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Bacteriology of chronic respiratory diseases in a tertiary care hospital in Assam

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ABSTRACT

Introduction: *Chronic respiratory diseases constitute a grave problem throughout the world and particularly in middle and low-income countries. The burden of these diseases leads to poor quality of life and disability of affected individuals leading to premature deaths and a great economic loss to their families and society.* ***Materials and Methods:*** *Bronchoalveolar lavage fluid samples of patients with chronic respiratory diseases undergoing bronchoscopy in a tertiary care hospital were collected under aseptic precautions after obtaining approval from the institutional ethical committee. Antibiotic sensitivity testing was performed for the bacterial isolates.* ***Results:*** *40 out of 110 cases (36.36%) showed the growth of pathogenic bacteria. The most common bacteria isolated were Klebsiella pneumoniae (14.54%) and Pseudomonas aeruginosa (10.90%). The other bacterial isolates were Staphylococcus aureus (2.72%), Enterococcus faecium (2.72%), Acinetobacter baumannii (1.18%), Enterobacter cloacae (1.18%), Escherichia coli (0.90%) and Streptococcus pneumoniae (0.90%). Most of the strains of Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Enterobacter cloacae were sensitive to antibiotics like piperacillin-tazobactam, cefepime, and ciprofloxacin. The gram-positive isolates showed 100% sensitivity to vancomycin and linezolid.* ***Conclusions:*** *Bronchoalveolar lavage has improved sensitivity and specificity in the diagnosis of pulmonary infections.*

Keywords: *Bronchoalveolar lavage (BAL); antibiotic sensitivity; bacterial isolate; Klebsiella pneumoniae; Staphylococcus aureus.*

INTRODUCTION

Chronic respiratory diseases constitute a grave problem throughout the world and particularly in middle and low-income countries. They comprise chronic diseases of the airways and other structures of the lung and account for 4 million deaths annually. The burden of these diseases leads to poor quality of life and disability of affected individuals leading to premature deaths and a great economic loss to their families and society.[1] Chronic respiratory diseases are prevalent among more than 500 million patients living in developing countries or deprived populations across the world.[2]

Bronchoalveolar lavage (BAL) is an invasive technique, used in the diagnosis of lower respiratory tract infections, where a saline wash of the bronchial tree is done. It was first introduced in 1970.[3] It has been observed by some investigators that the first aliquot represents predominantly the airway cells and secretions and is good for microbiological analysis.[4] One million alveoli are sampled from 1ml of secretions from the bronchoalveolar lavage fluid.[5] The main objective of the study was to determine the common aerobic bacterial pathogens in bronchoalveolar lavage fluid from patients with chronic respiratory diseases and to determine the antibiogram of the bacterial isolates.

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MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, Gauhati Medical College Hospital (GMCH), Guwahati, for a period of one year from June 2017 to May 2018 with a total of 110 samples. The study was commenced with ethical approval and clearance certificate from Institutional Ethics Committee, GMCH. Informed consent was taken from the patient and clinical details were recorded in a predesigned proforma. Adult patients with chronic respiratory diseases like bronchiectasis, COPD (Chronic Obstructive Pulmonary Disease) with respiratory tract infection, non-resolving pneumonia, interstitial lung disease, any case of hemoptysis, and any growth in endobronchial tree/lung undergoing bronchoscopy were included in the study. However, patients with unstable cardiac conditions, immunocompromised patients, pregnant women, patients who do not give consent for the procedure were excluded from the study.

Bronchoscopy procedure: The site to be lavaged was determined radiographically with the help of HRCT chest and the BAL fluid was recovered in 2-3 aliquots.[6]

Processing of the sample: The sample was centrifuged at 3000 rpm for 15-20 minutes. A smear was made from the sediment of the centrifuged BAL sample and it was subjected to Gram stain according to the methods described by Duguid JP et al.[7] The Gram-stained smear was observed to access the quality of the sample and also to differentiate gram-positive from gram-negative bacteria. On Gram staining, the presence of >10 squamous epithelial cells/low power field in the BAL fluid in the direct smear was considered as a criterion for sample rejection. A total of 110 BAL fluid samples that met the above quality control criteria were included in the study. For aerobic culture, the sediment of the centrifuged BAL sample was inoculated onto the Blood agar, Mac Conkey agar, and chocolate agar plates. The inoculated Blood agar and Chocolate agar plates were incubated under microaerophilic conditions with 5% CO_2 at 37^{0}C for 24-48 hours in a candle jar. The inoculated Mac Conkey agar plates were incubated under aerobic conditions at 37^{0}C for 24-48 hours.[8] For the isolation of *S.pneumoniae*, 5% Sheep Blood Agar with Optochin disk was used. The blood agar plates were incubated in a candle jar, in the presence of 5% CO_2 and incubated at 35-37^{0}C for 24-48 hours. The media used for the isolation of *H. influenzae* was Chocolate agar with a streak of *S. aureus* and incubated in an environment rich in 5% CO_2 using a candle jar at 35^{0}C for 24-72 hours.[6]

Colony count: As a loop with a volume of 10 microliters was used, the colonies were counted and multiplied by 100, and the colony-forming unit per ml was determined. A threshold of 10^4 CFU/ml was taken as diagnostic.[6,9,10]

Identification: Characterization and identification of organisms were done as per Collee et al[11] by interpretation of the colony characteristics, gram staining, motility, biochemical tests.

RESULTS

Most of the cases in the present study were between the ages of 51 - 60 years (21.81%) followed by 21-30 years (20.9%). There were no cases below 10 years of age. The median age is 48.5 years. **Figure 1** shows the age-wise distribution of cases.

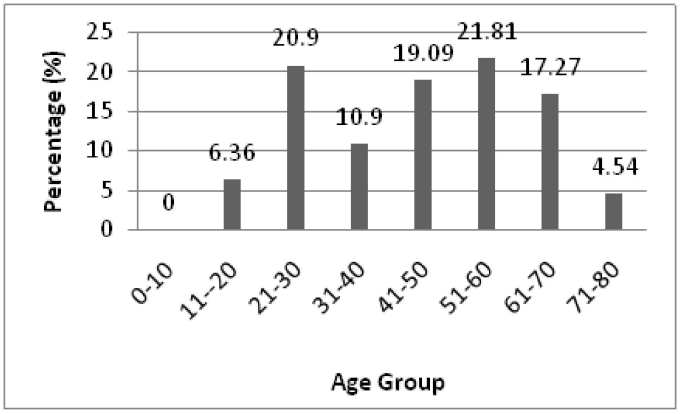

Figure 1 The age distribution of all the patients

The study shows that 71% of males and 29% of females were included in the study group. The male to female ratio was 2.5:1(M: F).

Most of the patients in this study belonged to lower (61.81%) and lower-middle (24.54%) socioeconomic status according to modified B.G. Prasad's socioeconomic status classification which applies to both urban and rural population.

Majority of the cases in this study include patients with bronchiectasis (20%), non-resolving pneumonia (19%), lung mass (17.27%), and pulmonary tuberculosis (16.36%). However, cases like carinal mass and sarcoidosis constitute a mere 0.9% of the cases. Details are shown in **Table 1.**

Table 1 The distribution of clinical diagnosis of all patients

Clinical diagnosis	No. of patients (n)	Percentage (%)
Bronchiectasis	22	20
Bronchitis	4	3.63
Interstitial lung disease	16	14.54
Carinal mass	1	0.90
Hilar mass	2	1.81
Sarcoidosis	1	0.90
Emphysema	6	5.45
Lung mass	19	17.27
Non-resolving pneumonia	21	19.09
Pulmonary tuberculosis	18	16.36
Total no of cases (N) = 110		

The study shows that 56% of the cases had a history of smoking.

Gram stain of the direct smears:

Out of 110-gram stained smears, 6.36% of the cases showed the presence of gram-positive bacteria and 30% showed gram-negative bacteria. Details are shown in **Table 2.**

Table 2 The prevalence of gram-positive and gram-negative bacteria in the direct smear

No. of smears examined.	Gram-Positive Organisms		Gram-Negative Organisms	
110	No.	Percentage (%)	No.	Percentage (%)
	7	6.36	33	30

Table 3 The distribution of bacterial isolates associated with chronic respiratory diseases

Bacterial isolates	**Number of organisms isolated**	**Frequency (%)**
Acinetobacter baumannii	2	1.18
Enterobacter cloacae	2	1.18
Enterococcus faecium	3	2.72
Escherichia coli	1	0.90
Klebsiella pneumoniae	16	14.54
Staphylococcus aureus	3	2.72
Streptococcus pneumoniae	1	0.90
Pseudomonas aeruginosa	12	10.90
Total number of cases with bacterial isolates	**40**	

The above table (**Table 3**) shows that *Klebsiella pneumoniae* (14.54%) was the most commonly isolated gram-negative pathogen whereas *Staphylococcus aureus* (2.72%) and *Enterococcus faecium* (2.72%) were the most commonly isolated gram-positive pathogen.

Number of gram-positive bacteria sensitive to commonly used antibiotics and their percentage (%)

Table 4 Gram-positive bacteria (GPB) sensitive to the commonly used antibiotics

GPB	No. of Pisolates	P	AMP	GEN	OF	CIP	LE	NX	CTR	AZ	E	CX	DO	TE	OP	TEI	VA	LZ
S.aureus	3	0(0)	2(66.6)	2(66.6)	2(66.6)	2(66.6)	2(66.6)	"-"	2(66.6)	–	0(0)	2(66.6)	3(100)	0(0)	–	–	3(100)	3(100)
E.faecium	3	0(0)	0(0)	–	–	3(100)	3(100)	3(100)	–	–	–	–	3(100)	3(100)	–	2(66.6)	3(100)	3(100)
S.pneumoniae	1	1(100)	–	–	1(100)	–	1(100)	–	–	1(100)	1(100)	–	1(100)	1(100)	1(100)	–	1(100)	1(100)

Table 4 shows the antibiotic sensitivity pattern of the gram-positive bacteria. The table shows the number of isolates sensitive to the respective antibiotic along with its percentage given in brackets.100% sensitivity was observed in doxycycline, vancomycin, and linezolid among all the gram-positive isolates. Resistance to penicillin was observed in 100% of the isolates of *S.aureus* and *E.faecium.*

The number of gram-negative bacteria (GNB) sensitive to commonly used antibiotics and their percentage (%):

Table 5 Gram-negative bacteria (GNB) sensitive to the commonly used antibiotics

GNB	No.of isolates	AMP	DO	TE	AT	TMP/SMX	CO	E	AZ	AK	GEN	OF	CIP	LE	NX	CTR	CAZ	CTX	CPM	AMC	PIT	MRP	IPM
K.pneumoniae	16	7 (43.7)	9 (56.2)	-	4 (25)	-	-	-	2 (12.5)	14 (87.5)	-	-	11 (68.7)	-	-	8 (50)	-	-	9 (56.25)	2 (12.5)	12 (75)	3 (18.75)	8 (50)
P.aeruginosa	12	-	-	-	4 (33.3)	-	-	-	-	12 (100)	12 (100)	11 (91.6)	11 (91.6)	11 (91.6)	11 (91.6)	-	12 (100)	-	7 (58.3)	-	12 (100)	1 (8.3)	6 (50)
A.baumannii	2	-	1 (50)	1 (50)	-	2 (100)	2 (100)	-	-	2 (100)	2 (100)	-	2 (100)	2 (100)	-	0 (0)	0 (0)	0 (0)	0 (0)	-	-	0 (0)	0 (0)
E.coli	1	1 (100)	0 (0)	-	0 (0)	-	-	-	0 (0)	1(100)	-	-	1 (100)	-	-	1 (100)	-	-	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
E.cloacae	2	0 (0)	1 (50)	0 (0)	2 (100)	-	-	-	1 (50)	2 (100)	-	-	2 (100)	-	-	2 (100)	-	-	2 (100)	0 (0)	2 (100)	1 (50)	2 (100)

Table 5 depicts the antibiotic sensitivity pattern of the gram-negative bacteria. Most of the isolates *of K pneumoniae* showed sensitivity to amikacin (87.5%) and ciprofloxacin (68.7%). 100% of the strains of *P aeruginosa* showed sensitivity to the aminoglycosides, ceftazidime, and piperacillin-tazobactam. *A baumannii* showed a multidrug resistance pattern with 100% of the strains being resistant to the cephalosporins, imipenem, and meropenem.

DISCUSSION

Chronic respiratory disease is a public health challenge across the world owing to the disability and economic burden associated with it. Keeping this in mind, the present study was conducted to identify the common bacterial pathogens associated with chronic respiratory diseases along with their antibiogram. The study shows that out of a total of 110 cases included in the study, most of the cases with chronic respiratory diseases were between the ages of 51 - 60 years (21.81%). Moreover, age above 65 years is a risk factor for pneumonia. The study included 78 (71%) male and 32 (29%), female patients. These findings were similar to the study by Bari SA et al.[6] The study showed most of the cases belonged to the lower and lower-middle socioeconomic scale according to modified BG Prasad's socioeconomic status classification. Out of 110 cases, the total number of bacterial pathogens identified was found to be 40 (36.36%) which resonated with the study by Sethi S et al.[12] which showed a prevalence of 34.6%. Out of the 40 bacterial isolates, 7 (6.36%) were gram-positive cocci and 33 (30%) were gram-negative bacilli. The number of gram-positive cocci in the study resonated with the findings of Bari SA et al[6] in which 7 (7%) of the isolates were gram-positive. However, the prevalence of gram-negative bacilli in the present study was 30% which was comparatively lesser than the findings of the study mentioned above. The reason for this could be due to the over-enthusiastic use of empirical antibiotic therapy by the clinicians before bronchoscopy. *Klebsiella pneumoniae* (14.54%) was the most commonly isolated gram-negative bacteria. Moreover, *Klebsiella pneumoniae* is most commonly associated with elderly patients and with pneumonia in hospitalized patients. Whereas, *Staphylococcus aureus* and *Enterococcus faecium* were the most commonly isolated gram-positive bacteria. Both these findings correlated with the study by Bari SA et al[6] and Vivek KU et al.[13] *Enterococcus species* are seen to be commonly associated with patients having underlying diseases. In this study, 100% of the gram-positive isolates were sensitive to doxycycline, vancomycin, and linezolid, which was in concordance with the study by Vivek KU et al.[13] and Galate LA et al.[14] Most of the gram-negative isolates in the present study showed sensitivity to aminoglycosides, fluoroquinolones, piperacillin-tazobactam, and imipenem, however, resistance was seen to aztreonam, azithromycin, amoxyclav, and meropenem. *Acinetobacter*

baumannii, being a hospital strain, was seen to be multidrug-resistant.

CONCLUSION

Chronic respiratory diseases are one of the leading causes of morbidity and mortality worldwide and especially in developing countries. The use of bronchoalveolar lavage as a diagnostic tool has proved to be a sensitive tool in diagnosing the lower respiratory tract infections associated with chronic respiratory diseases. Moreover, the abuse of antibiotics has led to the emergence of multidrug-resistant bacteria which are difficult to control. Hence it has become important to have the knowledge of the microbial flora causing lower respiratory tract infection and also to regularly monitor the antimicrobial susceptibility pattern of the microorganisms at local, regional and national levels as it would guide the physicians to prescribe the right antimicrobials and initiate the empirical therapy without leading to the emergence of multidrug-resistant strains.

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Contribution of authors: We declare that this work was done by the author(s) named in this article and all liabilities of claims relating to the content of this article will be borne by the authors. Dr Dina Raja conceived and designed the study and Dr Baishali Das was involved in collecting and analysing the data.

Author disclosure:

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3. All author(s) have contributed sufficiently to the article to take public responsibility for it.
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REFERENCES

1. Cruz AA. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. In: Bousquet J, Khaltaev N, editors. World Health Organization. Switzerland: WHO Press; 2007.
2. Aḷt-Khaled N, Enarson D, Bousquet J. Chronic respiratory diseases in developing countries: the burden and strategies for prevention and management. Bull World Health Organ 2001;79(10):971-9.
3. Radha S, Afroz T, Prasad S, Ravindra N. Diagnostic utility of bronchoalveolar lavage. J Cytol 2014;31(3):136-138.
4. Meyer KC. Bronchoalveolar lavage as a diagnostic tool. Semin Respir Crit Care Med 2007;28(5):546-60.
5. Charles MP, Kali A, Easow JM, et al. Ventilator-associated pneumonia. Australas Med J 2014;7(8):334-44.
6. Bari SA, Mustafa M. A study on bacterial isolates from bronchoalveolar lavage (Bal) fluid obtained from patients with pulmonary infections–in tertiary care hospital, Hyderabad. Int J Recent Sci Res 2018 June;9(6):27531-5.
7. Duguid J.P. Staining methods. In: Collee JG, Marmion BP, Fraser A, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. India: RELX India Pvt. Ltd.; 2006. p. 793-812.
8. Tille P. Bailey & Scott's Diagnostic Microbiology. 13th ed. St. Louis, Missouri: Elsevier Mosby; 2007. p. 878-91.
9. Baselski V, Klutts JS. Point-Counterpoint: Quantitative cultures of bronchoscopically obtained specimens should be performed for optimal management in patients with ventilator associated pneumonia. J Clin Microbiol 2013 Jan;2:03383.
10. Johanson WG, Dever LL. Nosocomial pneumonia. Intensive Care Med 2003;29(1):23-9.
11. Colle J.G., Marr W. Culture of bacteria. In: Collee JG, Marmion BP, Fraser A, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. India: RELX India Pvt. Ltd.; 2006. p. 113-29.
12. Sethi S, Maloney J, Grove L, Wrona C, Berenson CS. Airway inflammation and bronchial bacterial colonization in chronic obstructive pulmonary disease. Am J Respir Crit Care Med2006 May 1;173(9):991-8.
13. Vivek KU, Nutan Kumar DM. Microbiological profile of bronchoalveolar lavage fluid in patients with chronic respiratory diseases: a tertiary care hospital study. Int J Med Res Rev 2016;4(3):330-7.
14. Galate LA, Gajbhiye PS. Microbiological profile and antibiogram patterns of lower respiratory tract infection. Int J Humanit, Arts, Med Sci 2015;3(4):1-6.
15. Montaner AE, de Lomas JG, Asensi JR, de la Cruz OA, de la Serna Blįzquez O, Burruchaga MS, et al. Bacteria from bronchoalveolar lavage fluid from children with suspected chronic lower respiratory tract infection: results from a multi-center, cross-sectional study in Spain. Eur J Pediatr 2018 Feb 1;177(2):181-92.