus influenza (12.6%).[8] Our study showed a significant percentage of aerobic bacterial colonization and the commonest organism is Pseudomonas aeruginosa followed by klebsiella Pneumoniae. This result was similar to the studies of HUO Hai-yan et al, Dimakou et al and Ho et al. However the present study did not show anaerobic organisms in post TB bronchiectasis patients as anaerobic bacteriological culture techniques were not available in our institution. Therefore further studies are required to understand the distribution of these organisms.

In this study, bronchial colonization by fungi was uncommon with Aspergillus and Candida being isolated in 4%. In a study done by Sobti, KL et al and Sahoo RC et al, they found that tuberculosis of lung can be a predisposing factor in colonization of Aspergilli.[9] However in this study, bronchial colonization by fungi is uncommon in post tubercular bronchiectasis as in 48 subjects, out of 50, there was absence of fungal growth and further studies are required in this context. The limitation of the study was small sample size and short duration.

Antibiotic Susceptibility

The aerobic bacteria isolated in this study were most sensitive to imipenem (100%), piperacillin and tazobactam (97.4%) and ciprofloxacin (94.9%). In a study by conducted J Angrillet al they have shown that resistance to antibiotics was found among 30% of the isolated pathogens and also observed that 33% of the S pneumonia strains isolated were resistant to penicillin, 29% of the H influenza were beta-lactamase positive, and 33% of the Pseudomonas species were resistant to quinolones.[10] In a study done by HUO Hai-yan et al, pseudomonas aeruginosa, Ecoli and klebsiella pneumoniae were the common organisms. There was high sensitivity with piperacillin tazobactam, imipenem, cefazolin and quinolones. [7] As antibiotic susceptibility patterns vary according to geographical distribution of various strains, further studies are required to identify the causes of increased distribution of these resistant strains in various areas.

Fig 6

On HRCT, distribution of Post TB Brochiectasis was most commonly multilobar (28%) followed by left lower lobe (22%) and right upper lobe (20%). Studies by Brock RC, Fretheim B, Cohen AG, Bombarda S had shown that right middle lobe was commonly involved in post TB bronchiectasis. In a study done by S. Rajasekharanet al (90), of the 50 patients, 12(24%) had bilateral bronchiectasis and the rest had unilateral disease, with left lower lobe being predominantly affected in 30 patients (60%). They found that only 36% of post TB bronchiectasis was seen in upper lobes and 64% of them was in the lower lobes.[6] CK Rhee et al had found that most commonly involved sites of post TB bronchiectasis were right upper lobe (71.6%) and upper division of left upper lobe (67.6%).[11] In present study out of 50 patients 25 patients (50%) had cystic type of bronchiectasis, 12 patients (24%) had cylindrical type of bronchiectasis, 13 patients (26%) had traction bronchiectasis and no patient had varicose type

of bronchiectasis. S Rajasekharan et al had shown that 60% of patients of their study had cystic bronchiectasis, 36% of patients had cylindrical bronchiectasis and 4% of patients had both types of bronchiectasis.[6] RC Brock had found that post TB bronchiectasis develops as tractionbronchiectasis. This study had shown that cystic bronchiectasis was common followed by traction and cylindrical bronchiectasis.

FEV1 comparison with microbiological isolates

In Pseudomonas aeruginosa, the comparison of the FEV1 severity between isolated and non-isolated groups showed statistically significant. In case of Klebsiella pneumonia, streptococcus species no statistical significance was observed. Infection with Pseudomonas when compared with other aerobic bacteria lead to worsening of lung function and increased morbidity and mortality. In a study conducted by Barker AF et al, they stated that Pseudomonas species will promote disease progression, and the infection with this species can be related to worsening of lung function and increased morbidity and mortality of the patient. [12] Bogossian et al had found lower FEV1 (54.5±22.8% vs. 67.4±27.6%) and FVC (70.4±22.2% vs. 81.8 ± 24.2%) values in individuals with post tuberculosis bronchiectasis compared to those without a history of tuberculosis. [13]

G Davies et al had concluded in their study that infection by pseudomonas aeruginosa occurs in bronchiectasis patients with more severe impairment of pulmonary function but does not influence rate of decline in pulmonary function and thus pseudomonas aeruginosa is a marker of bronchiectasis severity but is not linked to an accelerated decline in pulmonary function. [14] The greater reduction of FEV1 and FVC in individuals with pos tuberculosis bronchiectasis might be due to a greater severity and intensity of the bronchial and parenchymal damage; in addition, the reduced FVC might denote an airway obstruction by mucus or an airway collapse. MA Martinez Garcia et al had shown that chronic colonization of sputum by Pseudomonas aeruginosa, frequency of severe exacerbations, and increased systemic inflammation are independently associated with a faster decline in FEV1.[15] Pseudomonas aeruginosa was the most common pathogen yielded in sputum cultures (43%) followed by haemophilus influenza (12.6%). Patients with P. aeruginosa had more long-standing disease and worsened lung function.[8] Our study had shown concurrence with the previous studies in regard to the decline in FEV1 in patients colonized with Pseudomonas aeruginosa with p value <0.01. It must be underlined that the intensification of anti Pseudomonas aeruginosa antibiotic treatment in these patients (eg, with increased doses, longer treatment, or combined therapy) possibly could slower even stop the impairment of lung function over the longer term.

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