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BACTERIOLOGICAL PROFILE OF BRONCHOALVEOLAR LAVAGE AND THEIR ANTIBIOGRAM BY VITEK-2 IN TERTIARY CARE HOSPITAL, BANGLADESH

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ABSTRACT

Background: Bronchoalveolar lavage (BAL) is a medical diagnostic procedure in which a bronchoscope is passed through the mouth or nose into an appropriate airway in the lung with a measured amount of fluid introduced and then collected for examination. BAL may have an important role in reaching a specific diagnosis, characterizing alveolitis, and monitoring patients during treatment and follow-up. **Methods:** To examine the bacterial etiology of BAL fluids among tertiary care patients in Bangladesh with pulmonary infections, a cross-sectional study was conducted over a period of one year from January to December 2022. A total of 245 BAL fluid samples were examined and antimicrobial susceptibility of isolates were carried out by VITEK® 2 automated culture system. **Result:** Out of the 245 samples, 161 samples were culture positive. Among 6 different isolates (4 Gram-negative and 2 Gram-positive), *Pseudomonas aeruginosa* (49.06 %) was predominant followed by *Klebsiella pneumoniae* (26.70 %). A higher infection rate was seen among males (55.90 %) and in the age group 51-60 years (30.0 %). Colistin was the most effective drug against all the gram-negative bacteria followed by amikacin, imipenem and netilmicin. High degree of resistance was seen against cephalosporins and ciprofloxacin. Among gram positive isolates the most effective drugs were vancomycin (100%) and linezolid (100%). *Staphylococcus aureus* isolates were 100% Methicillin sensitive (MSSA). **Conclusions:** Bronchoalveolar lavage has improved diagnostic efficacy of pulmonary infections. It facilitates the early detection of pulmonary diseases diagnosis and which helps to have an updated local antibiogram for each hospital. Thus, detection of bacterial agents from BAL fluid can be a basis for successful antimicrobial therapy for patients with pulmonary infections.

KEYWORDS: Bacteriological profile, BAL, MDR, Antimicrobial sensitivity, Respiratory infection.

INTRODUCTION

Pulmonary infections are alluded to the lethal infections presenting with symptoms namely expectorant, dyspnea, wheeze, discomfort of chest for the time ranging from 1-3 weeks.[1,2] Viruses and bacteria (75-80%) mostly are the causative agents in these patients. Variance in the use of antibiotics, environmental factors and ventilation in the critically ill patients influence the bacteriological profile of pulmonary infections within the same country. Increasing variety of emerging pathogens also keeping impact in this issue.[3] Which results in antimicrobial resistance in intensive care unit (ICU) and lack of prompt and appropriate access to treatment attributed to the high mortality rate of these patients.

About 20% to 25% patients of intensive care units (ICUs) are frequently affected with lower respiratory tract (LRT) infection resulting in High mortality (22-71%) and morbidity.[4,5]

Contributing factors to the emergence of resistance are mostly inappropriate use of antibiotics in hospitals, community along with severity of illness, length of ICU stays, exposure to invasive devices, increased contact with health care personnel.[6] Which results in spontaneous mutation and acquiring new resistant gene from other species by horizontal transfer.[5,7] Resistance pattern vary from region to region depending on antibiotic use.[8] Unnecessary use of broad-spectrum antibiotics can be controlled by an antibiotic policy thereby decrease the development of resistant strains.[9] Effective antimicrobial therapy depends on the identification of the etiologic agent. It is therefore necessary to obtain the appropriate material for

bacteriological diagnosis. Improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections has been achieved by the advent of bronchoscopy and quantitative invasive techniques like Bronchoalveolar lavage.[10]

Broncho alveolar lavage (BAL) is increasingly utilized as a diagnostic tool. BAL is a consummate sample that allows the recovery of pathogens cellular and non-cellular components from the epithelial surface of lower respiratory tract in which cells and fluids from bronchioles and lung alveoli are extracted for disease diagnosis.[11,12] Broncho alveolar lavage is performed by bronchoscope, the fiber optic wire that is wedged into a bronchus, and sterile saline is pumped in and then withdrawn along with the fluid and cells for analysis.[12] Diagnosis of various diseases like pulmonary infections, pulmonary malignancy, acute respiratory failure, diffuse infiltrative lung disease, occupational lung disease, pediatric lung disease, post-transplant monitoring of the lung allograft, pediatric lung disease, and others can be significantly performed from BAL sample.[13]

The aim of the study was performed to detect pathogenic organisms by microscopy of BAL fluid and isolate and identify various bacteria from BAL fluid in culture and analyze their antibiogram.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, from January 2022 to December 2022.

Sample Collection

Bronchial wash was done with the help of fiberoptic bronchoscope under local anesthesia (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 from 245 patients were sent to microbiology laboratory immediately for further processing.

Processing of samples

All BAL samples were cultured on three bacteriological media (Blood, Chocolate and MacConkey's) agar plates using a sterile 4mm nichrome loop (0.01ml), and incubated at 37^0 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.[14]

Identification and antibiotic susceptibility testing of bacterial isolates

The isolates were tested by Vitek-2 (Vitek 2, bioMérieux, Marcy L'Etoile, France), using VITEK® 2 Gram-Negative identification card (GN) card, VITEK® 2 Gram-Positive identification card (GP) card and VITEK® 2 Gram Negative Susceptibility Card VITEK® 2 AST-N280 and VITEK® 2 AST-GP67. Antimicrobial susceptibility results were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.[15]

2.1. Inclusion criteria

Patients between 18-85 years presenting with symptoms like fever with purulent sputum, breathing difficulty with physical findings suggestive of consolidation.

2.2. Exclusion criteria

Patients with cardiac diseases, and pregnant women.

Statistical analysis

Descriptive statistics was used for analysis. The collected data was entered in MS-Excel and statistical analysis was done using SPSS 22 software and were expressed as percentages.

Table 1: Age and gender distribution of patients n=161.

Age Group	Male No. (%)	Female No. (%)	Total No. (%)
18-30	6 (6.66)	1 (1.4)	7 (4.34)
31-40	12 (13.3)	11 (15.49)	23 (14.28)
41-50	13 (14.4)	14 (19.71)	27 (16.77)
51-60	27 (30.0)	20 (28.16)	47 (29.19)
61-70	23 (25.5)	17 (23.94)	40 (24.84)
>70	9 (10.0)	8 (11.26)	17 (10.55)
Total	90 (55.90)	71 (44.10)	161 (100)

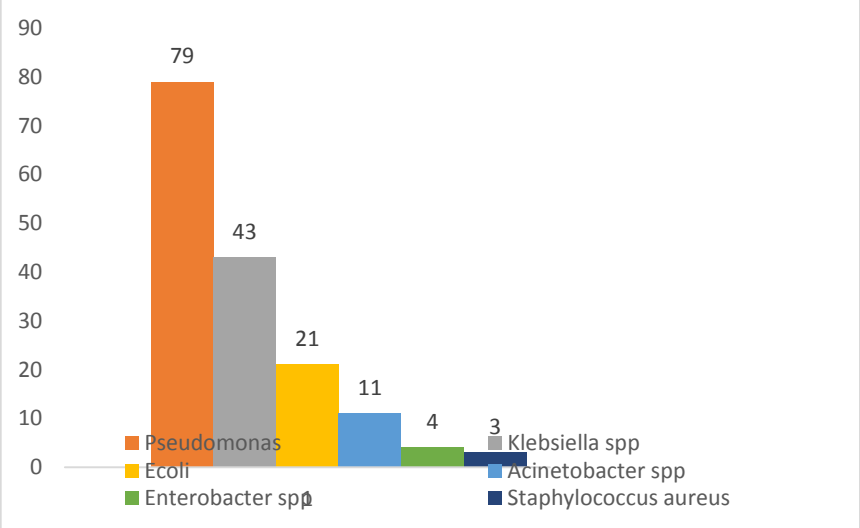

Figure 1: Spectrum of bacterial isolates from BAL fluids n=161

Pseudomonas aeruginosa	79	49.06
Klebsiella pneumoniae	43	26.70
Escherichia coli	21	13.04
Acinetobacter baumannii	11	6.83
Enterobacter cloacae	04	2.48
Staphylococcus aureus	03	1.86

Table II: Antibiotic Sensitivity of Gram-negative organism n=154.

Antibiotics	***Klebsiells spp* n=43**	***Pseudomonas spp* n=79**	***Acinetobacter spp* n=11**	***E coli* n=21**
Ciprofloxacin	44.18	35.44	36.36	38.09
Ceftriaxone	27.90	-	27.27	33.33
Gentamicin	67.44	39.24	54.54	52.38
Cefotaxime	25.58	-	27.27	33.33
Ceftazidime	20.93	-	27.27	28.57
Netilmycin	65.11	65.82	54.54	66.66
Amikacin	81.39	74.68	72.72	71.42
Imipenam	79.06	64.55	72.72	66.66
Cefepime	25.58	-	27.27	33.33
Tazobactum+ Pipercillin	55.81	65.82	45.45	61.90
Colistin	100	100	100	100

RESULT

A total of 245 Bronchoalveolar samples which met the inclusion criteria were included in this study. Age of the patients varied from 18-85 years. Out of 245 samples, 161 (65.71%) were found to be culture positive for bacterial isolates. Of those, 90 samples (55.90%) from among males and 71 samples (44.1%) from among females were culture positive, thus showing male predominance. The highest isolation rate was observed in the 51-60 years age group (29.19%) followed by 61-70 age group (24.84%) (Table 1).

Among the 161 bacterial isolates which were obtained, the predominant organism was *Pseudomonas aeruginosa* 79 (49.06%) followed by *Klebsiella pneumoniae* 43 (26.70%), *Escherichia coli* 21 (13.04%), *Acinetobacter baumannii* 11 (6.83%), *Enterobacter cloacae* 4 (2.48%) and *Staphylococcus aureus* 3 (1.86%) as shown in Figure 1.

The antibiotic sensitivity pattern of gram-negative isolates is provided in Table 2.

Colistin was the most effective drug against all the gram-negative bacteria followed by amikacin, imipenem and netilmicin. High degree of resistance was seen against cephalosporins and ciprofloxacin. Among gram positive isolates the most effective drugs were vancomycin (100%) and linezolid (100%). *Staphylococcus aureus* isolates were 100% Methicillin sensitive (MSSA).

DISCUSSION

Chronic respiratory tract diseases represent an important public health challenge, both in developing and

developed countries; they appear to be more serious when located in the Lower Respiratory Tract. The most common infections seen in the community and among hospitalized patients remains pneumonia. 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.

In our study, 161(65.71%) BAL specimens were found to be culture positive for bacterial isolates. This is in similar to other studies conducted by Velez *et al* and Kottmann *et al*, where the positive yield was 51.6% and 55.8% respectively.[16,17] The higher positivity rate in the present study might be because our study was done in selected (ICU) population, similar to other studies quoted above.

Maximum number of patients were in the age group of more than 51-60 years group in our study followed by 61-70 years age group. These findings correlate with findings conducted by Sánchez *et al* (60%), Mullerova *et al* (45%).[2,18] 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 defense and paves the way for microbial colonization.

Our study also showed that aerobic gram-negative bacilli (98.13%) 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.[19] Many other studies also found out considerable predominance of gram-negative bacilli among respiratory pathogens.[20,21] 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 *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter baumannii*. This is in concordance with the studies of Thomas *et al* and Salman et al.[22,23] But in some other studies the predominant pathogen was *Klebsiella pneumonia.*[24,25]

Against Gram negative bacilli, the most active antibiotic was Colistin (100%) followed by Amikacin, Imipenem, Netilmicin, and Piperacillin- tazobactam which shows similarity with Akter *et al.*[26] 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 (74.68%). *Pseudomonas 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 *Pseudomonas aeruginosa*, with the profound use of various antibiotics, has changed itself into a stubborn organism, resistant to almost all the antibiotics. *Klebsiella pneumoniae* showed 100% sensitivity to colistin, followed by Amikacin (81.39%) and Imipenem (79.06%). In our study Amikacin showed greater activity against majority of the isolates, which was similar to the study made by another investigator.[27]

In cases of Highly resistant strains to most of the frequently used broad spectrum antibiotics, Colistin 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 *Staphylococcus aureus* 1.86% was the most common pathogen isolated which was similar to study of Akter *et al.*[26] 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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ETHICAL MATTERS
Ethical approval was not required to carry out this work as the bacterial isolates were collected as part of routine patient care investigation in the hospital.

CONFLICT OF INTEREST
The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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