Journal of Kathmandu Medical College, Vol. 9, No. 4, Issue 34, Oct.-Dec., 2020

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

Jha B[1], Sharma M[1], Sapkota J[1], Pant S[2], Neopane A[2]

[1]Jha Beena, Assistant Professor; [1]Sharma Manisha, Assistant Professor; [1]Sapkota Jyotshna, Assistant Professor, Department of Microbiology; [2]Pant Subash, Assistant Professor; [2]Neopane Arpana, Professor Department of Medicine, Kathmandu Medical College, Kathmandu, Nepal.

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 Polymixin B followed by Colistin(92.85%). Cotrimoxazole was 100% resistant to these isolates followed by Azithromycin (92.85%.
Conclusion: *Klebsiella pneumomiae*, *Acinetobacter baumanii* were the most common bacterial pathogens isolated from BAL.

Key words: Antimicrobials; Bronchoalveolar lavage; Pneumonia

Access this article online

Website: www.jkmc.com.np

DOI: https://doi.org/10.3126/jkmc.v9i4.38091

HOW TO CITE
Jha B, Sharma M, Sapkota J, Pant S, Neopane A. Antibiotic susceptibility pattern of bacterial isolates from quantitative culture of bronchoalveolar lavage fluid in patients with clinical suspicion of pneumonia. J Kathmandu Med Coll. 2020;9(4):197-200.

Address for correspondence
Dr. Beena Jha
Department of Microbiology
Kathmandu Medical College
Kathmandu, Nepal.
E-mail: drbeenajha@gmail.com

ISSN: 2019-1785 (Print), 2091-1793 (Online)

INTRODUCTION

Early diagnosis and proper antimicrobial therapy are crucial for successful management of pneumonia[1]. The useful method of diagnosis of pneumonia is quantitative culture of bronchoalveolar lavage (BAL) samples[2]. Colony count of $>10^4$ CFU/ml is consistent with bacterial pneumonia, whereas counts below 10^4 CFU/ml is likely to indicate contamination with oronasal microbiota[3]. The advent of bronchoscopy and quantitative invasive techniques like BAL has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections[4]. 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) 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% CO_2. Gram staining was performed on 1 drop of undiluted BAL fluid. Final colony counts were determined at 48 hours. Potential pathogens present at $\geq 1 \times 10^4$ 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
Polymixin 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[5]. 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[5,6]. This study is in contrast to the study done by Velez *et al* and Kottmann*et al*, where positive yield was 51.6% and 55.8% respectively[7,8]. 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*[6]. 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. AfifyEnas A *et al* (20 of 40 samples) followed by *Acinetobacter spp* (17 of 40) and then *Pseudomonas spp* (11 of 40)[9]. In contrast to our study, Rajasekhar T. *et al* reported *Acinetobacter baumanii* (30.59%) as the most common pathogen[1]. Klebsiella is a part of normal flora of the mouth and most widely associated with pneumonia in a hospitalised patients and elderly[6]. 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%)[10]. 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[11]. 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[10]. 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[12]. 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.

ACKNOWLEDGMENTS

We sincerely thank the staff and faculty of the Medicine at Kathmandu Medical College for support and contributions to this investigation.

REFERENCES

1. Rajasekhar T, Anuradha K, Suhasini T, Lakshmi V. The role of quantitative cultures of non-bronchoscopic samples in ventilator associated pneumonia. *Indian J Med Microbiol.* 2006;24:107-13. [PubMed | Full Text]
2. Hummel M, Rudert S, Hof H, Hehlmann R, Buchheidt D. Diagnostic yield of bronchoscopy with bronchoalveolar lavage in febrile patients with hematologic malignancies and pulmonary infiltrates. Ann Hematol. 2008;87:291-7. [PubMed | Full Text | DOI]
3. Mandell GL, Bennett JE, Dolin R. Principles and practice of infectious diseases. 7th ed. Churchill Livingstone- Elsevier; 2010. 899 p.
4. Gomes JCP, Pedreira WL, Evangelina MP, Araujo A, Soriano FG, Negri EM *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.
5. Sistla R, Tameem A, Sudheer P, Nallagonda R. Diagnostic utility of bronchoalveolar lavage. *J Cytol.* 2014;31(3):136-8. [PubMed | Full Text | DOI]

6. Vivek KU, Nutan Kumar DM. Microbiological profile of bronchoalveolar lavage fluid in patients with chronic respiratory diseases: a tertiary care hospital study. *International J Med research and Review*. 2016;4:330-4. [Full Text | DOI]
7. Vélez L, Correa LT, Maya MA, Mejía 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. [PubMed] [PubMed | Full Text | DOI]
8. 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. [PubMed | Full Text | DOI]
9. Mohammad H, Enas A, Samy S, Hanady M. Comparison between bronchoscopic BAL and non-bronchoscopic BAL in patients with VAP. 2016;65:113-9. [Full Text | DOI]
10. Sowmya KN, Sevitha B, Vishwas K. Spectrum of bacteria isolated from bronchoalveolar lavage in a tertiary care centre. J of Evolution of Medical and Dental Sciences. 2014;3:7950-4. Full Text]
11. Mishra DR, Shah DS, Shah N, Prasad JN, Gupta PP, Agrawaal KK. Study of microbiological and antibiotic sensitivity pattern of ventilator associated pneumonia (VAP) in ICU of a tertiary care hospital in Nepal. J Family Med Prim Care. 2020;9:6171-6. [PubMed | Full Text | DOI]
12. Jackson SR, Ernst NE, Mueller EW, Butler KL. Utility of bilateral bronchoalveolar lavage for the diagnosis of ventilator-associated pneumonia in critically ill surgical patients. Am J Surg. 2008;195(2):159-63. [PubMed | Full Text | DOI]