

Microbiological profile of bronchoalveolar lavage fluid in patients with chronic respiratory diseases: a tertiary care hospital study

Vivek KU¹, Nutan Kumar DM²

¹Ex Senior Resident, Department of Pulmonary Medicine, ²Junior Resident, Department of Medicine, Both are affiliated with Kempegowda Institute of Medical Sciences (KIMS), Bangalore.

Address for correspondence: Dr. Vivek KU, Email: vivek.kotyal@gmail.com

Abstract

Objective: Chronic respiratory diseases account for 4 million deaths annually. Infections are most frequent cause of exacerbations. Bronchoalveolar lavage has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections. Hospital antibiograms are an important component of detecting and monitoring trends in antimicrobial resistance. **Materials and Methods:** Retrospectively BAL fluid reports of patients with chronic respiratory diseases undergoing bronchoscopy in KIMS Hospital were collected under aseptic precautions. Antibiotic and antifungal susceptibility testing was done for bacterial isolates and fungal isolates. **Results:** Among 100 BAL sample cultured for bacterial etiology, 38 samples showed growth, 56 samples showed no growth. Monomicrobial growth of the BAL culture was seen in 34 cases (89%) and polymicrobial growth was seen in 4 (11%). In the antimicrobial susceptibility testing, 100% sensitivity was noted to linezolid, levofloxacin, tetracycline, vancomycin, netilmycin and tobramycin. Piperacillin-tazobactam and imipenem show sensitivity of 96.2%. Antibiotics showing high resistance pattern were ampicillin (73%) and amoxicillin-clavulanic acid (52.3%). On fungal culture, 27 cases showed growth, of which candida albicans was the most common isolate (37%). Aspergillus accounted for 4 isolates (14.8%), aspergillus niger being predominant. All isolates of candida were 100% susceptible to fluconazole, itraconazole and voriconazole except candida albicans, 10% showed resistance to voriconazole. All isolates of aspergillus were 100% susceptible to voriconazole and 100% resistance to fluconazole. **Conclusion:** Bronchoalveolar lavage has improved sensitivity and specificity in diagnosis of pulmonary infections. An updated local antibiogram for each hospital based on local bacteriological patterns and susceptibilities is essential to guide initial empiric therapy.

Keywords: Bronchoalveolar lavage, Bacterial isolate, Fungal isolate, Antibiotic sensitivity, Candida, Klebsiella, aspergillus.

Introduction

Chronic respiratory diseases are a group of chronic disease affecting the airways and other structures of lungs. Common chronic respiratory diseases are asthma, bronchiectasis, chronic obstructive lung diseases, including chronic obstructive pulmonary disease, emphysema and bronchitis, chronic pleural disease, pneumoconiosis, pulmonary eosinophilia, pulmonary heart diseases and disease of pulmonary embolism, pulmonary hypertension, cor pulmonale, sarcoidosis, sleep apnoea syndrome [1]. Other diseases like cystic fibrosis, pulmonary fibrosis and occupational lung diseases are included [2]. They present with symptoms of cough, pain in throat or chest, abnormalities of

breathing, hemorrhages of respiratory passages, signs involving respiratory and circulatory system (asphyxia, pleurisy, cardio respiratory arrest, abnormal sputum) [1]. Most common causes of infections in these patients are viruses and bacteria (75-80%). Most frequent bacteria involved in exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis [3]. Chronic respiratory diseases account for 4 million deaths annually contributing to 5% of global deaths [1]. Measured in Disability Adjusted Life Years (DALY), in 2005 the burden of chronic respiratory diseases was projected to account for 4% of the global burden and 8.3% of the burden of chronic diseases [1]. In India, chronic respiratory disease accounted 7% deaths and 3% DALYs lost [3]. The course of these

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diseases is punctuated by exacerbations. Exacerbations are associated with greater and irreversible decline in lung function, significant mortality and morbidity. Infections are most frequent cause of exacerbations [4]. Early diagnosis and proper choice of antimicrobials is crucial for management of these patients. Sputum culture yields diagnosis in fewer than 50% of patients with pneumonia [5]. The advent of bronchoscopy and quantitative invasive techniques like Bronchoalveolar lavage has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections [6].

Aims and objectives

- To detect pathogenic organism by microscopy of BAL fluid.
- Isolate and identify aerobic bacteria and fungi from BAL fluid specimen.
- To determine antimicrobial susceptibility pattern of the isolates.

Material and methods

This was a retrospective collection of data from January 2014 to July 2015 of BAL fluid specimens of patients with chronic respiratory diseases undergoing bronchoscopy in KIMS Hospital, Bangalore. BAL fluid specimens were collected under aseptic precautions and transported to the laboratory immediately for further processing. The sample was inoculated on Blood agar, chocolate agar and Macconkey agar using a sterile 4mm nichrome loop (0.01ml), and incubated at 37 C for 72 hours for quantitative bacterial culture using standard laboratory techniques. Sample was also inoculated in brain heart infusion broth. For growth positive plates, the colony forming units was calculated [7]. Direct

Results

This study was conducted in the department of pulmonary medicine, Kempegowda Institute of Medical Sciences, Bangalore from January 2014 to July 2015. 100 BAL samples of patients with chronic respiratory diseases were studied to detect the presence of bacterial and fungal infections of lung. Age of the patients varied from 18-85 years. Out of 100 cases majority of cases 28 (28%) were in the age group of >60 years, followed by 22% in 51-60 years, 21% in 41-50 years and 3 % in the age group of 18-20 years. 61 cases were male and 39 cases were female.

Most of the cases in our study include patients with tuberculosis (16%), COPD (15%), asthma(15%), bronchiectasis (7%). Occupational lung diseases and chronic lung abscess accounted for only 2% of the cases respectively as shown in table1. In the study, cough with sputum production was the most common symptom present in 94% cases. Fever was the second most common symptom (60%), followed by dyspnea in 59%. 7 patients presented with hemoptysis who were diagnosed with bronchogenic carcinoma. Out of 100 cases 78% of the cases had radiological signs suggestive of lung infections. Among 100 BAL sample cultured for bacterial etiology, only 38 samples showed growth >10,000 CFU/ml, 56

microscopy with wet mount preparation (10% potassium hydroxide) was done on part of the sample to rule out fungal filaments, and remaining part of the sample was centrifuged at 3000rpm for 15-20 minutes [8]. Supernatant was resuspended in phosphate buffer saline or 1-5ml of the sample itself subjected to Gram's stain for Bacteria or fungus, acid fast staining techniques like Ziehl-Neelsen stain to rule out Mycobacteria, Kinyoun's technique for Nocardia [9], May-Grunwald- Giemsa stain to rule out cysts of parasites like Pneumocystis [10] and fungus [11]. A part of centrifuged sample was also inoculated on sabouraud's dextrose agar for fungal culture and incubated for 4 weeks at 370C. Antibiotic susceptibility testing was done for bacterial isolates by Kirby-Bauer's disc diffusion method. Using commercially available MIC test strips (HiMedia Laboratories) antifungal susceptibility testing was done for yeast like fungal isolates by E-test for fluconazole.

Inclusion criteria

1. Adult patients with chronic respiratory diseases undergoing bronchoalveolar lavage.

Exclusion criteria

1. Patients with unstable cardiac conditions (recent myocardial infarction, cardiac arrhythmias etc.
2. Pregnant women.
3. Patients who did not give consent for the procedure

Statistical Analysis: Data collected will be analyzed using descriptive statistical methods by computing percentage, mean and standard deviation. Wherever necessary the results were depicted in the form of percentages.

Table 1: Respiratory disease profile

Disease	Cases	Percentage (%)
Chronic obstructive respiratory disease	15	15
Asthma	12	12
Interstitial lung disease	13	13
Tuberculosis	16	16
Malignancy	10	10
Non resolving pneumonia	7	7
Pleural diseases	7	7
Sarcoidosis	4	4
Chronic lung abscess	2	2
Occupational lung disease	2	2
Bronchiectasis	7	7
Corpulmonale	7	7
Total	100	100

Samples showed no growth. Monomicrobial growth of the BAL culture was seen in 34/38 cases (89%) and polymicrobial growth was seen in 4/38 (11%).

Table 2: Profile of infections.

Types of organisms	Number of cases (%)	Number of isolates (%)
Monomicrobial	34 (89.4%)	34 (80.9%)
Polymicrobial	4 (10.5%)	8 (19%)
Total	38 (100%)	42 (100%)

Table 3: Organisms in monomicrobial distribution

Type of organisms	Number of isolates	Percentage (%)
Monomicrobial	34	80.9
Klebsiella pneumonia	7	16.6
Acinetobacter baumannii	6	14.2
Enterococcus faecalis	5	11.9
Escherichia coli	4	9
Streptococcus pneumonia	4	9
Staphylococcus aureus	3	7
Enterobacter aerogenes	2	4.7
Pseudomonas aeruginosa	1	2.3
Klebsiella oxytoca	1	2.3
Serratia marcescens	1	2.3

Table 4: Organisms in polymicrobial distribution

Type of organisms	Number of isolates	Percentage (%)
Polymicrobial	8	19
Klebsiella oxytoca+ Escherichia coli	2	4.7
Klebsiella pneumonia+ Enterococcus faecalis	2	4.7
Klebsiella oxytoca + Staphylococcus aureus	2	4.7
Enterobacter aerogenes+ Staphylococcus aureus	2	4.7

In the antimicrobial susceptibility testing done for isolates, 100% sensitivity was noted to linezolid, levofloxacin, tetracycline, vancomycin, netilmycin and tobramycin. Piperacillin-tazobactam and imipenem show sensitivity of 96.2%. Antibiotics showing high resistance pattern were ampicillin (73%) and amoxicillin-clavulanic acid (52.3%). Antibiotic resistance pattern shown in table 4.

Table 5: Antibiotic resistant pattern seen in different aerobic organisms

Antibiotics	Klebsiella pneumoniae(8)	Acinetobacter baumannii(6)	Enterococcus fecalis(6)	Staph aureus(5)	E.coli(5)	Streptococcus pneumoniae(4)	Enterobacter aerogenes (3)	Klebsiella oxytoca (3)	Pseudomonas aeruginosa(1)
Amikacin	0	1(16%)	0	0	1(20%)	-	1(33.3%)	0	0
Amoxicillin/clavulanic acid	5(62.5%)	3(50%)	1(16.7%)	2(66.6%)	5(100%)	1(25%)	3(100%)	3(100%)	0
Clindamycin	-	-	1(16.7%)	-	-	1(25%)	-	-	-
Cefoperazone	2(25%)	3(50%)	1(16.7%)	0	3(60%)	1(25%)	1(33%)	2(66.6%)	-
Cefepime			1(16.7%)	0	3(60%)	1(25%)	1(33%)	2(66.6%)	0
Imipenem	0	1(16%)	-	0	0	-	0	0	0
Linezolid	-	-	0	-	-	0	-	-	-
Vancomycin	-	-	0	-	-	0	-	-	-
Piperacillin/Tazobactam	0	1(16%)	-	0	0	-	0	0	0

Out of 100 cases studied, 9 cases were positive for acid fast bacilli with ziehl-neelson stain, 23 cases were positive for fungal elements with wet mount examination. None of them were positive for nocardia screened by modified kinyoun's stain with 1% sulphuric acid. On gram staining for fungus, 26 cases showed the presence of gram positive budding yeast like cells and hyphae structures. 77 cases showed no evidence of fungus on gram stain. On May-Grunwald –Geimsa staining, 14 cases showed budding yeast like cells and hyphal structures. On fungal culture, 27 cases showed growth on Sabouraud's dextrose agar and BACTEC mycosis IC/F bottles. Out of 27 species of fungus isolated, candida albicans was the most common isolate (37%). Aspergillus accounted for 4 isolates (14.8%), aspergillus niger being predominant. only one isolate of candida dublinensis was seen as shown in table 4

Table 6: Organisms isolated in fungal culture.

Organisms	Number
Candida albicans	10
Candida parapsilosis	4
Candida glabrata	4
Candida tropicalis	4
Candida dublinensis	1
Aspergillus niger	2
Aspergillus flavus	1
Aspergillus fumigates	1
Total	27

All isolates of candida were 100% susceptible to fluconazole, itraconazole and voriconazole except candida albicans, 10% showed resistance to voriconazole. Amphotericin B resistance was seen in candida albicans (10%), candida tropicalis(75%), candida glabrata and candida parapsilosis. 25 % of candida tropicalis were resistance to ketoconazole. All isolates of aspergillus were 100% susceptible to voriconazole and 100% resistance to fluconazole. Aspergillus niger and aspergillus fumigatus showed 100 % susceptibility to itraconazole , whereas aspergillus flavus was resistant. Aspergillus fumigatus was resistance to amphotericin B and ketoconazole.

Discussions

Chronic respiratory diseases represent a public health challenge in both industrialized and developing countries because of their frequency and economic impact. It is major cause of mortality and morbidity across the globe. This study was conducted to evaluate the bacterial and fungal agents causing infections in patients with chronic respiratory disease and with perspective of evaluating their sensitivity to different antibiotics. In this study, most of the chronic respiratory diseases belong to age group of more than 45 years which correlated with the study conducted by Mullerova et al [12] (45%) and Merino- Sanchez et al (60%). [13] Age above 65 years is the risk factor for developing pneumonia. Use of inhalational steroids lowers the oral defence and pay route for microbial colonization.

Bronchoalveolar lavage provides a very usefull tool for diagnosing lower respiratory tract infections. The present study yielded positive bacterial BAL cultures in 38% of the cases of suspected lung infections. This is in contrast to other studies like Velez et al [14] and Kottmann et al [15], where the positive yield was 51.6% and 55.8% respectively. The lower positivity rate in the present study might be because the study was done in general population, whereas other studies quoted above were done in immunocompromised patients. In the present study, aerobic gram negative and gram positive bacteria constituted 65.7% and 34.3% of the isolates respectively, which correlates with the study conducted by Groenewegen and Wouters in patients with COPD with suspected pneumonia, where aerobic gram negative and gram positive bacteria constituted 71% and 27% of the isolates respectively [16]. Klebsiella (26%) was the most common pathogen isolated from our study which correlates with studies conducted by Lin SH et al [17] (19.6%) and Singh AK et al.[18] Klebsiella is a part of normal flora of the mouth and most widely associated with pneumonia in a hospitalised patients and elderly. Hence, its predominance may be related to the predominate elderly population in our study. Comparision among different studies shown in table 5.

Table 7: Comparison of bacterial flora isolated from various studies on chronic respiratory disease.

Study	Total isolates	Percentage of isolates				
		klebsiella	Acinetobacter	S. aureus	S. pneumoniae	Pseudomonas aeruginosa
Torres et al [19]	73	-	-	-	43%	-
Lin SH et al [17]	328	19.6%	6.9%	6.1%	2.4%	16.8%
Bari et al [20]	60	13.3%	6%	-	3%	25%
Our study	42	26%	14.2%	11.9%	9.5%	2%

Enterococcal infections are common in those with underlying diseases such as after major surgery, immunosuppression, organ transplant patients and cardio-pulmonary disease. The present study showed 14.2% isolates of enterococcus faecalis, which is in agreement with study conducted by Bonten et al [21] (13.3%) and Hohenadel et al [22] (9.9%) on pneumonia patients. The present study showed maximum resistance of organisms to antibiotics like ampicillin (73%), amoxyclav (52%), cefuroxime (44%), cefaperazone (31.7%) and cotrimoxazole (39%). In the present study, 9% of the patients were diagnosed with mycobacterium tuberculosis. This is correlated to study conducted in immunocompromised patients with pneumonia, where 6.6% of the cases were due to tuberculosis. The increased incidence of fungal lung infection is due to increase in population of immunocompromised and susceptible patients like those on steroids, chronic diseases like COPD, cirrhosis of liver etc. The increase use of antibiotic and antifungal prophylaxis has also lead to colonization of fungi in the respiratory tract. All these factors contribute to the recent explosion in number of cases of

fungal infection. The prevalence of fungal infection in the present study was 27%, which was similar to study conducted by Hohenadel et al where fungal etiology accounted for 35% [22]. Among the isolates, 23 were of candida species and 5 were of aspergillus species. These findings are in concordance with a study conducted in patients with malignancy with suspected lung infections, where 23 isolates of candida species and 7 isolates of aspergillus species were demonstrated. Another study conducted in patients with chronic respiratory diseases in Himalayan region had 14 isolates of candida and 13 isolates of aspergillus [23]. *Candida albicans* (37%) was the most frequent isolate in the present study among candida species. This is in agreement with the study conducted by Phukan AC et al which showed 76% isolation of *Candida albicans* [24]. Similar to other studies *Aspergillus fumigatus* was the most common isolate among aspergillus species.[25], [26] The isolation of aspergillus in a healthy person would be contaminant, but population included in the present study already had decreased immunity as they had chronic lung disease and most of them were on steroid treatment for their exacerbations. Hence these cases need early initiation of antifungal treatment.

Available studies on fluconazole susceptibility of *Candida albicans* isolates from India have showed either no resistance or a very low percentage of resistance. The present study showed 100% susceptibility to fluconazole. The present study has recorded high resistance to amphotericin B (75%), which correlated with the study conducted by Changdeo S Aher which showed high resistance to amphotericin B in *C. tropicalis* and *C. glabrata* [27]. Emergence of azole resistance in aspergillus is demonstrated in various studies. Voriconazole is preferred therapy for invasive aspergillosis, high susceptibility to voriconazole in our study is a positive factor for the patients which correlated with studies conducted by Snelders et al and Denning DW et al [28], [29] There is emergence of multidrug resistant strains of fungus at an alarming rate. Hence it is necessary to determine the antifungal susceptibility to decrease the morbidity and mortality and also prevent the emergence of drug resistance to higher drugs. The limitation of the study were small sample size limits the generalization, Outcome of all the patients studied could not be monitored. Anaerobic organisms and all antibiotic groups could not be studied because of technical limitations.

Conclusion

Delays in initiation of antibiotic treatment may lead to poor outcomes. There is a risk of emergence of MDR pathogens with inadequate, inappropriate antibiotic treatment. To initiate an empiric antimicrobial therapy we should have the knowledge of microbial flora of the locality and their sensitivity and resistance patterns, such information needs to be analysed periodically and institution based antibiotic policies formed from time to time and made available to all consultants treating infectious diseases. Hospital antibiograms are an important component of detecting and monitoring trends in antimicrobial resistance. It would be ideal, through multicenter studies, to generate nationwide or more appropriately region-specific antibiograms,

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