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Research Article

A STUDY ON BACTERIAL ISOLATES FROM BRONCHOALVEOLAR LAVAGE (BAL) FLUID OBTAINED FROM PATIENTS WITH PULMONARY INFECTIONS –IN TERTIARY CARE HOSPITAL, HYDERABAD

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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.

Materials and Methods: BAL fluid samples of patients with chronic respiratory diseases undergoing bronchoscopy in Deccan College of medical sciences and Owaisi Hospital were collected under aseptic precautions.

Results: A total of 100 BAL fluid samples were studied which met the quality control criteria. Among these 60 were from male patients and 40 were from female patients. Among 100 BAL sample cultured for bacterial aetiology, Of the 100 samples processed 58 (58%) were positive for bacterial growth. Among the 58 bacterial isolates which were obtained the predominant organism was Klebsiella pneumoniae 28 (48%) followed by Acinetobacter baumannii 12 (20.6%), Pseudomonas aeruginosa 8 (13.7%), Escherichia coli 2 (3.4%), Enterobacter sps 1 (1.7%), Staphylococcus aureus 4 (6.8%), Streptococcus pneumoniae 3 (5.1%). The highest no. of isolates were in the age group of 51-60yrs followed by 61-70yrs, least no. of isolates were in the age group 18-30yrs.

Conclusion: Bronchoalveolar lavage has improved sensitivity and specificity in diagnosis of pulmonary infections.

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INTRODUCTION

Pulmonary infections (Lower Respiratory Tract infections) are among the most common infectious diseases of humans worldwide [1]. Pulmonary infections may be defined as those infections presenting with symptoms such as cough with expectoration, dyspnea, wheeze, chest pain/discomfort to potentially life threatening infections usually for period ranging from 1-3 weeks [3-4]. Pulmonary infections are a persistent and a pervasive health problem which impose an enormous burden on society. These bring common reasons for consultation and hospitalization. Patients present with self-limiting illnesses to potential life threatening infections [5].

Respiratory Tract Infection (RTI) is by large one of the leading causes of the morbidity and mortality in the world. By terminology, RTI is a term assigned not to a single disease, but to a spectrum of infections, each with a different epidemiology, clinical presentation, pathogenesis and prognosis. The etiology, signs and symptoms of respiratory diseases vary with age, sex,

season, the type of population at risk and various other factors. These are commonly the first infection to occur post birth and pneumonia is quite often the final illness to occur before death [1-2].

The bacteriological profile of pulmonary infections varies within the same country, with time due to differences in the frequency of use of antibiotics, environmental factors, and ventilation in the critically ill patients. Likewise, an expanded variety of emerging pathogens provide challenges for the microbiology laboratory [6].

It has been reported clinical microbiologists in diagnostic laboratories have a critical role to play in the diagnosis and management of LRTI as overtreatment of acute uncomplicated pulmonary infections led to unparalleled levels of multi drug resistance among pathogens [7].

Since the etiological agents of pulmonary infections (LRTI) cannot be determined clinically, microbial investigation is

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required for both treatment and management of individual case and for epidemiological purposes [8].

Broncho alveolar lavage (BAL) is an ideal sample that allows the recovery of pathogens cellular and non-cellular components from the epithelial surface of lower respiratory tract. It is increasingly utilized as diagnostic tool though in the past it remained as investigative and research tool. As the Sputum culture yields diagnosis in fewer than 50% of patients with pulmonary infections. Early diagnosis and proper choice of antimicrobials is crucial for management of these patients. The advent of bronchoscopy and quantitative analysis of BAL have improved sensitivity and specificity in diagnosis of pulmonary infections. The study focused on aerobic bacteriological analysis of BAL fluid in patients of pulmonary infections presenting with a cough, chest pain, fever and acute infiltrate on chest radiograph. Subjects who are critically ill on mechanical ventilation.

The main objective of this study was to identify the bacterial flora, the common causative bacterial agents among patients with pulmonary infections in our hospital.

MATERIALS AND METHODS

The study was carried out at the Department of Microbiology, Deccan College of Medical Sciences, and Hyderabad.

Study Period

January 2015 to June 2016 (18 Months).

METHODOLOGY

The Research protocol was approved by Institutional Review Board and Institutional Ethical Committee. After taking the consent clinical history of patient was obtained in all cases.

Subjects

Between 18 years to 70 years of age, from the inpatients (Medicine & Respiratory units) ICUs and outpatients (day care procedure patients) which comprise of both males and females.

Inclusion Criteria

Patients having at least two of the following symptoms.

1. Fever above 37°C, Cough, Production of purulent sputum, breathing difficulty, in association with physical findings suggestive of consolidation, Chest pain and Leukocytosis (W.B.C > 11,500/cumm).
2. Patients in whom clinical examination and routine laboratory findings could not clinch the diagnosis
3. Patients not responding to empirical treatment

Exclusion Criteria

1. Patients with HIV positive
2. Patients with fungal infections
3. Patients with cardiac diseases and those patients receiving Immunosuppressive therapy.

Sample Collection

BAL fluid samples were collected from patients suffering from LRTI after giving instructions to the patients regarding bronchoscopic procedure. Patients who were critically ill

having endotracheal intubation, BAL fluid was collected using Metra's catheter (miniBal).

Bronchoscopic procedure

Site of lavage

The site of lavage depends on the localization of the abnormalities, an infection with radio graphically apparent infiltrate or suspected malignancy of the involved segment.

In patients with diffuse lung diseases the middle lobe or lingual lobes were commonly lavaged sites. Since anatomically this is the most accessible site and the fluid obtained at one site is representative of the whole lung in diffuse lung disease, using this method approximately (1.5 to 3%) of the lung (10⁵ alveoli) are sampled.

Fluid Used

Usually the lavage is performed using sterile saline (0.9% NaCl) preferable at 37 degrees centigrade to help prevent cough. Or saline at room temperature can also be used. The volume of saline instilled varied between 100 to 300 ml [9-10].

Fluid instillation and recovery

The fibre optic bronchoscope is wedged into a sub segmental bronchus. The fluid instilled through the bronchoscope is almost immediately recovered by applying suction (25-100) mm hg. Fluid was collected in 2 to 3 aliquots usually 2nd aliquot was preferred for microbiological examination [11, 12].

Microscopy

Gram stained smears of BAL fluid were examined to access the quality of sample to detect predominant morphotypes and to differentiate gram positive from gram negative bacteria. Samples showing less than 10 squamous epithelial cells and more than 25 leucocytes or pus cells per low power field, indicates good quality of the specimen. All samples were subjected for Ziehl Neelsen (Z-N) staining for acid fast bacilli.

Laboratory processing of BAL fluid

BAL fluid is processed immediately upon arrival at the laboratory and is vortexed and was checked for quality control criteria such as, It is rejected if one of following criteria is observed:

1. Volume less than 20 ml.
2. Presence of >10 squamous epithelial cells/low power field.
3. Presence of extensive amount of debris.

Samples under study

A total of 100 BAL fluid samples were included in the study which met the above quality control criteria.

Culture of the specimen

BAL fluid samples that were satisfactory by microscopic examination were subjected for quantitative culture using a 4 mm loop (10 micro litre). Each sample was inoculated on Blood agar, MacConkey agar and chocolate agar. For isolation of *S. pneumoniae*, 5% Sheep Blood Agar with optochin disk were incubated in 5% CO₂ rich environment using candle jar at 35°C for 24-48hrs [13]. For isolation of *H. influenzae*,

chocolate agar with a streak of *S. aureus* was incubated in 5% CO₂ rich environment using candle jar at 35° C for 24-72 hours. In case if the isolate was suspected to be *H. influenzae*, a lawn of the test organism is streaked onto Blood Agar with impregnated disks (X, V) are placed directly on the confluent inoculation, at least 4 to 5 cm apart and incubated at 35°- 37°C for 24-48 hours. The organisms will grow only around the disk that provides the appropriate factor for growth of the organism [14].

Identification

After 24 hour incubation, the plates were observed for the following morphological characters growth, size, shape of the colony, elevation, odor, pigmentation and haemolysis; the colonies were counted for threshold. And the colonies were counted and multiplied by 100 as 0.01 ml of BAL fluid was used for inoculation and the number of CFU per ml is determined Diagnostic threshold for BAL was taken as 10⁴ CFU per ml followed by Microscopy and Gram staining.

The Gram Positive and Gram Negative Bacterial isolates were further identified and speciated by using the set of relevant biochemical reactions as per standard reference. The following biochemical tests were put up for identification of Gram Positive isolates.-Catalase Test, Coagulase test-(Slide and Tube method), Optochin Disc test, Bile solubility test, O.F Sugars (Oxidative Fermentative Sugars). Mannitol Fermentation tests. The following tests were performed for the Gram Negative isolates- Catalase test, Hanging Drop test for Motility, Oxidase test. IMViC Reaction (Indole test, Methyl red test, Voges Proskauer test, Citrate test) and Urease test. Sugar Fermentation tests using 1% primary sugars & TSI [15].

RESULTS

A total of 100 BAL fluid samples were studied which met the quality control criteria. Among these 60 were from male patients and 40 were from female patients. The maximum numbers of patients were in the age group between 51-60 years, followed by the age group 61-70 years. Least number of patients belonged to age group between 18-30 years of age.

Age and sex distribution of the subjects included in the study shown in Table-1

S. No	Age group (in years)	Male	Female	Total
1.	18-30	6	8	14
2.	31-40	8	10	18
3.	41-50	6	6	12
4.	51-60	28	8	36
5.	61-70	12	8	20

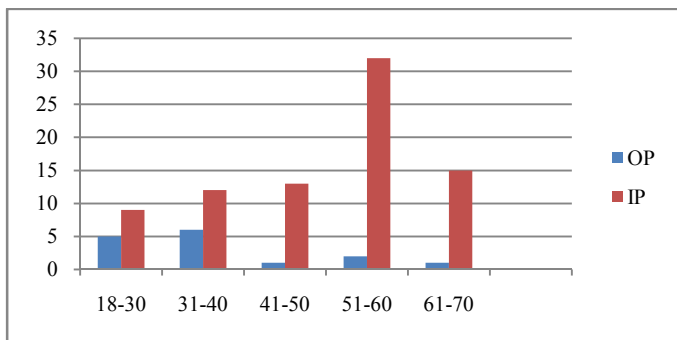


Fig 1 Distribution of patients (Day care & Inpatients) enrolled in the study

15 Patients were stable following bronchoscopic procedure that were discharged on the same day and were considered as out patients (O.P) & remaining 85 were Inpatients

Table 2 Spectrum of bacterial isolates from BAL fluids

S.No	Isolates	No. of isolates	Percentage
1.	Klebsiella pneumoniae	28	48.2%
2.	Acinetobacter baumannii	12	20.6%
3.	Pseudomonas aeruginosa	8	13.7%
4.	Escherichia coli	2	3.4%
5.	Enterobacter sps	1	1.7%
6.	Staphylococcus aureus	4	6.8%
7.	Streptococcus pneumoniae	3	5.1%

Of the 100 samples processed 58 were positive for bacterial growth. Among the 58 bacterial isolates which were obtained the predominant organism was Klebsiella pneumoniae 28 (48%) followed by Acinetobacter baumannii 12 (20.6%), Pseudomonas aeruginosa 8 (13.7%), Escherichia coli 2 (3.4%), Enterobacter sps 1 (1.7%), Staphylococcus aureus 4 (6.8%), Streptococcus pneumoniae 3 (5.1%).

Table 3 Occurrence of bacterial isolates in different age groups under study

S.No	Bacteria	18-30 Yrs (n=14)	31-40 Yrs (n=18)	41-50 Yrs (n=12)	51-60 Yrs (n=36)	61-70 Yrs (n=20)	Total
1.	Klebsiella pneumoniae	3(21.4%)	5(27.7%)	4(33.3%)	10(27.7%)	6(30%)	28
2.	Acinetobacter baumannii	2(14.2%)	3(16.6%)	2(16.6%)	2(5.5%)	3(15%)	12
3.	Pseudomonas aeruginosa	3(21.4%)	1(5.5%)	0	3(8.3%)	1(5%)	8
4.	Escherichia coli	0	2(11.1%)	0	0	0	2
5.	Enterobacter sps	0	0	0	0	1(5%)	1
6.	Staphylococcus aureus	0	0	0	1(2.7%)	3(15%)	4
7.	Streptococcus pneumoniae	0	0	0	1(2.7%)	2(10%)	3

The highest no. of isolates were in the age group of 51-60yrs followed by 61-70yrs, least no. of isolates were in the age group 18-30yrs.

Distribution of co-morbid conditions

Table 4 Positive Culture isolates in relation to co morbid conditions

S. No	Co- morbid condition	Total No. of cases	Positive culture (%)
1.	Diabetes Mellitus	24	18(75%)
2.	Hypertension	22	15(68.8%)
3.	Bronchial asthma	08	3(37.5%)
4.	COPD	05	2(40%)

The comorbidities seen in study population were Diabetes Mellitus (24%), hypertension 22%), bronchial asthma (8%), C.O.P.D ((5%). Chest radiographs of subjects showed features suggestive of consolidation bilateral (42%), unilateral (50%).

Table 5 Distribution pattern of isolates from BAL fluid samples obtained by bronchoscopic method and METRAS technique (minibal)

Bacterial isolates	Total isolates	BAL fluid isolates by bronchoscopy	BAL fluid isolates by metras technique
Klebsiella pneumonia	28	18	10
Acinetobacter baumannii	12	5	7
Pseudomonas aeruginosa	8	5	3
Ecoli	2	1	1
Enterobacter	1	-	1
Staphylococcus aureus	4	4	-
Streptococcus pneumonia	3	3	-
Total	58	36	22

The empirical antibiotic therapy received by the subjects prior to bronchoscopy included in the study. Ceftriaxone-61%, Augmentin 32%, Azithromycin 45%, Levofloxacin 10%, Amikacin 20%. Two antibiotics were prescribed to 74% of subjects out of which 82% received cephalosporin in combination with other antibiotic.

DISCUSSION

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 agents causing infections in patients with chronic respiratory disease. The likelihood of diagnosis of Pneumonia is high when fever, Leucocytosis & purulent tracheal secretions develop in association with an abnormal chest radiograph [16]. However symptoms suggesting pneumonia may be muted in debilitated and elderly patients and a variety of other non infectious conditions may mimic Pneumonia. Clinical findings alone, then, are not sufficient for definitive diagnosis. A variety of invasive and non invasive tests have been proposed as guides for diagnosis and treatment. Lack of specificity in clinical findings and the poor reproducibility of chest radiography warrant the performance of additional procedures, such as culture of specimens from the lower respiratory tract.

The present study was conducted to find the aerobic bacterial etiology in LRTI patients with a prospective of evaluating whose BAL fluid was submitted to the lab & their diagnosis couldn't be clinched by clinical, radio graphical, sputum examination by smear & culture. Maximum number of patients belongs to the age group of 51-60yrs. Our present study correlated with previous findings conducted by Merino-Sánchez *et al.*[4]. This is possible due to the more number of pneumonia cases was observed with the increasing age, and use of inhalational steroids. As it lowers the host defence & pay route for microbial colonization.

Broncho alveolar lavage of lung sub segment samples a large area of alveolar surface and sensitive specimen in diagnosing pulmonary infections.

It has double advantage of being appropriate for all microbiologic procedures and to perform multiple tests. BAL provided a useful tool for diagnosis of LRTI's. Fluid collected varied from 10-100ml depending on the technique used (bronchoscopic or metras technique). Quality of specimen (BAL fluid) was evaluated based on Gram stain finding, followed by quantitative culture & susceptibility testing.

In 48% of smears the organism observed could be correlated, out of 58% of BAL fluid samples positive for culture. In 10% cases organisms could not be observed that could be due to paucity in the number of bacteria due to prior antibiotic usage. According to the study by JC Bhatia smear examination was found to have 83% sensitivity as compared to culture, false negative smear examination could be due to concomitant antibiotic use & such patients have to continue antibiotics till the culture results are obtained [17].

Among the 100 BAL fluid specimens subjected for Z-N smear examination 5% were positive for AFB though these were

negative for sputum smear examination (Z-N technique). This indicated BAL fluid specimen provided better results in sputum smear negative pulmonary cases which is in accordance with the study done by Cunha *et al* [18].

In the present study, 58% of bacterial isolates were recovered from 100 BAL fluid samples which were subjected to quantitative culture (> 10,000CFU/ ml). Quantitative culture technique was adopted to differentiate colonization from infection [19].

Of the 58 isolates recovered 7 were gram positive & remaining 51 were gram negative isolates, the less number of gram positive isolates could be due to broad spectrum empirical antibiotic therapy and inhibitory affect of solution (normal saline or ringer lactate) used in bronchoscopy & specimen transport. As per the recorded history of patients in the proforma all were on prior antibiotic therapy, the most common combination therapy which they received was 3rd generation cephalosporin with aminoglycoside & many individuals were from treatment failure group, this could be the reason for isolation of many gram negative isolates 51(n). Our study showed predominance of Gram negative bacterial cause (87.93%) among the RTI's. A similar finding was observed by a recent study from Nepal by SK Mishra *et al* who reported 84.1% occurrence. Incidentally, the same study reported Haemophilus influenza (21%) as the most common Gram negative bacterium isolated in contrast to our study, which showed Klebsiella pneumonia (41.66%) as predominant Gram negative isolate [1]. In their study, SK Mishra *et al* stated Streptococcus pneumoniae as the predominant Gram positive bacterial (8.6%) cause in contrast to the our study, which showed Staphylococcus aureus (12.86%) as predominant Gram positive bacterial cause[1]. Among other notable studies, Kaul *et al.* [23] and Akingbade OA [24] too found Klebsiella pneumonia as predominant pathogen which resonated with our study which showed similar findings.

A study published by GC Bhatia *et al*, showed overall isolation rate of 51%. 38% [17]. In other study, occurrence of pathogens were reported by S. Radha *et al* in 2014 [20]. A study by Vivek KU *et al* showed an isolation rate of 42%, which is low when compared to the present study [8]. This variable percentage could be due to technical factors such as specimen collection methods, transport, media, adequacy of incubation, antibiotics & other toxic components and the calculation of thresholds which affect the recovery of pathogens [22].

CONCLUSION

However, the main objective of the study was to identify the microbiologic profile of the BAL fluid isolated from the pulmonary infections. Results of the present study clearly demonstrated the predominance of gram negative bacteria. Regular anti-microbial susceptibility monitoring is essential for local, regional and national level isolates. This would help and guide the physicians in prescribing the right combinations of anti-microbial to limit and prevent the emergence of multi-drug resistant strains of Bacteria.

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Conflict of Interest: Nil

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