

Quantitative Culture of Bronchoalveolar Lavage Fluid for the Diagnosis of Bacterial Pneumonia at a Tertiary Care Centre

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ABSTRACT

Background: Pneumonia remains the leading cause of infectious disease mortality globally, necessitating early diagnosis and appropriate antimicrobial management. Bronchoalveolar lavage (BAL) has been established as a crucial diagnostic tool in both immunocompetent and immunocompromised patients, offering advantages over conventional sputum samples by reducing contamination and providing accurate antimicrobial susceptibility data.

Objectives: This study aimed to evaluate the diagnostic yield of quantitative BAL fluid culture in identifying bacterial pathogens responsible for pneumonia and to assess the antimicrobial susceptibility of these pathogens to guide effective treatment.

Methods: Conducted at the Department of Microbiology, Government Medical College, Maharashtra, the study included patients over 18 years undergoing BAL via fiberoptic bronchoscopy for pneumonia diagnosis from June 2018 to May 2019. Quantitative cultures were grown on Blood Agar, MacConkey Agar, and Chocolate Agar, with a positive culture defined at $\geq 10^4$ CFU/mL. Pathogen identification and antibiotic susceptibility testing were performed using standard microbiological techniques.

Results: Of the 63 patients studied, 57.14% showed a positive quantitative BAL culture. *Staphylococcus aureus* was the most frequently isolated pathogen, followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. Notably, Methicillin-resistant *Staphylococcus aureus* (MRSA) constituted a significant portion of *S. aureus* cases. Gram-negative bacteria showed high resistance to standard antibiotics but were sensitive to Imipenem and Piperacillin-Tazobactam.

Conclusion: Quantitative BAL fluid culture is a valuable diagnostic method for effectively identifying and managing bacterial pneumonia. The procedure not only confirms the pathogen but also informs targeted antibiotic therapy, thereby minimizing the use of broad-spectrum antibiotics and reducing the emergence of resistant bacterial strains.

Keywords: Bronchoalveolar lavage, Pneumonia, Antimicrobial susceptibility.

INTRODUCTION

Pneumonia causes more deaths worldwide than any other infectious disease.^[1] Early diagnosis and proper choice of antimicrobials are crucial for successful management of pneumonia.^[2] Bronchoalveolar lavage (BAL) and protected specimen brush are standard methods for identifying causative organisms.^[3] The usefulness of endoscopic procedures, especially BAL, is well-established in both immunocompetent and immunocompromised patients with pneumonia.^[4]

Bronchoalveolar lavage (BAL) was introduced as a research-oriented procedure way back in 1970.⁵ It was originally used as a tool for obtaining secretions and cells from the lower respiratory tracts of patients with interstitial,

occupational, or both types of pulmonary diseases⁶. It was used for first time in India in 1994 for its important role in diagnosis of infections and malignancies⁷. Lower Respiratory tract infections (LRTI), caused by various organisms like bacteria and fungi, can be isolated by culturing BAL samples⁸. Sputum samples are still considered effective in diagnosing infective lung conditions in our country, as they are easy to obtain. Although they have 24% diagnostic yield, contamination of sample from oral flora during expectoration makes it unsatisfactory for culture⁹. BAL has an advantage compared to repeat sputum sample collections as the contamination by oral flora can be avoided easily. Further, the quantitative culture with proper colony cut off provides a greater insight for therapy. Another distinct

advantage of BAL is that being an outpatient procedure, it is very much useful in suspected cases of community acquired pneumonia (CAP).¹⁰

The aim of this study was to determine the yield of quantitative BAL fluid bacterial culture for the early and accurate diagnosis of pneumonia and the antimicrobial sensitivity testing for appropriate and early management of the patients.

MATERIALS AND METHODS

The study was performed in the Department of Microbiology at Government Medical College of Maharashtra from June 2018 to May 2019. Patients over 18 years of age in whom BAL through fiberoptic bronchoscopy was performed to identify pneumonia-causing organisms were included in the study. A

detailed history of the patients were taken. Quantitative Culture of BAL fluid was performed by calibrated loop techniques on Blood Agar, MacConkey Agar and Chocolate Agar. A positive quantitative culture was defined when bacteria were cultured from BAL samples at a concentration of 1×10^4 CFU/mL or more.¹¹ Identification of the isolates were done by conventional methods. Antibiotic susceptibility was performed by Kirby-Bauer disc diffusion method.¹²

RESULTS

Quantitative culture of BAL fluid was performed on 63 patients with pneumonia, comprising 45[71.43%] men and 18[28.57%] women. The mean Age of the patients was 47.5 years(range 18-71years). A positive quantitative culture ($>10^4$ CFU/mL) of BAL was documented in 36(57.14%) patients as shown in figure 1.

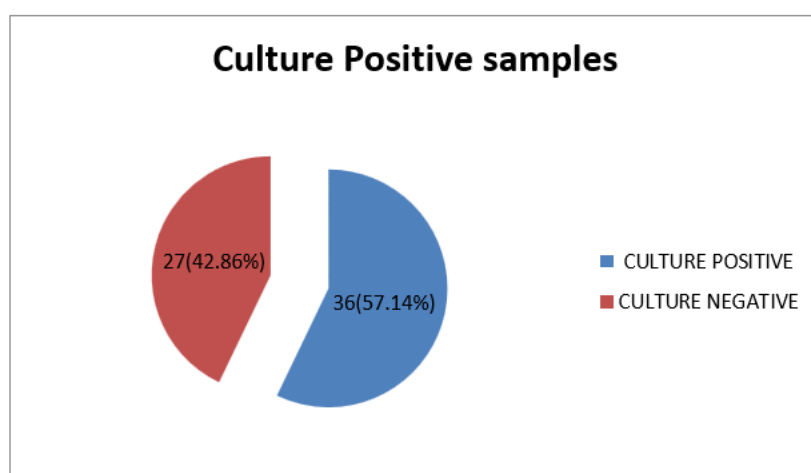


Figure 1: Culture positive samples.

The total number of bacterial isolates were 42 from 36 culture positive samples of which 6 samples were culture positive for two organisms each.

The most frequently isolated organism was *Staphylococcus aureus* 19(45.24%) followed by *Pseudomonas aeruginosa* 14(33.33%), *Klebsiella pneumoniae* 5(11.90%) and *Escherichia coli* 4(11.90%).

Table 1: Number of bacterial isolates.

Organism	No. of isolates	%
<i>Staphylococcus aureus</i>	19	45.24
<i>Pseudomonas aeruginosa</i>	14	33.33
<i>Klebsiella pneumoniae</i>	5	11.9
<i>Escherichia coli</i>	4	9.53
Total	42	100

Most of the *Staphylococcus aureus* isolates showed resistance to Penicillin, Erythromycin and Ciprofloxacin whereas they were sensitive to Cotrimoxazole, Tetracycline and Gentamicin.

Staphylococcus aureus isolates were 100% sensitive to Linezolid and Vancomycin. Methicillin resistant *Staphylococcus aureus* accounted for 36.84% (7/19) of the isolates.

Pseudomonas aeruginosa was found to be least sensitive to Cephalosporins but was 100% sensitive to Imipenem and Piperacillin-Tazobactam. *Klebsiella pneumoniae* and *Escherichia coli* documented resistance to Cephalosporins, Ampicillin and Amoxicillin-

Clavulanic acid but were sensitive to Amikacin, Imipenem and Piperacillin-Tazobactam. The antimicrobial susceptibility pattern of Gram Positive Isolates and Gram Negative Isolates is Shown in Table 2 and Table 3 respectively.

Table 2: Sensitivity Percentage of Antibiotics for the Gram Positive Isolates

Antibiotic	<i>Staphylococcus aureus</i>	Methicillin resistant <i>S. aureus</i>
Vancomycin	100(12/12)	100(7/7)
Linezolid	100(12/12)	100(7/7)
Cotrimoxazole	91.66(11/12)	100(7/7)
Tetracycline	83.33(10/12)	71.42(5/7)
Gentamicin	75(9/12)	71.42(5/7)
Ciprofloxacin	33.33(4/12)	57.14(4/7)
Erythromycin	33.33(4/12)	28.57(2/7)
Penicillin	16.67(2/12)	0(0/7)

Table 3: Sensitivity Percentage of Antibiotics for Gram Negative Isolates

Antibiotic	<i>Pseudomonas aeruginosa</i> (n=14)	<i>Klebsiella pneumoniae</i> (n=5)	<i>E. coli</i> (n=4)
Imipenem	100(14)	100(5)	100(4)
Piperacillin-Tazobactam	100(14)	100(5)	100(4)
Amikacin	64.28(9)	100(5)	100(4)
Ciprofloxacin	71.42(10)	40(2)	50(2)
Piperacillin	64.28(9)	60(3)	75(3)
Gentamicin	57.14(8)	40(2)	50(2)
Ceftazidime	35.71(5)	20(1)	0(0)
Amoxicillin-clavulanate	28.53(4)	20(1)	25(1)
Ampicillin	14.28(2)	0(0)	0(0)
Cefotaxime	7.14(1)	0(0)	25(1)
Ceftriaxone	7.14(1)	0(0)	0(0)

DISCUSSION

BAL is a diagnostic method used in many studies to determine the cause of VAP, pneumonia in immunocompromised patients, nosocomial pneumonia, and CAP of severe nature or refractory to treatment.¹³

The number of culture positive patients in our study accounted for 36(57.14%) which correlates with the study conducted by GJC Pereira et al.¹³ The study conducted in Brazil in 62 patients with pneumonia, BAL fluid was positive 45 out of 62 patients(72.6%) with 58 of 62 BAL performed under antibiotics. Quantitative culture of BAL allows exclusion of even low level of contamination of virtue of culture growth below diagnostic threshold ($>10^4$ colony forming units/ml). Low colony

counts suggest oropharyngeal contaminants and high colony counts suggest pathogen.¹⁴ In a study conducted by Kahn and Jones in 94 immunocompromised patients to analyze the laboratory protocol of BAL fluid analysis found BAL microscopy and culture both were diagnostic in 12 out of 18 cases (66%).

In our study *Staphylococcus aureus* (45.24%) was the most predominant isolate. The study conducted by E S Kim *et al*/correlates with the findings of our study which showed *Staphylococcus aureus* as the major pathogen.¹⁵ Other studies conducted such as Bhatia et al showed *Klebsiella* species as the predominant organism followed *Escherichia coli* and *Staphylococcus aureus*.¹⁶

Methicillin resistant *Staphylococcus aureus* accounted for 36.84% (7/19) of the isolates. Recent trend with spread of MRSA strains is documented in various studies and is also shown in the study conducted by E S Kim *et al.*¹⁵

The gram negative organisms were accounted for in 54.76% of the isolates. The predominance of gram-negative bacilli as aetiological agents in contrast to gram-positive bacteria in the present study was typical of the hospital-acquired infections.¹⁶ The other predominant isolate was *Pseudomonas aeruginosa* 14(33.33%) which correlates with the findings of Magazine *et al.*¹⁷ The other organisms which were isolated were *Klebsiella pneumoniae* and *Escherichia coli*.

The *Staphylococcus aureus* isolates were resistant to Penicillin, Erythromycin and Ciprofloxacin which correlates with the findings of Magazine *et al.* whereas they were sensitive to Cotrimoxazole, Tetracycline and Gentamicin. *Pseudomonas aeruginosa* was found to be least sensitive to Cephalosporins but was 100% sensitive to Imipenem and Piperacillin-Tazobactam which correlates with the study conducted by Magazine *et al.*¹⁷ The study conducted by Ranjan N *et al* also showed maximum sensitivity to Carbapenems and Piperacillin-Tazobactam combination.¹⁸ *Klebsiella pneumoniae* and *Escherichia coli* documented resistance to Cephalosporins, Ampicillin and Amoxicillin-Clavulanic acid but were sensitive to Amikacin, Imipenem and Piperacillin-Tazobactam. The multi drug resistant ESBL producing strains contribute to the failure of the antimicrobial therapy in pneumonia. Multiple antibiotic resistance to useful antibiotics, including the penicillins, cephalosporins, aminoglycosides, and fluoroquinolones, has gradually increased among a number of gram negative pathogens, especially *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other gram negative isolates.¹⁹

CONCLUSION

BAL quantitative culture is performed to establish the bacterial etiologic diagnosis and to guide therapy specifically, minimizing the selection of resistant strains while reducing the side effects of empirically used antibiotics. Many studies including our study conclude that BAL may help to reduce inappropriate antibiotic

use in cases of pneumonia. BAL examination offers valuable information on the diagnosis of pyogenic infections and also other diseases.

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