Antibiotic susceptibility pattern of bacterial isolates from quantitative culture of bronchoalveolar lavage fluid in patients with clinical suspicion of pneumonia

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Abstract

**Background:** The common bacterial pathogens isolated from bronchoalveolar lavage (BAL) include *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Coagulase negative Staphylococcus*.

**Objectives:** This study was conducted to identify pneumonia causing organisms and to determine their antibiogram.

**Methodology:** A descriptive cross-sectional study was carried out at Kathmandu Medical College Teaching Hospital, Kathmandu, Nepal over a period of one year (Aug 2019-Jul 2020). Ethical approval (Reference Number:1004201810) was taken and convenience sampling was done using Clinical and Laboratory Standards Institute (CLSI) guidelines. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 19.

**Results:** Out of 32 BAL samples, 14 were culture positive. The commonest bacterial pathogens isolated were *Klebsiella Pneumoniae* 6(42.85%), followed by *Acinetobacter baumanii* 5(35.71%), *Pseudomonas aeruginosa* 2(14.28%) and *Coagulase negative Staphylococcus* 1(7.14%). The isolates were 100% sensitive to Tigecycline and Polymyxin B followed by Colistin(92.85%). Cotrimoxazole was 100% resistant to these isolates followed by Azithromycin (92.85%.

**Conclusion:** *Klebsiella pneumoniae*, *Acinetobacter baumanii* were the most common bacterial pathogens isolated from BAL.

**Key words:** Antimicrobials; Bronchoalveolar lavage; Pneumonia

INTRODUCTION

Early diagnosis and proper antimicrobial therapy are crucial for successful management of pneumonia1. The useful method of diagnosis of pneumonia is quantitative culture of bronchoalveolar lavage (BAL) samples2. Colony count of >10⁴ CFU/ml is consistent with bacterial pneumonia, whereas counts below 10⁴ CFU/ml is likely to indicate contamination with oronasal microbiota3. The advent of bronchoscopy and quantitative invasive techniques like BAL has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections4. This study provides clinicians knowledge about the common organisms isolated from BAL samples and their susceptibility pattern towards different antibiotics.

METHODOLOGY

A cross-sectional prospective study was carried out at Kathmandu Medical College and Teaching Hospital, Kathmandu, Nepal. A total of 32 BAL samples were collected over a period of one year (August 2019- July 2020).
2020) from patients over 18 years of age who were undergoing bronchoscopy in order to identify the organism that caused pneumonia. Convenient sampling technique was used. Institutional ethical clearance, reference no. 1004201810 was obtained from the Institutional Review Committee before this study was conducted.

Bronchoscopic BAL specimens were collected by physicians by wedging the tip of a fiberoptic bronchoscope into a segment of the airway, sequentially instilling sterile physiological saline, and aspirating each aliquot. First aliquot of samples was discarded and the remaining fluid was pooled for microbiological analysis. Samples were being transported to the microbiology laboratory within 2 hours of collection. Quantitative culture was processed according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

All samples received were inoculated on blood agar, MacConkey agar and chocolate agar, then incubated at 37°C with 5% CO₂. Gram staining was performed on 1 drop of undiluted BAL fluid. Final colony counts were determined at 48 hours. Potential pathogens present at ≥1 × 10⁶ CFU/ml were considered clinically significant. Subsequent identifications were based on colony characteristics and biochemical tests performed. Antimicrobial susceptibility tests were performed using modified Kirby Bauer disc diffusion method on Mueller Hinton Agar; zone sizes were measured and interpreted as sensitive and resistant. Antibiotics used were from HiMedia; Meropenem (10μg), Amikacin (30μg), Ciprofloxacin (1μg), Azithromycin (15μg), Cotrimoxaole (23.75/1.25μg), Piperacillin/Tazobactam(100/10μg), Cefotaxime(5μg), Polymixin B (0.016-256 μg), Colistin (10μg), and Tigecycline (15μg).

Raw data obtained from laboratory investigation were tabulated and presented in defined tables to explore the findings. The data was expressed in percentage. Data were analyzed using Statistical Package for Social Sciences (SPSS) 19.0 version. Inferential statistics i.e. Chi-square test was used to find the association.

**RESULTS**

Out of 32 BAL samples, 14 (43.75%) were culture positive.

Maximum number of isolated organisms were from male patients i.e. 8 (57.14%) and 6 (42.85%) were female patients.

Most of the culture results were positive among the age group above 60 years, followed by age group 41-60 years, 21-40 years and below 20 years respectively as shown in table 1.

The most frequently isolated species were *Klebsiella pneumoniae* 6 (42.85%), followed by *Acinetobacter baumanii* 41 (35.71%), *Pseudomonas aeruginosa* 2 (14.28%), *Coagulase negative Staphylococcus aureus* 1 (7.14%), respectively as shown in (Table 2).

The isolates were 100% sensitive to Tigecycline and Polymyxin B followed by Colistin (92.85%). Organisms were 100% resistant to Cotrimoxazole followed by Azithromycin (92.85%) as shown in table 1 by disc diffusion method.

**Multidrug resistant organism among the isolates**

7 (50%) isolates were multidrug resistant. Out of these 7 MDR strains, 3 (21.48%) were *K. pneumoniae*, 2 (14.28%) *A. baumanii* and 2 (14.28%) were *P. aeruginosa* respectively.

**Table 1: Age wise distribution of positive cultures**

| Age in years | No. of Positive culture |
|--------------|-------------------------|
| <20          | 1 (7.14%)               |
| 21-40        | 1 (7.14%)               |
| 41-60        | 3 (21.42%)              |
| >61          | 9 (64.26%)              |

**Table 2: Organism wise distribution**

| Isolated organism                  | Total number | % of isolates |
|------------------------------------|--------------|---------------|
| *Klebsiella pneumoniae*            | 6            | 42.85%        |
| *Acinetobacter baumanii*           | 5            | 35.71%        |
| *Pseudomonas aeruginosa*           | 2            | 14.28%        |
| *Coagulase negative Staphylococcus*| 1            | 1.28%         |

**Table 3: Antibiotic susceptibility pattern in isolates from BAL sample**

| Antibiotics                  | Sensitive (%) | Resistant (%) |
|------------------------------|---------------|---------------|
| Tigecycline                  | 100           | 0             |
| Polymyxin B                  | 100           | 0             |
| Colistin                     | 92.85         | 7.14          |
| Amikacin                     | 42.85         | 57.14         |
| Piperacillin/Tazobactam      | 21.42         | 78.57         |
| Meropenam                    | 14.28         | 85.71         |
| Cefotaxime                   | 14.28         | 85.71         |
| Ciprofloxacin                | 14.28         | 85.71         |
| Azithromycin                 | 7.14          | 92.85         |
| Cotrimoxazole                | 0             | 100           |
DISCUSSION

BAL is a preferred investigative tool over invasive techniques like needle biopsies and thoracoscopy. This study was conducted to evaluate quantitative bacterial culture from patients who underwent bronchoscopy to identify pneumonia causing organisms.

The present study yielded positive bacterial BAL cultures in 43.75% of the cases of suspected pneumonia which is similar to other studies done by Sistla R and Vivek KU, Nutun Kumar DM which showed positive bacterial cultures in 38-39% of the cases of suspected lung infection. This study is in contrast to the study done by Velez et al and Kottmannet al, where positive yield was 51.6% and 55.8% respectively. There was a male preponderance in the study group with a male:female ratio of 4:3. In this study most of the suspected cases of pneumonia belonged to the age group of more than 61 years, which correlated well with the study conducted by Vivek KU et al. Age above 65 years is a risk factor for developing pneumonia.

Klebsiella pneumoniae was the most common organism isolated from our study which is in agreement with the study done by Mohammad H. AffyEnas A et al (20 of 40 samples) followed by Acinetobacter spp (17 of 40) and then Pseudomonas spp (11 of 40). In contrast to our study, Rajasekhar T. et al reported Acinetobacter baumannii (30.59%) as the most common pathogen. Klebsiella is a part of normal flora of the mouth and most widely associated with pneumonia in a hospitalised patients and elderly. Hence, its predominance may be related to the predominant elderly population in our study. In another study done by Swomya K.N. et al most common organism isolated was Pseudomonas spp (21.8%). The present study showed maximum resistance of organism to Cotrimoxazole (100%) and 100% susceptibility to Tigecycline and Polymyxin B. Resistance to Cotrimoxazole could be due to resistant transferable dhfr and sul genes.

In a study conducted by Mishra DL in Nepal exhibited 62% sensitivity towards Colistin, as compared to our study which showed 92% sensitive. The primary purpose of quantitative culture of BAL fluid was to identify potential pathogens and determine their antimicrobial susceptibility patterns. This information allows clinicians to de-escalate the initial empirical regimen if bacterial agents are detected or to discontinue therapy in their absence. Limitations of this study were small sample size as, bronchoscopic sampling is costly and requires highly trained personnel to perform the procedure and anaerobic culture method was not included. Newer methods like VITEK, molecular study were not used in this study.

CONCLUSION

The study provides data regarding the incidence rate of various bacterial pathogens isolated from BAL in our setup along with their antibiotic susceptibility pattern. Any delay in initiation of antibiotic treatment may lead to poor outcomes. So there is a risk of emergence of MDR pathogens with inadequate, inappropriate antibiotic treatment. To initiate an empiric antimicrobial therapy all consultants should have the knowledge of microbial flora of the locality and their antibiotic susceptibility pattern. Such information needs to be analyzed periodically and institution based antibiotic policies formed from time to time.

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