

Original Research Article

A study on identification of common aerobic bacterial isolates and their sensitivity pattern from bronchoalveolar lavage in patients with lower respiratory tract infections in a tertiary care hospital

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Abstract: Broncho alveolar lavage is a deeper sampling and has considerable value in diagnosing pulmonary infections. Antimicrobial susceptibility tests are mandatory to monitor the efficiency of available antimicrobial agents and the emergence of drug resistance among bacterial isolates. The aim of the study is to isolate and identify the common aerobic bacterial agents in broncho alveolar lavage and to evaluate the sensitivity pattern of all the bacterial agents. Grams staining, acid fast staining and culture was done for all the samples. All the isolates were identified by biochemical reactions. Antimicrobial susceptibility testing was done. The isolates were screened and confirmed for ESBL production. 87.1% of isolates were gram negative bacilli with *Klebsiella spp.* as the most common isolates (33.3%). 17% of the gram negative isolates was ESBL producers and 66.7% of staphylococcus aureus was MRSA. 11% samples were found to be positive for Acid Fast Bacilli. Precise identification of the causative organisms and timely institution of appropriate antimicrobial therapy based on the prevailing sensitivity pattern of the bacterial isolates could reduce the morbidity and mortality of lower respiratory tract infections.

Keywords: Klebsiella Spp. Antimicrobial Therapy, Bronchoalveolar Lavage

INTRODUCTION:

Lower respiratory tract infections are the major cause of morbidity and mortality worldwide. They remain the leading cause of deaths among all infectious diseases and they account for 3.9 million deaths worldwide and 6.9% of all deaths [1]. Although rapid determination of the etiologic agents is of paramount importance in managing respiratory infections, the responsible pathogens are not determined in 50% of patients despite extensive diagnostic tests [2]. The specimens used for the diagnosis of lower respiratory tract infections can be sputum, endotracheal aspirate, trans tracheal aspirations, bronchoscopy specimens, lung puncture or biopsy. Though many specimens are available for the diagnosis of LRI, Broncho alveolar lavage is a deeper sampling of desquamated host cells and secretions and now reported to have considerable value in diagnosing pulmonary infections. The value of this technique in conjunction with quantitative cultures for the diagnosis of most of the major respiratory tract pathogens, including bacterial pneumonia, has been documented [3, 4]. Direct microscopic evaluation of

smears provides immediate information about the causative organism and is helpful in starting antimicrobial therapy, but culture of the microbial pathogens is considered to be the gold standard. Antimicrobial susceptibility tests are mandatory to monitor the efficiency of available antimicrobial agents and the emergence of drug resistance among bacterial isolates. Considering the importance of bronchoalveolar lavage in the diagnosis of lower respiratory tract infections, the present study was conducted to identify the common aerobic bacterial isolates and their antimicrobial susceptibility profile in patients with lower respiratory tract infections attending a tertiary care hospital in Chennai.

AIM OF THE STUDY:

To isolate and identify the common aerobic bacterial agents in bronchoalveolar lavage in patients with lower respiratory tract infections. To evaluate the sensitivity and resistance pattern of all the bacterial agents isolated.

MATERIALS AND METHODS:

This is a cross sectional study undertaken over a period of one year from May 2015 to April 2016. Bronchoalveolar lavage samples from patients with lower respiratory tract infections were collected by fiberoptic bronchoscopy. Two sets of samples were taken, centrifuged at 3000rpm for 15 mts and the deposit was processed as follows. 1. First set of sample was used for microscopic examination. a) Gram's stain procedure b) Ziehl-Neelsen staining .2. Second set of sample was inoculated onto solid media like blood agar, chocolate agar and MacConkey agar and incubated at 37°C for 24 hrs. Bacterial isolates were identified by means of Gram's staining, motility and biochemical reactions by standard microbiological techniques as recommended by Clinical and Laboratory Standards Institute (CLSI). The isolates were subjected to antibiotic sensitivity by the Kirby-Bauer's Disc Diffusion technique on Mueller Hinton agar plates as recommended by CLSI. Peptone water culture of the bacterial isolates corresponding to 0.5 McFarland's turbidity was used as inoculum. The entire dried agar surface was evenly streaked in three different directions with a sterile cotton swab dipped into the inoculum [5]. Commercial Hi-Media Antibiotic discs were used. Maximum six antibiotic discs were used for each 9cm diameter petridish. These plates were incubated at 37°C for 16–18 hours in ambient air. The diameters of zones of inhibition were interpreted according to CLSI standards [6] for each organism. Media and discs were tested for quality control using standard strains. The antibiotic discs used for gram negative bacilli were Amikacin 30µg, Amoxicillin/clavulanic acid 20/10µg, Ceftazidime 30 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Cotrimoxazole 1.25/23.75 µg, Gentamicin 10 µg and Imipenem 10 µg. The antibiotic discs used for Gram Positive Cocci were Amikacin 30 µg, Amoxicillin/clavulanic acid 20/10 µg, Ampicillin 10 µg, Ceftriaxone 30 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Cotrimoxazole 1.25/23.75 µg, Erythromycin 15µg, Oxacillin 1µg and Vancomycin 30 µg. MIC was performed with Oxacillin by broth microdilution method for *Staphylococcus aureus* isolates to detect Methicillin Resistant *Staphylococcus aureus* (MRSA). Screening was done for ESBL strains with Cefotaxime. According to CLSI guidelines, strains

showing zones of inhibition ≥ 27 mm for cefotaxime were selected for conformational tests of ESBL. ESBL confirmatory test was done with Double Disc Synergy test (DDST) and Phenotypic Confirmatory Disc Diffusion Test (PCDDT) [7] according to CLSI guidelines.

RESULTS:

Among the 100 patients, 67% of the cases with lower respiratory tract involvement were males and 33% were females. 52% of cases were in the 41-60 years age group and 23% of cases in the 21-40 years of age. Pneumonia (33%) was the most common LRTI evaluated by bronchoalveolar lavage sampling, followed by lung abscess (16%) and tumours (15%). Gram staining was done as screening test for all the 100 samples and among them 47 smears showed positivity for the organisms. Gram's staining showed sensitivity of 89.5% and specificity of 83%. Out of the total 100 samples processed, 54 bacterial agents were isolated both as pure and mixed growth from the patients with lower respiratory tract infection. The organisms isolated were *Klebsiella pneumoniae* 12 isolates (22.2%), *Klebsiella oxytoca* 6 isolates (11.1%), *Pseudomonas aeruginosa* 9 isolates (16.7%), *Acinetobacter spp.* 9 isolates (16.7%), *Staphylococcus aureus* 6 isolates (11.1%), *Escherichia coli* 5 isolates (9.3%), *Proteus vulgaris* 4 isolates (7.4%), *Streptococcus pneumoniae* 1 isolate (1.9%), *Proteus mirabilis* 1 isolate (1.9%) and *Citrobacter koseri* 1 isolate (1.9%). Among the 54 bacterial cultures, 47 (87.1%) were gram negative bacilli and 7 (12.9%) were gram positive cocci. Majority of the isolated agents were gram negative bacilli (87.1%). *Klebsiella spp.* were the most common bacterial isolates in bronchoalveolar lavage accounting for 33.3% of infection followed by *Pseudomonas aeruginosa* and *Acinetobacter spp.* (16.7%). Acid fast staining was done for all the 100 samples, and among them 11(11%) samples were found to be positive for Acid Fast Bacilli. The one isolate of *Strep.pneumoniae* was sensitive to all the antibiotics. Among the six isolates of *Staphylococcus aureus*, 4 isolates (66.7%) were methicillin resistant and 2 isolates (33.3%) were methicillin resistant. All the *Staphylococcus aureus* were sensitive to vancomycin.

Table-1: Antibacterial Sensitivity Pattern of Gram-Negative Organisms

Organism	Ampicillin	Cotrimoxazole	Cefotaxime	Amikacin	Ceftazidime	Gentamicin	Ciprofloxacin	Imipenem
<i>Klebsiella pneumoniae</i> (n=12)	3(25%)	6(50%)	7(58.3%)	10(83.3%)	8(66.7%)	9(75%)	11(91.6%)	12(100%)
<i>Klebsiella oxytoca</i> (n=6)	3(50%)	2(33.3%)	3(50%)	5(83.3%)	4(66.7%)	3(50%)	4(66.7%)	6(100%)
<i>Pseudomonas aeruginosa</i> (n=9)	2(22.2%)	3(33.3%)	5(55.6%)	8(88.9%)	7(77.8%)	6(66.7%)	8(88.9%)	9(100%)
<i>Acinetobacter spp.</i> (n=9)	6(66.7%)	8(88.9%)	6(66.7%)	9(100%)	8(88.9%)	8(88.9%)	8(88.9%)	9(100%)
<i>Escherichia coli</i> (n=5)	3(60%)	4(80%)	4(80%)	5(100%)	5(100%)	5(100%)	3(60%)	5(100%)
<i>Proteus vulgaris</i> (n=6)	4(66.7%)	5(83.3%)	5(83.3%)	6(100%)	5(83.3%)	4(66.7%)	4(66.7%)	6(100%)
<i>Proteus mirabilis</i> (n=1)	1(100%)	0	1(100%)	1(100%)	1(100%)	0	0	1(100%)
<i>Citrobacter koseri</i> (n=1)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	0	0	1(100%)

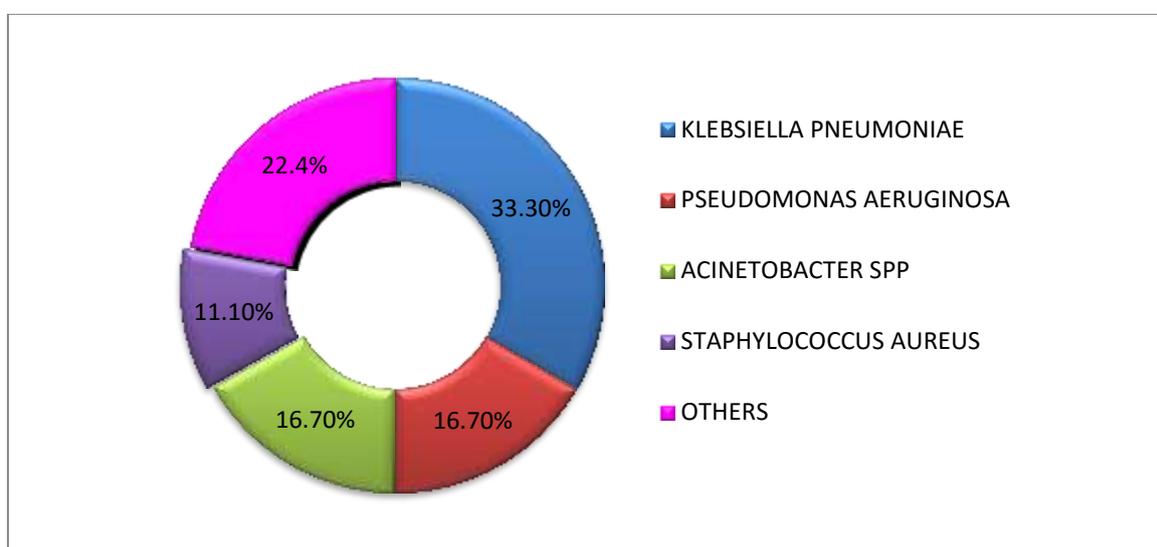


Fig-1: Bacterial Isolates

All the Gram Negative Isolates were sensitive to Imipenem. All the isolates were subjected to screening test for ESBL. Five isolates of *Klebsiella pneumoniae* (42%), three isolates of *Klebsiella oxytoca* (50%), one of *Escherichia coli* (20%) and one of *Proteus vulgaris* (25%) were resistant to third generation cephalosporins. 10 isolates were selected by screening test and subjected for confirmatory tests for ESBL. 8 isolates were confirmed as ESBL Producers by PCDDT.

DISCUSSION:

In this study the occurrence of lower respiratory tract pathology was found to be more common in males (67%) than females (33%). This is similar to the study by Karen C.Caroll *et al.*; in 2002, which revealed 62% cases in males.[8] Similar findings

were observed in the study by Reimer *et al.*; and Leatherman *et al.*; with 65% and 70% of predominance in males respectively [9,10]. The distribution of cases was found to be more common in the age group of 41-60 years (52%) in this study. This observation correlates with the study by Karen *et al.*; in 2002, who reported higher prevalence among patients more than 40 years of age. The study by Grayston *et al.*; in 1994, also showed a higher prevalence in 41-60 years age group [11]. The most common LRTI that required bronchoalveolar lavage analysis in this study was found to be pneumonia (33%). This was similar to the study by Reimer *et al.*; in 1998 and Kahn *et al.*; which showed higher prevalence of pneumonia among the lower respiratory infections.

In the present study, aerobic gram negative bacilli were found in 87.1% of isolate. The predominant

isolate was *Klebsiella spp.* (33.3%) followed by *Pseudomonas aeruginosa* (16.7%) and *Acinetobacter spp.* (16.7%). Gram positive cocci were found in 12.9% of cases. *Staphylococcus aureus* was the predominant isolate. A study conducted by Crystal *et al.*; in 1996 showed the predominant isolates in bronchoalveolar lavage were gram negative bacilli particularly in hospital acquired infections [12]. Among those, *Klebsiella pneumoniae* was found in 30%. These observations were similar to the study by Bhatia *et al.*; in 2006, where the percentage of *Klebsiella spp.* was 32% [13]. The next common isolates following *Klebsiella spp.* were *Pseudomonas aeruginosa* (16.7%) and *Acinetobacter spp.* (16.7%). This correlates well with the study by Frederick *et al.*; in 1998 [14].

In the present study, all the samples of Bronchoalveolar lavage fluids were screened for *Mycobacterium tuberculosis* by Ziehl Neelsen staining method. Among them, 11 samples (11%) were found to be positive for Acid Fast Bacilli. In a study by Purohit *et al.*; in 2000, an early diagnosis of pulmonary tuberculosis was made in 13% by positive microscopy for AFB on BAL [15]. In another study by Getachew *et al.*; *Mycobacterium tuberculosis* was isolated in 19% sample of BAL [16]. The AntibioGram was performed for all the bacterial isolates in this study by Kirby-Bauer Disc Diffusion Method. All the isolates were 100% sensitive to Imipenem. The next most effective drug was found to be Amikacin with 94% sensitivity. These observations were similar to the studies of Bartlett JG *et al.*; in 2002 and Sharma *et al.*; in 2006, where Imipenem showed 100% sensitivity and Amikacin was effective against 93 to 95% isolates [17, 18].

Out of the 6 strains of *Staphylococcus aureus*, 2 strains (33.3%) showed sensitivity to Oxacillin (1 μ g disc) by disc diffusion method. The other 4 strains (66.7%) of *Staphylococcus aureus* showed resistance to Oxacillin (1 μ g disc) (MRSA). All the 6 isolates of *Staphylococcus aureus* were sensitive to Vancomycin (100%). Among the gram negative bacilli, 42% of *Klebsiella pneumoniae*, 50% of *Klebsiella oxytoca*, 20% of *Escherichia coli* and 25% of *Proteus vulgaris* were found to be Extended Spectrum β Lactamase producers by screening method. By Phenotypic Confirmatory Disc Diffusion Test, 33.3% of *Klebsiella pneumoniae*, 33.3% of *Klebsiella oxytoca*, 20% of *Escherichia coli* and 25% of *Proteus vulgaris* were confirmed as ESBL producers. Jarlier *et al.*; reported that *Klebsiella pneumoniae* (48%) was the most frequent ESBL producing organism followed by *Escherichia coli* (16.8%) in Bronchoalveolar lavage which correlates well with our study. In another study

by Baker *et al.*; in 2006, *Klebsiella pneumoniae* was found to be the most common ESBL producing organism. In evaluating the screening tests for rapid diagnosis of the bacterial in bronchoalveolar lavage, Gram's stain examination of the BAL fluid was analysed. In the present study, the Gram's stain showed sensitivity of 89.5% and specificity of 86%. Prekates *et al.*; in 1998, reported 77% sensitivity and 87% specificity of Gram's stain examination of Bronchoalveolar lavage [19]. In a study by Allaouchiche *et al.*; in 2007, the sensitivity of Gram's stain was 90.2% and specificity was 73.7% [20]. The results of the present study showed the vital role of Gram's stain examination in the diagnosis of Lower respiratory tract infections. Although culturing of microbial pathogens is considered to be the gold standard, direct microscopic evaluation of smears provide immediate information about the etiological agents and aid in early initiation of antimicrobial therapy.

CONCLUSION:

Lower respiratory tract infections are the major cause of morbidity and mortality worldwide. But the etiological agents were not determined in 50% of cases despite extensive diagnostic testings. Nowadays, analysis of Bronchoalveolar lavage plays a definite role in diagnosing pulmonary infections. On analysing the BAL fluid, *Klebsiella spp.* were found to be the most common bacterial isolated in lower respiratory tract infection. From the present study, the vital role of microbiological analysis of BAL fluid is clearly evident since the clinical features alone are not adequate to confirm infections. A simple Gram's stain was highly beneficial as rapid screening test for diagnosis. Antimicrobial susceptibility testing was done for the bacterial isolates. Precise identification of the causative organisms and timely institution of appropriate antimicrobial therapy based on the prevailing sensitivity pattern of the bacterial isolates could reduce the morbidity and mortality of lower respiratory tract infections.

REFERENCES

1. World Health Organization. The World health report: 2004: changing history.
2. Bailey and Scott's: Diagnostic Microbiology page 807
3. Cantral DE, Tape TG, Reed EC, Spurzem JR, Rennard SI, Thompson AB. Quantitative culture of bronchoalveolar lavage fluid for the diagnosis of bacterial pneumonia. The American journal of medicine. 1993 Dec 1; 95(6):601-7.

4. Kahn FW, Jones JM. Analysis of BAL from immunocompromised patients J Clin Microbiol 1988; 26:1150.
5. Mackie & McCartney, Practical Medical Microbiology, Chapter 6, 14th edition, 2007; 125.
6. Clinical Laboratory Standards Institute. (CLSI). Performance standards for antimicrobial susceptibility testing; seventh informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Pennsylvania, USA.2007.
7. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β -lactamases conferring transferable resistance to newer β -lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Review of Infectious Diseases. 1988 Jul 1; 10(4):867-78.
8. Karen C.Caroll et al. lab diagnosis of lower respiratory tract infections, Journal of clinical microbiology sep 2002: 3115-3120.
9. Reimer LG, Carroll KC. Role of the microbiology laboratory in the diagnosis of lower respiratory tract infections. Clinical infectious diseases. 1998 Mar 1; 26(3):742-8.
10. Leatherman JW, Iber C, Davies SF. Cavitation in bacteremic pneumococcal pneumonia. Causal role of mixed infection with anaerobic bacteria. The American review of respiratory disease. 1984 Feb; 129(2):317-21.
11. Grayston JT, Kuo CC, Wang SP, Altman J. A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. New England Journal of Medicine. 1986 Jul 17; 315(3):161-8.
12. Crystal RG, Reynolds HY, Kalica AR. Bronchoalveolar lavage. The report of an international conference. CHEST Journal. 1986 Jul 1; 90(1):122-31.
13. Bhatia, N.Hemvani, D.S.Chitris et al. Role of bronchoalveolar lavage in pulmonary infections. Lung India 2006;23:147-150
14. Frederic Duflo MD, Bernard Allaouchiche Richard Debon, Fabienne Bordet. Anaesthesia & Analgesia, Feb 2001; 92(2): 442-447
15. Purohit SD, Sisodia RS, Gupta PR, Sarkar SK, Sharma TN, Fiberoptic bronchoscopy in the diagnosis of smear negative pulmonary tuberculosis. Lung India 2000;1:143-6
16. Getachew Aderaye, Haimanot G/Egziabher, Abraham Aseffa, Alemayehu Worku, and Lars Lindquist. Comparison of acid-fast stain and culture for Mycobacterium tuberculosis in pre- and post-bronchoscopy sputum and bronchoalveolar lavage in HIV-infected patients with atypical chest X-ray in Ethiopia. Ann Thorac Med. 2007 Oct–Dec; 2(4): 154–157
17. Bartlett JG, Alexander J, Mayhew J, Sullivan-Sigler N, Gorbach SL. Should Fiberoptic Bronchoscopy Aspirates Be Cultured? 1, 2. American Review of Respiratory Disease. 1976 Jul; 114(1):73-8.
18. Sharma SK, Pande JN; Bronchoalveolar lavage; application in pulmonary diseases; India J Chest Dis: 2006;32(3):157-76
19. Prekates A, Nanas S, Argyropoulou A, Margariti G, Kyprianou T, Papagalos E, Paniara O, Roussos C. The diagnostic value of gram stain of bronchoalveolar lavage samples in patients with suspected ventilator-associated pneumonia. Scandinavian journal of infectious diseases. 1998 Jan 1; 30(1):43-7.
20. Allaouchiche B, Jaumain H, Chassard D, Bouletreau P. Gram stain of bronchoalveolar lavage fluid in the early diagnosis of ventilator-associated pneumonia. British journal of anaesthesia. 1999 Dec 1; 83(6):845-9.