

Characterization of Bacterial Isolates from Bronchoalveolar Lavage and Their Antimicrobial Resistance Patterns in a Tertiary Care Hospital in Bangladesh: A VITEK 2-Based Cross-Sectional Study

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ABSTRACT

Background: Lower respiratory tract infections (LRTIs) cause significant morbidity and mortality. Bronchoalveolar lavage (BAL) helps identify pathogens and guide therapy. This study determined the bacteriological profile and antimicrobial resistance patterns of BAL isolates in a Bangladeshi tertiary care hospital. **Methods & Materials:** This retrospective laboratory-based study was conducted at Bangabandhu Sheikh Mujib Medical University, Dhaka, from January 2024 to December 2024. A total of 245 BAL specimens from patients with suspected pulmonary infections were processed. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK 2 automated system and interpreted per CLSI guidelines. **Results:** Of 245 BAL specimens, 161 (65.7%) showed significant bacterial growth. Culture positivity was higher in males (55.9%) and in the 51–60 years age group (29.2%). Gram-negative bacteria comprised 98.1% of isolates. *Pseudomonas aeruginosa* (49.1%) was most common, followed by *Klebsiella pneumoniae* (26.7%), *Escherichia coli* (13.0%), *Acinetobacter baumannii* (6.8%), *Enterobacter cloacae* (2.5%), and *Staphylococcus aureus* (1.9%). Colistin showed 100% susceptibility among Gram-negatives. Amikacin and imipenem were the most active non-polymyxin agents. High resistance was seen to cephalosporins and ciprofloxacin. All *S. aureus* isolates were methicillin-sensitive and fully susceptible to vancomycin and linezolid. **Conclusions:** Gram-negative bacteria, especially *P. aeruginosa*, predominate in BAL isolates from pulmonary infection patients. High antimicrobial resistance highlights the need for routine surveillance, local antibiograms, and antimicrobial stewardship. BAL remains valuable for targeted therapy.

Keywords: Bronchoalveolar lavage; Lower respiratory tract infection; *Pseudomonas aeruginosa*; Antimicrobial resistance; Antibiogram.

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INTRODUCTION

Pulmonary infections are among the leading causes of morbidity and mortality worldwide and commonly present with symptoms such as productive cough, dyspnea, wheezing, and chest discomfort lasting for one to three weeks [1,2]. These infections are predominantly caused by bacterial and viral pathogens, with bacteria accounting for approximately 75–80% of cases. The bacteriological profile of pulmonary infections varies considerably within and across countries due to differences in antibiotic utilization practices, environmental conditions, healthcare infrastructure, and the use of mechanical ventilation among critically ill patients. In addition, the emergence and dissemination of novel and multidrug-resistant pathogens continue to alter the epidemiology of respiratory infections [3].

The growing burden of antimicrobial resistance has become a major challenge in the management of pulmonary infections, particularly in intensive care units (ICUs). Inappropriate or delayed antimicrobial therapy, coupled with increasing resistance among respiratory pathogens, contributes significantly to prolonged hospitalization, treatment failure, and increased mortality.

Lower respiratory tract (LRT) infections represent one of the most common healthcare-associated infections among

critically ill patients, affecting approximately 20–25% of individuals admitted to intensive care units (ICUs). These infections are associated with substantial morbidity and mortality, with reported mortality rates ranging from 22% to 71% [4,5].

The emergence and spread of antimicrobial resistance among respiratory pathogens have become a major global healthcare concern. Several factors contribute to the development of resistance, including inappropriate and excessive antibiotic use in both hospital and community settings, severity of underlying illness, prolonged ICU stay, exposure to invasive medical devices, and increased contact with healthcare personnel [6]. These selective pressures facilitate the development of resistance through spontaneous genetic mutations as well as the acquisition of resistance determinants from other bacterial species via horizontal gene transfer [5,7]. Furthermore, antimicrobial susceptibility patterns vary considerably across different geographical regions, largely reflecting local prescribing practices and antibiotic consumption patterns [8]. The implementation of evidence-based antibiotic stewardship programs and institutional antibiotic policies can help reduce unnecessary use of broad-spectrum antimicrobials and limit the emergence of resistant bacterial strains [9].

Successful management of pulmonary infections relies heavily on the accurate identification of the causative pathogen. Therefore, obtaining appropriate lower respiratory tract specimens for microbiological analysis is essential for establishing a definitive diagnosis. Advances in bronchoscopic procedures and quantitative invasive diagnostic techniques, particularly bronchoalveolar lavage (BAL), have significantly improved the sensitivity and specificity of microbiological diagnosis in pulmonary infections [10].

Bronchoalveolar lavage has increasingly become an important diagnostic modality in respiratory medicine. BAL provides a representative sample from the lower respiratory tract, enabling the recovery of microorganisms as well as cellular and non-cellular components from the epithelial surfaces of the bronchioles and alveoli [11,12]. The procedure is performed using a flexible bronchoscope that is advanced into a selected bronchial segment, where sterile saline is instilled and subsequently aspirated along with cells, microorganisms, and respiratory secretions for laboratory evaluation [12]. BAL specimens play a crucial role in the diagnosis of a wide range of pulmonary conditions, including respiratory infections, pulmonary malignancies, acute respiratory failure, diffuse infiltrative lung diseases, occupational lung disorders, pediatric pulmonary diseases, and post-transplant monitoring of lung allografts [13].

The present study was undertaken to evaluate the bacteriological profile of bronchoalveolar lavage specimens by microscopic examination, culture isolation, and bacterial identification, as well as to determine the antimicrobial susceptibility patterns of the recovered bacterial pathogens.

METHODS & MATERIALS

Study Design and Setting

This retrospective laboratory-based study was conducted in the Department of Microbiology, Bangladesh Medical University (BMU), Dhaka, Bangladesh, between January 2024 and December 2024. Bronchoalveolar lavage (BAL) specimens received during the study period were analyzed to determine the bacteriological profile and antimicrobial susceptibility patterns of respiratory pathogens.

Sample Collection

Bronchoalveolar lavage specimens were collected using a fiberoptic bronchoscope under local anesthesia. Approximately 10–30 mL of sterile normal saline was instilled into the affected bronchopulmonary segment or lobe and subsequently aspirated into sterile collection containers. The recovered lavage fluid was transported immediately to the microbiology laboratory for processing.

An initial microscopic examination was performed using wet mount preparation and Gram staining to assess specimen quality and detect the presence of inflammatory cells, epithelial cells, and microorganisms. BAL specimens yielding bacterial growth below 10^3 colony-forming units (CFU)/mL were considered representative of colonization or contamination and were excluded from further analysis. A total of 245 BAL specimens met the study criteria and were included in the analysis.

Processing of Samples

All BAL specimens were cultured quantitatively using standard microbiological techniques. Briefly, 0.01 mL of each specimen was inoculated onto Blood agar, Chocolate agar, and MacConkey agar using a calibrated 4-mm nichrome loop. In addition, samples were inoculated into Brain Heart Infusion

(BHI) broth to enhance the recovery of bacterial pathogens. Culture plates were incubated aerobically at 37°C and examined periodically for up to 72 hours. For culture-positive specimens, colony-forming units were calculated to determine the quantitative bacterial load [14].

Identification and Antimicrobial Susceptibility Testing

Bacterial isolates recovered from BAL specimens were identified using the automated VITEK 2 Compact system (bioMérieux, Marcy l'Etoile, France). Species identification was performed using VITEK 2 Gram-Negative Identification (GN) cards and VITEK 2 Gram-Positive Identification (GP) cards, according to the manufacturer's instructions.

Antimicrobial susceptibility testing (AST) was carried out using VITEK 2 AST-N280 cards for Gram-negative bacteria and VITEK 2 AST-GP67 cards for Gram-positive bacteria. The antimicrobial susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and breakpoints current at the time of analysis [15].

Inclusion Criteria

Patients aged 18–85 years who underwent bronchoalveolar lavage for suspected lower respiratory tract infection and presented with clinical features suggestive of pulmonary infection, including fever, purulent sputum production, dyspnea, and radiological or physical findings consistent with pulmonary consolidation, were included in the study.

Exclusion Criteria

Patients with known cardiac diseases and pregnant women were excluded from the study.

Statistical Analysis

Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, whereas continuous variables were expressed as mean \pm standard deviation (SD).

Ethical Considerations

The study utilized anonymized laboratory records collected as part of routine clinical care. As no patient identifiers were accessed and no direct patient contact occurred, the requirement for informed consent was waived in accordance with institutional policy and IVI IRB Standard Operating Procedure D-RB-4-003. All data were handled confidentially and analyzed in compliance with applicable ethical standards.

RESULTS

A total of 245 bronchoalveolar lavage (BAL) specimens collected from patients with suspected lower respiratory tract infections were analyzed during the study period. Of these, 161 (65.7%) specimens yielded significant bacterial growth and were included in the final analysis. The age of the patients ranged from 18 to 85 years.

Among the culture-positive cases, 90 (55.9%) were males and 71 (44.1%) were females, resulting in a male-to-female ratio of 1.27:1. The highest proportion of culture-positive patients belonged to the 51–60 years age group (29.2%), followed by the 61–70 years age group (24.8%). Patients aged 18–30 years accounted for the lowest proportion of positive cultures (4.3%). The age and sex distribution of the study population is presented in *Table 1*.

Table I: Age and sex distribution of patients with culture-positive bronchoalveolar lavage specimens (n = 161).

Age group (years)	Male, n (%)	Female, n (%)	Total, n (%)
18–30	6 (6.7)	1 (1.4)	7 (4.3)
31–40	12 (13.3)	11 (15.5)	23 (14.3)
41–50	13 (14.4)	14 (19.7)	27 (16.8)
51–60	27 (30.0)	20 (28.2)	47 (29.2)
61–70	23 (25.6)	17 (23.9)	40 (24.8)
>70	9 (10.0)	8 (11.3)	17 (10.6)
Total	90 (55.9)	71 (44.1)	161 (100)

*Values are presented as number (%).

Distribution of bacterial isolates from BAL specimens

Frequency of bacterial pathogens recovered from culture-positive bronchoalveolar lavage specimens (n=161).

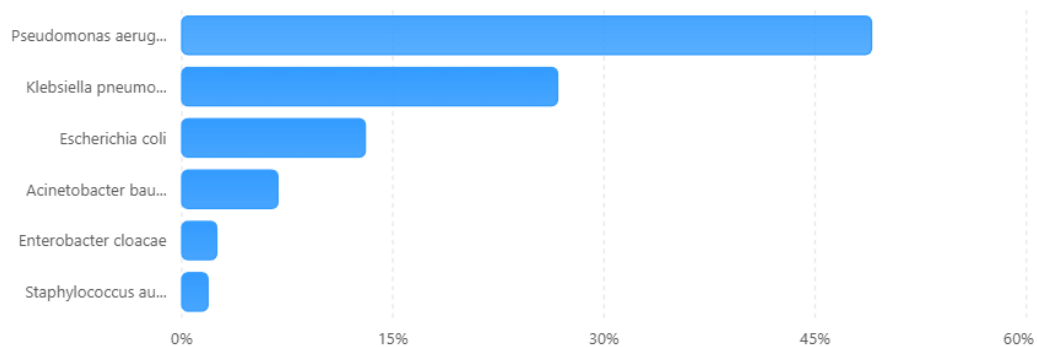


Figure 1: Distribution of bacterial isolates recovered from bronchoalveolar lavage (BAL) specimens.

A total of 161 bacterial isolates were recovered from BAL specimens. Gram-negative bacteria constituted 158 (98.1%) of all isolates, whereas Gram-positive bacteria accounted for only 3 (1.9%) isolates. *Pseudomonas aeruginosa* was the most frequently isolated pathogen, representing 79 (49.1%) isolates, followed by *Klebsiella pneumoniae* 43 (26.7%), *Escherichia coli* 21 (13.0%), and *Acinetobacter baumannii* 11 (6.8%). Less commonly isolated organisms included *Enterobacter cloacae* 4 (2.5%) and *Staphylococcus aureus* 3 (1.9%) (Figure 1).

The antimicrobial susceptibility patterns of Gram-negative isolates are summarized in Table II. Colistin exhibited 100% susceptibility against all Gram-negative pathogens tested, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli*. Among *Klebsiella pneumoniae* isolates, the highest susceptibility rates after colistin were observed for amikacin (81.4%) and imipenem (79.1%), followed by gentamicin (67.4%) and netilmicin (65.1%). Susceptibility to third- and fourth-generation cephalosporins was low, ranging from 20.9% to 27.9%.

For *Pseudomonas aeruginosa*, amikacin demonstrated the highest susceptibility rate after colistin (74.7%), followed by netilmicin (65.8%), piperacillin-tazobactam (65.8%), and

imipenem (64.6%). Ciprofloxacin susceptibility was observed in only 35.4% of isolates.

Among *Acinetobacter baumannii* isolates, susceptibility was highest to colistin (100%), followed by amikacin and imipenem (72.7% each). Moderate susceptibility was observed to gentamicin and netilmicin (54.5% each), whereas susceptibility to cephalosporins and ciprofloxacin remained low (27.3–36.4%).

Similarly, *Escherichia coli* isolates showed complete susceptibility to colistin (100%). Amikacin demonstrated the highest activity among the non-polymyxin agents (71.4%), followed by imipenem (66.7%), netilmicin (66.7%), and piperacillin-tazobactam (61.9%). Susceptibility to ciprofloxacin and cephalosporins ranged from 28.6% to 38.1%.

The three *Staphylococcus aureus* isolates recovered in this study were uniformly susceptible to vancomycin and linezolid (100%). All isolates were methicillin-sensitive *Staphylococcus aureus* (MSSA).

Overall, Gram-negative bacteria predominated among BAL isolates, with *Pseudomonas aeruginosa* being the most common pathogen. Colistin retained excellent activity against all Gram-negative isolates, whereas substantial resistance was observed against fluoroquinolones and cephalosporins.

Table II: Antimicrobial susceptibility profile of Gram-negative bacterial isolates recovered from bronchoalveolar lavage specimens (n=154).

Antimicrobial agent	<i>K. pneumoniae</i> (n=43)	<i>P. aeruginosa</i> (n=79)	<i>A. baumannii</i> (n=11)	<i>E. coli</i> (n=21)
Ciprofloxacin	44.2	35.4	36.4	38.1
Ceftriaxone	27.9	NA	27.3	33.3
Gentamicin	67.4	39.2	54.5	52.4
Cefotaxime	25.6	NA	27.3	33.3
Ceftazidime	20.9	NA	27.3	28.6
Netilmicin	65.1	65.8	54.5	66.7
Amikacin	81.4	74.7	72.7	71.4
Imipenem	79.1	64.6	72.7	66.7
Cefepime	25.6	NA	27.3	33.3
Piperacillin-Tazobactam	55.8	65.8	45.5	61.9
Colistin	100	100	100	100

Values represent percentage susceptibility. Abbreviations: NA, not applicable/not tested; BAL, bronchoalveolar lavage.

DISCUSSION

Lower respiratory tract infections (LRTIs) continue to represent a significant public health burden worldwide and are associated with substantial morbidity, mortality, and healthcare costs. These infections are particularly severe among hospitalized and critically ill patients, where pneumonia remains one of the most common infectious syndromes. The bacteriological profile and antimicrobial susceptibility patterns of respiratory pathogens vary considerably across geographical regions and healthcare settings, emphasizing the importance of local epidemiological surveillance to guide empirical antimicrobial therapy.

In the present study, 161 (65.71%) of 245 bronchoalveolar lavage (BAL) specimens yielded significant bacterial growth. This culture positivity rate is higher than that reported by Velez et al. (51.6%) and Kottmann et al. (55.8%) [16,17]. The comparatively higher yield observed in our study may be attributed to the inclusion of a predominantly hospitalized and critically ill patient population, many of whom were managed in intensive care settings where the burden of bacterial respiratory infections is typically greater.

The majority of culture-positive cases in our study were observed among patients aged 51–60 years, followed by those aged 61–70 years. Similar age distributions have been reported by Sánchez et al. and Mullerova et al., who documented a higher prevalence of lower respiratory tract infections among older adults [2,18]. Advanced age is a well-recognized risk factor for respiratory infections because of age-related decline in immune function, the presence of multiple comorbidities, impaired mucociliary clearance, and frequent exposure to healthcare environments. Furthermore, the use of inhaled corticosteroids and repeated courses of antibiotics in patients with chronic respiratory diseases may facilitate microbial colonization and infection.

A notable finding of the present study was the overwhelming predominance of Gram-negative bacteria, which accounted for 98.13% of all isolates. This observation is consistent with the findings of Mishra et al., who reported an 84.1% prevalence of Gram-negative pathogens among BAL isolates [19]. Similar trends have also been documented in several other studies investigating respiratory infections in hospitalized patients [20,21]. The predominance of Gram-negative bacilli may reflect the increasing burden of healthcare-associated and ventilator-associated respiratory infections, prolonged hospital stays, extensive antimicrobial exposure, and the emergence of multidrug-resistant organisms in tertiary care settings.

Among the recovered pathogens, *Pseudomonas aeruginosa* was the most frequently isolated organism (49.06%), followed by *Klebsiella pneumoniae* (26.70%), *Escherichia coli* (13.04%), and *Acinetobacter baumannii* (6.83%). These

findings are in agreement with those reported by Thomas et al. and Salman et al., who also identified *Pseudomonas aeruginosa* as the predominant respiratory pathogen [22,23]. However, several investigators have reported *Klebsiella pneumoniae* as the leading pathogen in lower respiratory tract infections [24,25]. Such variations may be attributed to differences in patient demographics, healthcare practices, antibiotic prescribing patterns, infection control measures, and local microbial ecology.

Analysis of antimicrobial susceptibility patterns demonstrated that colistin retained excellent activity against all Gram-negative isolates, with 100% susceptibility observed across all major pathogens. Among the non-polymyxin agents, amikacin, imipenem, netilmicin, and piperacillin-tazobactam exhibited the highest levels of activity. These findings are comparable to those reported by Akter et al. [26]. In contrast, a high degree of resistance was observed against third- and fourth-generation cephalosporins as well as ciprofloxacin. The widespread resistance to β -lactam antibiotics observed in this study may be attributed to the extensive and often empirical use of cephalosporins in both hospital and community settings, resulting in strong selective pressure favoring resistant strains.

Pseudomonas aeruginosa demonstrated complete susceptibility to colistin, while amikacin showed the highest susceptibility among the routinely used antimicrobial agents (74.68%). *Pseudomonas aeruginosa* is recognized as one of the most clinically significant opportunistic pathogens because of its remarkable ability to develop intrinsic and acquired resistance mechanisms, including efflux pumps, β -lactamase production, biofilm formation, and porin channel modifications. Consequently, infections caused by this organism are often associated with limited therapeutic options and adverse clinical outcomes.

Similarly, *Klebsiella pneumoniae* demonstrated 100% susceptibility to colistin, followed by amikacin (81.39%) and imipenem (79.06%). These findings indicate that aminoglycosides and carbapenems remain valuable therapeutic options for severe infections caused by this pathogen in our setting. The relatively high activity of amikacin against most Gram-negative isolates in the present study is consistent with observations reported by other investigators [27].

The universal susceptibility of Gram-negative isolates to colistin is encouraging; however, this finding should be interpreted cautiously. Colistin remains one of the last-line therapeutic options for infections caused by multidrug-resistant Gram-negative bacteria. The emergence of colistin resistance would significantly compromise treatment options and pose a serious public health threat. Therefore, stringent

antimicrobial stewardship programs and judicious use of reserve antibiotics are essential to preserve their effectiveness.

Among Gram-positive organisms, *Staphylococcus aureus* accounted for only 1.86% of isolates, a finding comparable to that reported by Akter et al [26]. All *S. aureus* isolates were methicillin-sensitive (MSSA) and demonstrated complete susceptibility to vancomycin and linezolid. Although Gram-positive pathogens were relatively uncommon in the present study, these agents remain reliable therapeutic options for serious staphylococcal infections.

The increasing prevalence of antimicrobial resistance among respiratory pathogens remains a major concern, particularly in developing countries where inappropriate antimicrobial use, over-the-counter availability of antibiotics, inadequate infection control practices, and limited antimicrobial stewardship programs contribute to the problem. Effective management of lower respiratory tract infections requires an integrated approach that combines accurate microbiological diagnosis, appropriate specimen collection, timely antimicrobial susceptibility testing, rational antibiotic prescribing, robust antimicrobial stewardship, and strict adherence to infection prevention and control measures. Continuous surveillance of local pathogen distribution and resistance patterns is essential for optimizing empirical therapy and improving patient outcomes.

LIMITATIONS

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

This study highlights the bacteriological profile and antimicrobial susceptibility patterns of pathogens isolated from bronchoalveolar lavage specimens in patients with suspected lower respiratory tract infections. Gram-negative bacteria predominated among the isolates, with *Pseudomonas aeruginosa* emerging as the most frequently recovered pathogen, followed by *Klebsiella pneumoniae* and *Escherichia coli*. A high level of resistance to commonly used cephalosporins and fluoroquinolones was observed, whereas colistin, amikacin, and imipenem retained substantial activity against most Gram-negative isolates. These findings underscore the growing challenge of antimicrobial resistance in respiratory pathogens and emphasize the importance of routine microbiological surveillance, antimicrobial susceptibility testing, and evidence-based antimicrobial stewardship programs. Knowledge of local pathogen distribution and resistance patterns can facilitate timely and appropriate empirical therapy, optimize patient management, and contribute to improved clinical outcomes.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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