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Original Research Article

Aerobic bacteriological analysis of bronchoalveolar lavage fluid in patients with pulmonary infection: a tertiary care hospital study

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ABSTRACT

Background: Respiratory tract infection are an important cause of mortality and morbidity worldwide. The prevalent bacterial agents and their antimicrobial resistance patterns differs, both geographically and over time. Bronchoalveolar lavage has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections. The present study aimed to determine the current aerobic bacterial isolates and their sensitivity pattern obtained from the bronchoalveolar lavage (BAL) fluid of patients with pulmonary infection.
Methods: BAL samples received from the patients of suspected respiratory tract infections over a period of one year, from June 2018 to May 2019 were processed by standard methods for isolation and identification. The antimicrobial susceptibility was done by the Kirby-Bauer disc diffusion method as per the CLSI guidelines.
Results: Out of 322 BAL samples, 84 (26.08%) were found to be culture positive for bacterial isolates. Of those, 44 samples (52.38%) from among males and 40 samples (47.61%) from among females were culture positive .The predominant organism isolated was *Pseudomonas aeruginosa* 46 (54.76%) followed by *Acinetobacter baumanii* 13 (15.47%), *Escherichia coli* 10 (11.90%), *Klebsiella pnuemoniae* 6 (7.14%) *Enterobacter sp* 3 (3.57%), *Staphylococcus aureus* 3 (3.57%), *Enterococcus sp* 2 (2.38%) and *Sphingomonas sp* 1 (1.19%). The Gram-negative organisms showed maximum sensitivity to colistin (100%) while as vancomycin and linezolid were the most effective drugs against Gram positive organisms.
Conclusions: Bronchoalveolar lavage has improved sensitivity and specificity in diagnosis of pulmonary infections. It is important to have an updated local antibiogram for each hospital and regular surveillance and monitoring of antibiotic resistance and the changing patterns of the bacterial pathogens is a must for better patient management.

Keywords: Bronchoalveolar lavage, Respiratory infection, Antimicrobial sensitivity, Bacterial isolates

INTRODUCTION

Chronic respiratory diseases are a group of diseases affecting the airways and other structures of lungs. Chronic respiratory diseases, as per WHO estimates (2004), account for 4 million deaths annually, contributing to 5% of global deaths.[1] The burden of chronic respiratory diseases in terms of Disability Adjusted Life Years (DALY), in 2005 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. (2005).[2]

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] 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*.[2]The bacteriological profile of pulmonary infections varies within the same country due to differences in the frequency of use of antibiotics, environmental factors, and ventilation in the critically ill patients. Also, increasing variety of emerging pathogens provide challenges for the microbiology laboratory.[5] In these patients the high mortality rate of these infections is attributed, in most part, to bacterial etiological agents as well as to the lack of prompt and appropriate access to treatment. Effective antimicrobial therapy depends on the identification of the etiologic agent. It is therefore necessary to obtain the appropriate material for bacteriological diagnosis. 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]

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.[7] It is increasingly utilized as diagnostic tool though in the past it remained as investigative and research tool. Early diagnosis and proper choice of antimicrobials is crucial for management of these patients and as the sputum culture yields diagnosis in fewer than 50% of patients with pulmonary infections. Further if the sputum culture report is inconclusive or the patient shows little response to the antibiotics reported as sensitive the situation gets complicated.

Therefore, in hospitals with bronchoscopy facilities, it is possible to obtain bronchoalveolar lavage (BAL) samples and isolate the specific bacterial pathogen(s) to guide therapy.[6,7] With this study we aimed to do aerobic bacteriological analysis of BAL fluid in patients of pulmonary infections and to know their antibiotic sensitivity profile.

METHODS

The cross-sectional prospective study was conducted in the Department of Microbiology, Government Medical College, Srinagar. The study was carried out during the period from June 2018 to May 2019. The study included 322 BAL samples taken from all consecutive patients referred with suspicion of pneumonia.

Inclusion criteria

Patients with progressive infiltrates on chest roentgenogram 48 hours or more after ICU admission with or without ventilatory support along with fever, purulent secretions, patients in whom clinical examination and routine laboratory findings could not clinch the diagnosis and patients not responding to empirical treatment were included in the study.

Exclusion criteria

Patients with Pulmonary Tuberculosis, Chronic Kidney Disease, Pulmonary oedema, Recent cardiac manifestations were excluded from the study.

Sample collection:

Bronchial wash was done with the help of fibreoptic bronchoscope under local anaesthesia (transtracheal). Around 10-30 mL of sterile normal saline was instilled into the infected lung lobe/ bronchopulmonary segments. Instilled saline was suctioned back and collected into sterile containers. Initial microscopic examination consisted of wet mount and Gram staining to observe the presence of pus cells and epithelial cells, bacteria. Bronchial secretions with less than 10^3 CFU/ml were regarded as commensals or contaminants and were excluded from the study. Collected samples of 322 patients were sent to microbiology laboratory immediately for further processing.

Processing of samples

All BAL samples were cultured on three bacteriological media (Nutrient, Chocolate and MacConkey's) agar plates 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.[8]

Identification and antibiotic susceptibility testing of bacterial isolates

Bacterial isolates were identified by performing standard microbiological procedures such as study of colony morphology, Gram staining and standard biochemical tests.[9] Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results were read and interpreted as per CLSI guidelines.[10]

Statistical analysis

Descriptive statistics was used for analysis. The collected data was entered in MS-Excel and statistical analysis was done using SPSS 17 software and were expressed as percentages.

RESULTS

A total of 322 Bronchoalveolar samples which met the quality control criteria were included in this study. Out of 322 samples, 84 (26.08%) were found to be culture positive for bacterial isolates. Of those, 44 samples (52.38%) from among males and 40 samples (47.61%) from among females were culture positive, thus showing male predominance. The highest isolation rate was

observed in the 50-59 years age group (36.9%) followed by 60-69 age group (22.61%) (Table 1).

Table 1: Age and gender distribution of patients.

Age group	Male No. (%)	Female No. (%)	Total No. (%)
20-29	1 (2.2)	-	1 (1.19)
30-39	4 (9.09)	4 (10)	8 (9.52)
40-49	7 (15.9)	8 (20)	15 (17.8)
50-59	17 (38.6)	14 (35)	31 (36.9)
60-69	10 (22.7)	9 (22.5)	19 (22.61)
>70	5 (11.3)	5 (12.5)	10 (11.90)
Total	44 (50.38)	40 (47.61)	84 (100)

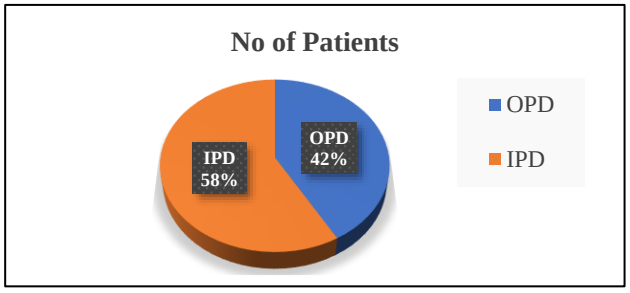

Figure 1: Distribution of patients (day care and Inpatients) enrolled in the study (no= 84).

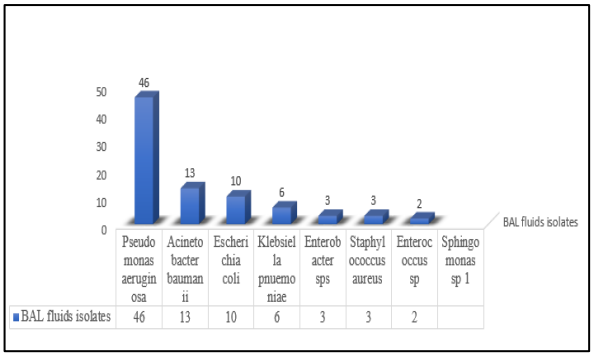

Figure 2: Spectrum of bacterial isolates from BAL fluids.

Maximum patients were inpatients (58.33%) whereas 35 patients (41.61%) were discharged the same day and were considered as outpatients (Figure 1).

Among the 84 bacterial isolates which were obtained, the predominant organism was *Pseudomonas aeruginosa* 46 (54.76%) followed by *Acinetobacter baumanii* 13 (15.47%), *Escherichia coli* 10 (11.90%), *Klebsiella pnuemoniae* 6 (7.14%) *Enterobacter sps* 3 (3.57%), *Staphylococcus aureus* 3 (3.57%), *Enterococcus sp* 2 (2.38%) and *Sphingomonas sp* 1 (1.19%) as shown in Figure 2. The antibiotic sensitivity pattern of gram-negative isolates is provided in Table 2.

Table 2: Antibiotic sensitivity of gram-negative isolates.

Name of Antibiotic	*Pseudomonas aeruginosa*	*Acinetobacter baumanii*	*Escherichia coli*	*Klebsiella pnuemoniae*
Amikacin	60.5%	15.3%	50%	16%
Gentamicin	36%	46.5%	50%	66%
Cefazolin	NT	20.1%	15.7%	15.9%
Ceftazidime	30.4%	NT	20.7%	20.9%
Cefepime	25.5%	15.3%	20.8%	16.7%
Ciprofloxacin	35.5%	35.9%	36.9%	45.6%
Tigecycline	NT	65.4%	85.7%	80.2%
Imipenem	15.4%	16.7%	23.9%	20.9%
Meropenem	11.2%	18.1%	45%	58%
Piperacillin/tazobactum	45.3%	7.6%	34.2%	35.3%
Colistin	100%	100%	100%	100%

Colistin was the most effective drug against all the gram-negative bacteria followed by amikacin, gentamicin and ciprofloxacin. High degree of resistance was seen against cephalosporins and carbapenems. Among gram positive isolates the most effective drugs were vancomycin (100%) and linezolid (100%). *S. aureus* isolates were 100% Methicillin resistant (MRSA).

DISCUSSION

Chronic respiratory diseases represent an important health challenge, both in developing and developed countries because of their frequency and economic impact. Respiratory tract infections are the second most common cause of hospital acquired infections. The etiological agents of Lower respiratory tract infections and their susceptibility patterns vary from area to area and these are a major cause of mortality and morbidity across the globe. Also, clinical findings alone may not be sufficient for definitive diagnosis. A variety of invasive and non-invasive tests have been proposed as guides for diagnosis and treatment. Bronchoalveolar lavage provides a very useful tool for diagnosing lower respiratory tract infections. The aim of this study was to find out the bacterial aetiology and antimicrobial sensitivity patterns in Bronchoalveolar lavage samples of patients with

pulmonary involvement, with a prospective of evaluating the BAL fluids submitted to the lab.

In our study, 84 (26.08%) BAL specimens were found to be culture positive for bacterial isolates. This is in contrast to other studies conducted by Velez et al and Kottmann et al, where the positive yield was 51.6% and 55.8% respectively.[11,12] The lower positivity rate in the present study might be because our study was done in general population, whereas other studies quoted above were done in selected (immunocompromised) patients.

Maximum number of patients were in the age group of more than 50 years in our study. These findings correlate with findings conducted by Merino-Sánchez et al (60%), Mullerova et al (45%).[4,13] This may be possibly due to the greater number of pneumonia cases observed with the increasing age, and use of inhalational steroids which lowers the host defence and paves the way for microbial colonization. The variable percentage could be also due other factors such as specimen collection methods, transport, media, adequacy of incubation, antibiotics etc.

Our study also showed that aerobic gram-negative bacilli (75%) were more frequently isolated than gram positive bacteria. A similar finding was observed by a recent study from Nepal by Mishra et al who reported 84.1% occurrence.[14] Many other studies also found out considerable predominance of gram-negative bacilli among respiratory pathogens.[15-17] The gram negative predominance might partly be due to the unequal distribution of patients with community acquired and hospital acquired infections and also due to the spread of antibiotics resistance in hospital settings. The most common organism isolated in our study was *Pseudomonas aeruginosa* followed by *Acinetobacter baumanii, E.coli* and *Klebsiella pneumoniae*. This is in concordance with the studies of Thomas et al and Salman et al.[18,19] But in some other studies the predominant pathogen was *Klebsiella pneumonia*.[20-22] Against Gram negative bacilli, the most active antibiotic was Colistin (100%) followed by Amikacin, Gentamicin and Piperacillin- tazobactam. High degree of resistance against all the generations of cephalosporins was seen among the gram-negative isolates. The reason for such high a percentage of beta lactam-resistant organisms could be the frequent use of cephalosporins in the empirical antibiotic regimens.

Pseudomonas aeruginosa showed 100% sensitivity to Colistin followed by Amikacin (60.5%) and Piperacillin plus tazobactam (45.3%). *P. aeruginosa* is one of the most important microorganisms in clinical settings which cause problems as a result of its high resistance to antimicrobial agents and therefore it is a dangerous and dreaded bug *P. aeruginosa*, with the profound use of various antibiotics, has changed itself into a stubborn organism, resistant to almost all the antibiotics. *Acinetobacter baumanii* showed 100% sensitivity to colistin, followed by tigecycline (65.4%). In our study Amikacin showed greater activity against majority of the isolates, which was similar to the study made by another investigator.[23] Both *E. coli* and *Klebsiella* isolates from our study showed high degree of resistance to carbapenems and 2nd and 3rd generation cephalosporins. Similar findings were also noted by other researchers.[24,25]

In such cases of highly resistant strains to most of the frequently used broad spectrum antibiotics, Colistin/ Polymyxin B remains the last option for treatment. As such all health care personnel should be trained in proper hygiene techniques and aseptic precautions for all therapeutic and diagnostic procedures done, which can go a long way in preventing nosocomial infections to an extent. Among gram positive organisms *S. aureus* 3.2% was the most common pathogen isolated followed by *Enterococci sp* 2.38%. Gram positive organisms showed highest sensitivity towards vancomycin followed by linezolid. The increasing antibiotic resistance problems, mainly due to wide spread and irrational use of antimicrobial agents in hospitals and community is of great concern, especially in developing countries. Hence it is very necessary that robust measures be adopted. A combined clinical, microbiological and infection control approach which include proper diagnosis, appropriate specimen collection, strict antimicrobial stewardship and hospital infection control should be adopted and stringently implemented.

Limitation

Limitation of the study were anaerobic organisms and all antibiotic groups could not be studied because of technical limitations. Also, small sample size limited the generalization and outcome of all the patients studied could not be monitored.

CONCLUSION

The main aim of the study was to identify the microbiologic profile of the BAL fluid isolated from the pulmonary infections. As was evident, the study demonstrated the predominance of gram-negative bacteria among the BAL isolates. Antibiotic resistance among respiratory bacterial pathogens was an alarming trend. Strict implementation of the concept of ‘antibiotic stewardship’ has become necessary to conserve the already available antibiotics. Proper identification of the probable pathogens and their antibiotic susceptibility pattern can help our health professionals to choose the right antibiotic therapy and improve the outcome.

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REFERENCES

1. WHO. Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2007:5-14.
2. Koul PA. Chronic obstructive pulmonary disease: Indian guidelines and the road ahead. Lung India. 2013;30:175-7.
3. Srivastav P, Kumar P, Nirwan PS, Sharma M. Bacteriological profile and antibiogram pattern of lower respiratory tract infections in a tertiary care hospital in Northen India. IJPRBS. 2013;2(3):225-33.
4. Sánchez MM, Michavila AI, Núñez N, Alvarez LJ. Prognosis in patients with pneumonia and chronic obstructive pulmonary disease. Arch Bronconeumol. 2005;41(11):607-11.
5. Ahmed MS, Jakribettu RP, Meletath SK, Arya B, Vpa S. Lower respiratory tract infections (LRTIS) :An insight into the prevalence and the antibiogram of the Gram negative, respiratory bacterial agents. J Clin Diag Res. 2013;7(2):253-56.
6. Carlos J, Gomes P, Wilson L, Pedreira J, Evangelina MP, Araujo A, et al. Impact of BAL in the management of pneumonia with treatment failure-positivity of BAL culture under antibiotic therapy. Chest. 2000;118:1739-46.
7. Bhatia RS. Bronchoalveolar lavage: indications and present status. Lung India. 1994;12:27-9.
8. York MK, Gilligan P, L Church. Processing and Interpretation of Lower respiratory tract specimens. In Garcia LS, Isenberg HD. Clinical Microbiology Procedure Handbook. 2nd edition. ASM Press, Washington DC. 2007:3-11.
9. Collee, JG, Fraser AG, Marmion BP, Simmons A. McCartney M. Practical medical microbiology. 14th edition. New Delhi, India: Elsevier; 2006.
10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. CLSI Document M100-S25. Wayne PA: Clinical and Laboratory Standards Institute. 2015.
11. Vélez L1, Correa LT, Maya MA, Mejia P, Ortega J, Bedoya V, et al. Diagnostic accuracy of bronchoalveolar lavage samples in immunosuppressed patients with suspected pneumonia: analysis of a protocol. Respir Med. 2007;101(10):2160-7.
12. Kottmann RM, Kelly J, Lyda E, Gurell M, Stalica J, Ormsby W, et al. Bronchoscopy with bronchoalveolar lavage: determinants of yield and impact on management in immunosuppressed patients. Thorax. 2011;66(9):823.
13. Müllerová H, Shukla A, Hawkins A, Quint J. Risk factors for acute exacerbations of COPD in a primary care population: a retrospective observational cohort study. BMJ Open. 2014;4(12):e006171.
14. Mishra SK, Kattel HP, Acharya J, Shah NP, Shah AS, Pokhrel BM, et al. Recent trend of bacterial aetiology of lower respiratory tract infections in a tertiary care center of Nepal. Int J Infect Microbiol. 2012;1(1):3-8.
15. Egbe CA, Ndiokwre1 C, Omoregie R. Microbiology of lower respiratory tract infections in Benin City, Nigeria. Malaysian J Med Sci. 2011;18(2):27-31.
16. Amutha C, Suganthi M, Radhika K, Leela KV, Jayachitra J. Bacterial profile of lower respiratory tract infections in adults and their antibiotic susceptibility pattern with detection of MRSA, ESBLs and MBLs. Int J Curr Microbiol App Sci. 2017;6(3):631-9.
17. Sharma AK, Prakash A, Kumar M. Bacterial profile, antibiotic sensitivity and resistance of lower respiratory tract infections in RIMS, Ranchi: A Tertiary Care Hospital. Int J Med Res Prof. 2016;2(1):149-54.
18. Thomas AM, Jayaprakash C, Amma GMR. The pattern of bacterial pathogens and their antibiotic susceptibility profile from lower respiratory tract specimens in a rural tertiary care centre. J Evolution Med Dent Sci. 2016;5(40):2470-6.
19. Khan S, Singh P, Sachan A. Bacterial etiological agents causing LRTIs and their resistance patterns. Ir Biomed J. 2015;19(4):240-6.
20. Ratna S. Bacteriological profile and antibiotic susceptibility pattern of lower respiratory tract infection in a tertiary hospital in north-east India. Int J Recent Sci Res. 2017;8(9):20337-40.
21. Verma D, Kumar P, Saxena N. Bacteriological profile and antibiogram pattern in lower respiratory tract infection in Kota Region (Raj). Research and Reviews. J Microbiol Virol. 2016;6(2):13-7.
22. Madhavi S, Ramarao MV, Janardhanrao R. Bacterial etiology of acute exacerbations of chronic obstructive pulmonary disease. J Microbiol Biotech Res. 2012;2(3):440-44.
23. Bajpai T, Shrivastav G, Bhatambare GS, Deshmukh AB, Chitnis V. Microbiological profile of lower respiratory tract infections in neurological intensive care unit of tertiary care center from central India. J Basic Clin Pharm. 2013;4(3):51-5.
24. Kumari VHB, Agarathna SN, Chandramuki A. Antimicrobial resistance pattern among Aerobic gram-negative bacilli of lower respiratory tract specimens of intensive care unit in a Neuro centre. Indian J Chest Allied Dis. 2007;49:19-22.
25. Sofianou DC, Constandinidis TC, Yannacou M, Anastasiou H, Sofianos E. Analysis of risk factors for Ventilator associated pneumonia in a multidisciplinary intensive care unit. Eur J Clin Microbiol Infect Dis. 2000;19:460-3.

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