



Original Article

## Clinico-microbiological profile and antimicrobial resistance patterns of bacterial isolates from bronchoalveolar lavage specimens in Central India

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### ABSTRACT

**Introduction** Lower respiratory tract infections (LRTIs) are a major cause of morbidity and mortality worldwide, particularly among critically ill patients. Bronchoalveolar lavage (BAL) is an important diagnostic specimen used for identifying aetiological agents directly from the lower respiratory tract. The increasing prevalence of multidrug-resistant (MDR) organisms and biofilm-forming bacteria has further complicated the management of LRTIs, especially in tertiary care hospitals. Continuous surveillance of bacterial pathogens, resistance patterns, and virulence factors such as biofilm formation is essential for guiding appropriate antimicrobial therapy.

**Aim & Objective of the Study** The study aimed to identify bacterial pathogens from BAL specimens, analyse their antimicrobial susceptibility patterns, detect resistance mechanisms such as ESBL, AmpC, MBL, and MRSA, and assess biofilm-forming ability among the isolates.

**Materials & Methods** This prospective study was conducted in the Department of Microbiology at a tertiary care hospital (IGGMC) in Nagpur, Maharashtra, Central India from September 2023 to February 2025. A total of 280 BAL samples were processed using standard microbiological techniques. Bacterial identification was done based on culture characteristics and biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method as per CLSI M100 guidelines. ESBL, AmpC, MBL, and MRSA were detected using phenotypic methods. Biofilm production was evaluated using quantitative microtiter plate and qualitative tube methods.

**Results** Out of 280 BAL samples, 45 (16.07%) were culture positive. Male patients (64.45%) and individuals aged 41–60 years were most commonly affected. The majority of isolates were obtained from the Respiratory Intensive Care Unit. Gram-negative bacteria predominated (88.9%), with *Klebsiella pneumoniae* (33.33%) being the most frequent isolate, followed by *Pseudomonas aeruginosa* (20%) and *Acinetobacter* spp. High resistance to commonly used antibiotics were observed. ESBL, AmpC, and MBL producers constituted 22.2%, 20%, and 11.1% respectively, while MRSA accounted for 8.8% of isolates. Nearly 49% of isolates demonstrated biofilm-forming ability.

**Conclusion** The study highlights a high burden of Gram-negative MDR and biofilm-forming pathogens in BAL specimens, especially among ICU patients. Regular BAL-based culture, antimicrobial susceptibility testing and surveillance of resistance

mechanisms are crucial for optimizing therapy and strengthening antibiotic stewardship in tertiary care settings.

**Keywords:** Bronchoalveolar Lavage; Respiratory Tract Infections; Drug Resistance, Bacterial; Multidrug Resistance; Gram-Negative Bacteria; Biofilms; Intensive Care Units; Antimicrobial Susceptibility Testing.

## INTRODUCTION

Lower Respiratory Tract Infections (LRTIs) are among the most common infectious diseases worldwide and are associated with significant morbidity and mortality, including in India. These infections can lead to a range of conditions, from acute bronchitis to pneumonia.<sup>1</sup> LRTIs account for approximately 6% of all infectious disease cases seen in outpatient departments of tertiary care hospitals and 4.4% of hospital admissions.<sup>2</sup> Among these, chronic respiratory conditions are responsible for around 4 million deaths annually, contributing to 5% of global mortality.<sup>3</sup>

In healthcare settings equipped with bronchoscopy facilities, Bronchoalveolar Lavage (BAL) samples can be obtained to isolate and identify causative bacterial pathogens for targeted therapy.<sup>1</sup> Common respiratory pathogens include Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas aeruginosa*, and Gram-positive bacteria like *Staphylococcus aureus* and *Streptococcus pneumoniae*.<sup>4</sup> The emergence of multidrug-resistant (MDR) Gram-negative organisms, including extended-spectrum beta-lactamase (ESBL) producers, has become an increasing concern in healthcare environments. ESBLs are enzymes that provide resistance to beta-lactam antibiotics, often leading to treatment failure and adverse clinical outcomes.<sup>5</sup>

Antibiotic resistance in respiratory infections is a growing global issue, largely driven by the inappropriate use of antibiotics.<sup>6</sup> In addition to resistance, biofilm production significantly enhances bacterial virulence,<sup>7</sup> contributing to nearly 80% of bacterial infections.<sup>8</sup> A biofilm is a polymicrobial community that adheres to surfaces or other microbial aggregates. These biofilms are frequently found on medical devices and can serve as physical barriers, protecting bacteria from various external stresses such as dehydration, antibiotics, and biocides.<sup>9</sup> Moreover, failure to adjust antibiotic therapy based on culture and sensitivity results further exacerbates resistance.<sup>6</sup>

BAL is a crucial diagnostic tool for identifying lung infections and evaluating lower respiratory tract conditions. The fluid retrieved during the procedure can reveal white blood cell profiles and allow isolation of pathogens from the epithelial surface of the lower airways.<sup>10</sup> The bacterial spectrum of pulmonary infections may vary within a country, influenced by patterns of antibiotic usage, environmental conditions, and the ventilation needs of critically ill patients. Consequently, BAL is an ideal specimen for identifying pathogens to guide empirical and targeted treatment strategies.<sup>10</sup>

This study was conducted to detect pathogenic organisms in BAL fluid via microscopy, isolate and identify bacterial species through culture, analysed their antibiotic susceptibility patterns, and determine the prevalence of biofilm-forming organisms. It also aimed to assess the occurrence of ESBL-producing and MDR pathogens in BAL samples collected from a tertiary care hospital in Central India.

## MATERIAL & METHODS:

This prospective study was conducted in the Department of Microbiology at a tertiary care hospital (IGGMC) in Nagpur, Maharashtra, Central India from September 2023 to February 2025. Bronchoalveolar lavage (BAL) fluid specimens were collected under aseptic precautions and immediately transported to the laboratory for processing. A total of 280 BAL fluid samples were included in the study. Each sample was carefully examined for consistency, colour, and odour. Initial microscopic examination involved Gram staining and wet mount preparation, which were evaluated for the presence of pus cells, epithelial cells, and bacteria.

Subsequently, the samples were inoculated on MacConkey agar, Blood agar, and Chocolate agar using a sterile 4 mm nichrome loop. The inoculated media were incubated at 37°C for 24–48 hours using standard laboratory protocols. The Petri dishes were examined the following day for bacterial growth, and the isolates were identified based on their morphological, cultural, and biochemical characteristics.

Necessary biochemical tests were done for the identification of bacteria. The following tests were performed as per the requirements: catalase test, slide coagulase test, tube coagulase test, oxidase nitrate, motility reduction test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, urease production test, triple sugar iron test, and mannitol motility test.

Antibiotic susceptibility testing was performed on the bacterial isolates using the Kirby-Bauer disc diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Zone diameters were measured in millimetres and interpreted accordingly.<sup>11</sup>

**Inclusion criteria:**

All BAL fluids received from clinically diagnosed cases, irrespective of age and gender.

**Exclusion criteria:**

Other respiratory samples such as tracheal aspirates, sputum & nasal swabs, etc.

In addition to routine microbiological analysis, the study was undertaken to evaluate the prevalence of biofilm-forming organisms in bronchoalveolar lavage (BAL) fluid samples. Furthermore, it aimed to investigate the incidence of *extended-spectrum beta-lactamase* (ESBL)-producing and *Metallo-β-lactamase* (MBL) producer isolated from patients admitted to a tertiary care hospital in Central India.

**Detection of methicillin-resistant *Staphylococcus* :-**

The test was done on a Muller Hinton agar with cefoxitin a disc in our laboratory. If zone size  $\geq 22$ mm was considered positive and zone size  $\leq 21$ mm was negative.

**ESBL test:-**

Ceftazidime 30 $\mu$ g and Ceftazidime+Clavulanic acid (30 $\mu$ g+10 $\mu$ g) disc were put on a Muller Hinton agar plate culture of inoculum equal to 0.5 Mc Farland opacity standard for land turbidity standard. An increase in zone size of Ceftazidime+Clavulanic acid by  $\geq 5$ mm component to ceftazidime disc alone is considered as ESBL positive.

**Detection of *Metallo beta-lactamase* by combined disc diffusion method:-**

A lawn culture of test isolate (0.5 Mc Farland opacity standard) was done on MHA plate. Two 10 $\mu$ g imipenem discs were placed on inoculated plates. To one of the imipenem discs, 10 $\mu$ L of 0.5M EDTA solution was added. After overnight incubation, if the zone of inhibition of imipenem + EDTA discs compared to imipenem alone is  $> 7$ mm, then the test was considered positive.

**Detection of Amp-C through disc- diffusion method:-****i) Cefoxitin screening test -**

The test organism inoculated in peptone water and incubated for 2 hours was taken out and with the help of sterile swab stick the MHA plates were swabbed according to standard technique. The cefoxitin disk (30 mcg) was then placed. The plate was then incubated at 37°C for 16- 18 hours and resistance was said to be positive when cefoxitin breakpoint  $\leq 18$ mm is observed and suspected to be Amp-C producer.

**ii) Ceftazidime - Imipenem disk antagonism test Confirmatory test-**

The test is used to detect inducible Amp-C production .0.5 Mcfarland inoculum of test organism was swabbed on MHA plate. Ceftazidime (30 $\mu$ g) and Imipenem (10 $\mu$ g) were placed 20mm apart from center to center which was incubated overnight at 37 °C. If there is blunting of Ceftazidime zone of inhibition adjacent to Imipenem disk is confirmatory for inducible Amp-C production.

**Biofilm Formation in Bacterial Isolates from BAL Samples:-**

Two phenotypic methods were used for the purpose of biofilm formation evaluation: the quantitative microtiter plate method and the qualitative evaluation method.

**i) Quantitative microtiter plate method:-** The first of these was the 96-well microtiter plate method, and the second was the tube test. The former approach was first described by *Stepanovi c et al.* (2007), whereas the latter was initially defined by *Christensen et al.* (1995).<sup>12,13</sup> The capacity of the isolates to form biofilms was evaluated using the crystal violet staining method in a 96-well microtiter plate. Briefly, 100 $\mu$ L of each culture at a concentration of 10<sup>6</sup> CFU/ml was pipetted into the wells of a 96-well polystyrene microplate. After incubation for 24 h at 37<sup>0</sup>C, the microplates were fixed in absolute methanol for 15 min and air-dried before 200  $\mu$ L of 1% crystal violet (CV) solution (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. After 15 min of incubation at room temperature, 300  $\mu$ L of acetic acid (30% v/v) was added to each well. Plates were then read at 570 nm using a plate reader (DAS, Rome, Italy). Between each step, the wells were washed three times with PBS. The quantification of biofilms in microplates was performed in accordance with the previously established methodology.<sup>14</sup>

**ii) Qualitative evaluation method:-**

For the test tube, the samples were plated in Tryptic Soy Agar (TSA) and left to incubate overnight at 37 °C. The colonies were used to prepare a suspension of 3 OD McFarland (3 mL of saline), of which 100 $\mu$ L was inoculated into a tube with 2.9mL of Tryptic Soy Broth (TSB) and incubated at 37 °C for 24 h. The tubes, whose broth showed evident turbidity, were decanted and washed with PBS (or saline) and left to dry upside down, and 1 mL of methanol was added to the tubes to fix the sample for 15 min and then removed. At this point, the dried test tubes were stained with 1 mL of crystal violet (1%) for 15 min. The excess colour was eliminated by washing with deionized water, and the tubes were left to dry again. The formation of the biofilm was determined by the addition of 1 mL of acetic acid (33% v/v) and subsequent reading of the respective OD. The tubes were analysed and rated for strong, moderate and no biofilm formation. One tube containing only culture broth (TSB) was run as a negative control.<sup>14</sup>

## RESULTS:

A total of 280 BAL samples were processed, of which 45 (16.07%) were culture positive. Male patients constituted 64.45% of positive cases, showing clear male predominance (Fig. 1).

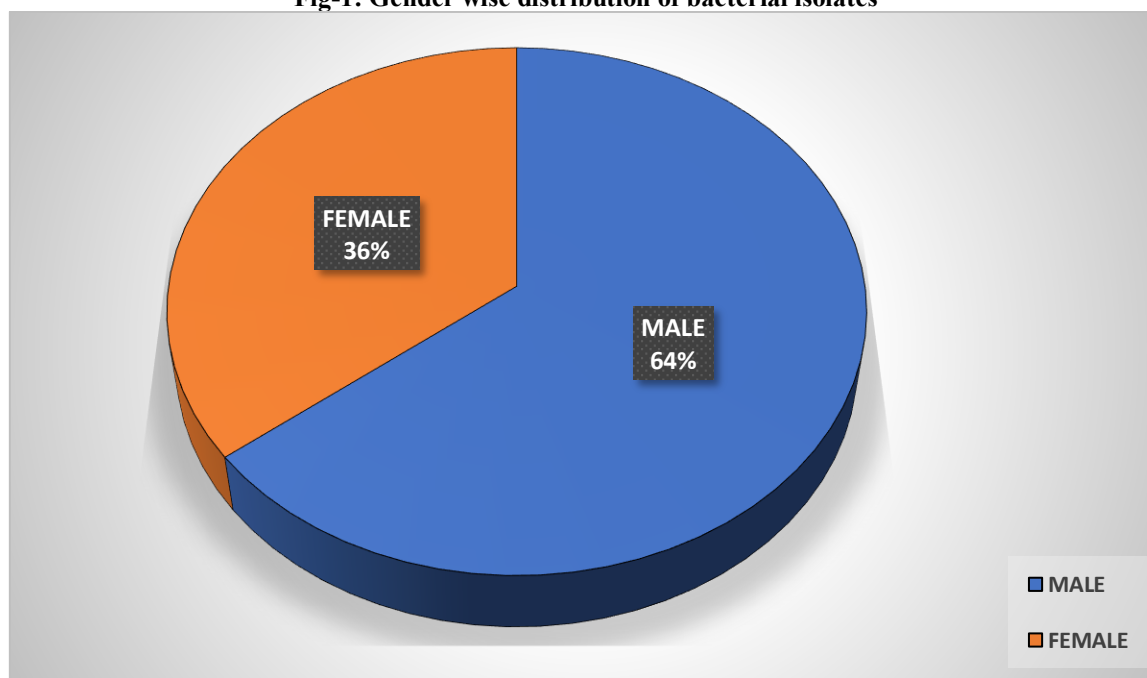
Culture-positive cases were observed across all age groups, with the highest bacterial isolation rate in the 41–60 years age group (42.2%), followed by 61–80 years (31.1%) (Table 1). Both sexes showed maximum positivity in the 41–60 years group. Most isolates were recovered from the Respiratory Intensive Care Unit (RICU) (68.9%), followed by the Respiratory Medicine ward (11.1%), while the least were from PICU (Table 2).

Gram-negative bacteria predominated (88.9%). *Klebsiella pneumoniae* was the most common isolate (33.33%), followed by *Pseudomonas aeruginosa* (20%) and *Acinetobacter* spp. (11.11%). Among Gram-positive organisms, *Staphylococcus aureus* (11.11%) was most frequent (Table 3). These pathogens were predominantly isolated from patients aged 41–60 years (Table 4).

Gram-positive isolates showed high resistance to penicillin, fluoroquinolones, and macrolides, while linezolid and vancomycin remained 100% effective (Table 5). MRSA accounted for 8.8% of isolates. Gram-negative organisms demonstrated high resistance to  $\beta$ -lactams, cephalosporins, and fluoroquinolones; however, carbapenems and tigecycline retained better activity (Table 6).

Overall, 22.2% of isolates were ESBL, 20% AmpC, 11.1% MBL, and 8.8% MRSA (Fig. 2). Biofilm production was detected in 49% of isolates, predominantly among *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. (Table 7).

**Fig-1: Gender wise distribution of bacterial isolates**



**Table-1: Age wise and sex wise distribution of bacterial isolates**

Age wise group	Male	Female	Total
0-20	1	1	2
21-40	6	3	9
41-60	13	6	19
61-80	8	6	14
80 + yr	1	0	1
<b>Total {n (%)}</b>	<b>29 (64.45%)</b>	<b>16 (35.55%)</b>	<b>45 (100%)</b>

**Table-2: Distribution of bacterial isolates by ward in the study population**

Hospital Ward	No. of Isolates
RICU	31
MICU	2
SICU	2

ICCU	2
PICU	1
General Medicine	2
Respiratory Medicine	5
<b>Total (n)</b>	<b>45</b>

**Table-3: Spectrum of bacterial isolates from BAL fluids**

Isolates	Male	Female	Total (%)
<i>Klebsiella pneumoniae</i>	11	4	15 (33.33)
<i>Pseudomonas aeruginosa</i>	7	2	9 (20)
<i>Staphylococcus aureus</i>	1	4	5 (11.11)
<i>Acinetobacter spp.</i>	2	3	5 (11.11)
<i>Escherichia coli</i>	3	-	3 (6.67)
<i>Citrobacter spp.</i>	1	2	3 (4.45)
<i>Enterococcus spp.</i>	2	-	2 (4.45)
<i>Serratia spp.</i>	1	1	2 (4.45)
<i>Non-fermenter spp.</i>	1	-	1 (2.21)
<b>Total (n)</b>	<b>29</b>	<b>16</b>	<b>45 (100)</b>

**Table-4: Bacterial isolates in different age groups**

Isolates	0-20	21-40	41-60	61-80	80 + yrs	Total
<i>Klebsiella pneumoniae</i>	4	3	3	3	2	15
<i>Pseudomonas aeruginosa</i>	2	3	2	1	1	9
<i>Staphylococcus aureus</i>	-	2	2	1	-	5
<i>Acinetobacter spp.</i>	-	2	2	1	-	5
<i>Escherichia coli</i>	-	1	1	1	-	3
<i>Citrobacter spp.</i>	-	1	1	1	-	3
<i>Enterococcus spp.</i>	-	1	1	-	-	2
<i>Serratia spp.</i>	-	-	1	1	-	2
<i>Non-fermenter spp.</i>	-	-	1	-	-	1
<b>Total (n)</b>	<b>6</b>	<b>13</b>	<b>14</b>	<b>9</b>	<b>3</b>	<b>45</b>

**Table-5: Antimicrobial resistance (%) of various Gram-positive isolate**

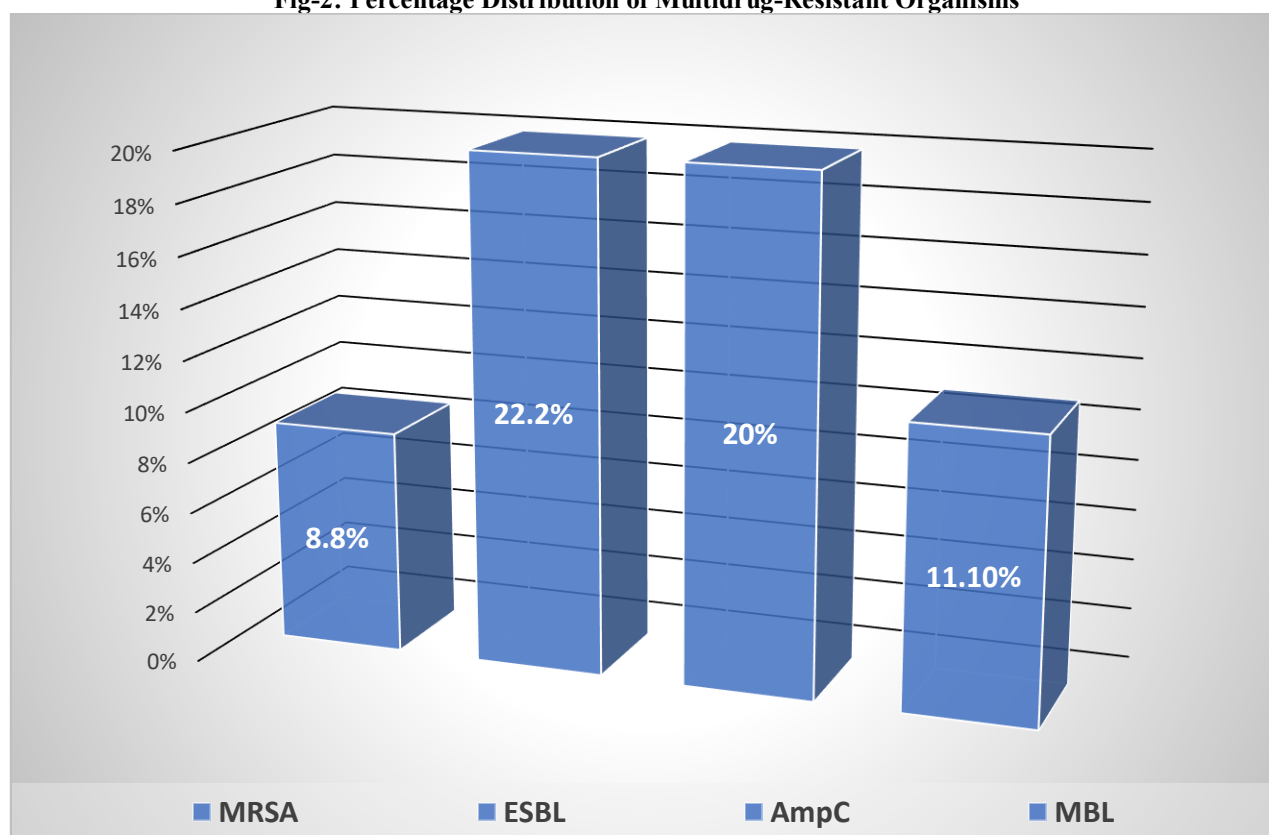
Organism	<i>Staphylococcus aureus</i> (n=5)		<i>Enterococcus spp.</i> (n=2)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Ampicillin	-	-	1(50)	1(50)
Penicillin	0	5(100)	1(50)	1(50)
Gentamycin	3(60)	2(40)	-	-
Ciprofloxacin	0	5(100)	-	-
Levofloxacin	0	5(100)	-	-
Erythromycin	1(20)	4(80)	1(50)	1(50)
Tetracycline	1(20)	4(80)	1(50)	1(50)
Doxycycline	1(20)	4(80)	1(50)	1(50)
Clindamycin	3(60)	2(40)	-	-
Cotrimoxazole	2(40)	3(60)	2(100)	0
chloramphenicol	-	-	1(50)	1(50)
Cefoxitin	1(20)	4(80)	-	-
Linezolid	5(100)	0	2(100)	0
Vancomycin	5(100)	0	2(100)	0
Teicoplanin	-	-	1(50)	1(50)
High level Gentamycin	-	-	1(50)	1(50)

**Table-6: Antimicrobial resistance (%) of various Gram-negative isolates**

Organism	<i>Klebsiella pneumoniae</i> (n=15)	<i>E.coli</i> (n=3)	<i>Pseudomonas aeruginosa</i> (n=9)	<i>Acinetobacter spp.</i> (n=5)	<i>Citrobacter spp.</i> (3)	<i>Serratia spp.</i> (2)	<i>Non-fermenters</i> (1)

Antibiotics	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
Ampicillin	-	-	33.4	66.6	-	-	-	-	-	-	-	-	-	-
Amoxicillin Clavulanate	26.6	73.4	0	100			-	-	33.4	66.6	50	50	-	-
Piperacillin-Tazobactam	66.6	33.4	0	100	100	33.4	60	40	33.4	66.6	50	50	0	100
Cefazolin	20	80	33.4	66.6	-	-	-	-	33.4	66.6	-	-	-	-
Cefuroxime	40	60	34.4	66.6	-	-	-	-	33.4	66.6	50	50	-	-
Cefoxitin	40	60	34.4	66.6	-	-	-	-	-	-	50	50	-	-
Ceftriaxone	33.4	66.6	0	66.6	-	-	20	80	33.4	66.6	50	50	0	100
Cefotaxime	33.4	66.6	100	0	-	-	20	80	33.4	66.6	50	50	0	100
Cefixime	33.4	66.6	100	0	-	-	20	80	33.4	66.6	50	50	0	100
Ceftazidime	46.6	53.4	100	0	44.5	55.5	40	60	33.4	66.6	50	50	0	100
Cefepime	46.6	53.4	0	100	66.6	33.4	40	60	33.4	66.6	50	50	0	100
Aztreonam	53	47	34.4	66.6	66.6	33.4	-	-	33.4	66.6	50	50	-	-
Imipenem	66.6	33.4	34.4	66.6	88.9	11.1	60	40	100	0	50	50	0	100
Meropenem	66.6	33.4	34.4	66.6	88.9	11.1	60	40	100	0	50	50	0	100
Gentamicin	66.6	33.4	34.4	66.6	66.6	33.4	20	80	100	0	50	50	0	100
Amikacin	46.6	53.4	34.4	66.6	55.6	44.4	20	80	100	0	50	50	0	100
Tetracycline	46.6	53.4	34.4	66.6	-	-	20	80	100	0	50	50	0	100
Doxycycline	46.6	53.4	34.4	66.6	-	-	20	80	100	0	100	0	100	0
Ciprofloxacin	60	40	0	100	66.6	33.4	20	80	66.6	33.4	0	100	0	100
Levofloxacin	60	40	0	100	55.6	44.4	20	80	33.4	66.6	0	100	0	100
Cotrimoxazole	60	40	34.4	66.6	-	-	20	80	33.4	66.6	50	50	100	0
Tigecycline	100	0	100		-	-	100	0	100	0	100	0	100	0

Fig-2: Percentage Distribution of Multidrug-Resistant Organisms



**Table-7: Distribution of biofilm production among isolated pathogens.**

Organism	Biofilm		Total n (%)
	Nonproducer (%)	Producer (%)	
<i>Klebsiella pneumoniae</i>	10 (22.2)	5 (11.1)	15 (33.3)
<i>Pseudomonas aeruginosa</i>	5 (11.1)	4 (8.8)	9 (19.9)
<i>Staphylococcus aureus</i>	3 (6.6)	2 (4.4)	5 (11.0)
<i>Acinetobacter spp.</i>	0	5 (11.1)	5 (11.1)
<i>Escherichia coli</i>	2 (4.4)	1 (2.3)	3 (6.7)
<i>Citrobacter spp.</i>	2 (4.4)	1 (2.3)	3 (6.7)
<i>Enterococcus spp.</i>	1 (2.3)	1 (2.3)	2 (4.6)
<i>Serratia spp.</i>	0	2 (4.4)	2 (4.6)
<i>Non-fermenter spp.</i>	0	1 (2.3)	1 (2.3)
<b>Total n (%)</b>	<b>23 (51)</b>	<b>22 (49)</b>	<b>45 (100)</b>

**DISCUSSION:**

The present study evaluated the bacteriological profile, antimicrobial resistance patterns, and biofilm production among BAL isolates in a tertiary care hospital in Central India. The present study findings are compared with national and international studies to highlight similarities, differences, and emerging trends.

The culture positivity rate in this study was 16.07%, which lies within the range reported by similar Indian studies. *Hammad et al.*(2019) reported a positivity of 18%,<sup>15</sup> while *Patel et al.*(2018) observed 15.3% positivity.<sup>16</sup> Studies from Western countries report slightly higher positivity rates, from 20–30%,<sup>17,18</sup> possibly due to differences in sampling technique, prior antibiotic use and disease severity. This study result is thus consistent with most Indian data but slightly lower than global figures.

In this study, predominately males accounted for 64.45% of culture-positive cases.(Fig-1) This is comparable to findings by *Sharma et al.*(2018) (68%)<sup>19</sup> and *Afolabi et al.*(2021) (62%).<sup>20</sup> Many studies attribute this male predominance to lifestyle factors such as higher smoking rates, occupational exposure, and environmental pollutants. However, lower male predominance (55–58%) has been reported in UK and US studies,<sup>21</sup> suggesting regional differences in risk exposure and healthcare access. Thus, the present study results align more closely with South Asian studies showing higher male susceptibility.

The highest positivity in this study was among the 41–60-year age group, followed by the elderly (Table-1) & (Table-4). This pattern is similar to findings by *Kumar et al.*(2019) (45–65 years)<sup>22</sup> and *Torres et al.*(2021) (50–70 years).<sup>23</sup> In contrast, a study by *Kollef et al.* (2017) reported older age groups (>65 years) as most affected.<sup>24</sup> These differences may be due to variations in the prevalence of chronic lung diseases and ICU admissions. Hence, this study findings are more consistent with most regional studies but differ slightly from Western literature where elderly dominance is more pronounced.

Most isolates were obtained from RICU followed by Respiratory Medicine.(Table-2) This correlates with the findings of *Gandra et al.*(2019) who also reported ICU predominance of BAL pathogens in India.<sup>25</sup> International studies similarly show higher bacterial recovery rates in ICU patients due to invasive ventilation and severe illness.<sup>26,27</sup> The low positivity from PICU in this study aligns with paediatric studies showing lower BAL yield in children.<sup>28</sup> Overall, in present study ward distribution is consistent with both Indian and global ICU-based findings.

Gram-negative organisms constituted 84.4% of isolates in the present study. This finding closely matches the proportions reported in study by *Gupta et al.*(2017) (85%)<sup>29</sup> and *Taneja et al.* (2019) (82%).<sup>30</sup> Western studies, however, report a slightly lower gram-negative prevalence (60–70%),<sup>31</sup> likely due to better infection control and lower antibiotic pressure. Our study found *Klebsiella pneumoniae* as the dominant pathogen (33.3%). This is comparable to *Gupta et al.*(2017)<sup>29</sup> 32% & *Khurana et al.*(2021)<sup>32</sup> 35%. However, some studies report *Pseudomonas* as the predominant organism,<sup>33</sup> reflecting geographical variations. In this study observed *Pseudomonas* in 20%, similar with *Taneja et al.*(2019) 18%<sup>30</sup> and *Gandra et al.*(2019) 21%.<sup>25</sup> But lower compared to Western ICU studies reporting 25–30% prevalence.<sup>26</sup> (Table-3)

Gram-positive organism especially *S. aureus* found 11.11%, similar to findings by *Miller et al.*(2014) (10–12%).<sup>34</sup> (Table-3) MRSA rates vary worldwide; our lower MRSA proportion (8.8%) mirrors some Indian centers.<sup>28</sup> Thus, the overall isolate pattern in our study similar with most Indian publications and reflects a strong Gram-negative predominance in ICU respiratory infections.

In our study *S. aureus* found high resistance to penicillin, ciprofloxacin & cefoxitin observed (80-100%), consistent with *Gadepalli et al.*(2019) 80–100%<sup>35</sup> & *Taneja et al.* (2019) 75–85%.<sup>30</sup> MRSA prevalence in our study 8.8% was comparatively lower than the 12–25% range reported by other Indian studies.<sup>35,36</sup> This difference might be due to smaller sample size or better infection control.(Table-5)

Our study demonstrated high multidrug resistance in *Klebsiella*, *Pseudomonas*, and *Acinetobacter*, aligning with Shebl *et al.*(2020) 60–80% resistance to cephalosporins<sup>37</sup> and Livermore *et al.*(2012) widespread  $\beta$ -lactam resistance.<sup>38</sup> (Table-6) Carbapenem resistance in our isolates is similar to the 30–40% range reported in regional ICUs.<sup>32,39</sup> The detection of ESBL (22.2%), AmpC (20%) and MBL (11.1%) similar to data from Indian tertiary hospitals.<sup>30–32</sup> Thus, our antimicrobial resistance patterns are consistent with both Indian and worldwide trends showing rising MDR Gram-negative pathogens.(Fig-2)

Biofilm production was seen in 49% of isolates. Similar rates were documented by Singh *et al.*(2017) 45–55%.<sup>40</sup> *Klebsiella* and *Pseudomonas* were major biofilm producers, also matching previous research.<sup>40</sup> Biofilm-positive isolates typically exhibit higher MDR, which was also reflected in our findings. Thus, the biofilm trends in our study align strongly with global evidence. (Table-7)

## CONCLUSION:

The present study highlights a substantial burden of bacterial pathogens and antimicrobial resistance among bronchoalveolar lavage specimens from patients with lower respiratory tract infections in a tertiary care hospital of Central India. Gram-negative organisms predominated, with *Klebsiella pneumoniae* emerging as the most frequently isolated pathogen, followed by *Pseudomonas aeruginosa* and *Acinetobacter* species. Male patients and individuals belonging to the middle-aged group were more commonly affected. Most isolates were recovered from intensive care units, underscoring the increased susceptibility of critically ill patients to lower respiratory tract infections.

A high level of antimicrobial resistance was observed among the isolates, with a substantial proportion exhibiting multidrug resistance including ESBL, AmpC, and MBL production. Although MRSA prevalence was comparatively low, resistance to commonly used antibiotics among Gram-positive organisms remained a concern. Carbapenems and agents such as linezolid and vancomycin retained better efficacy, underscoring their role as last-line therapies.

Nearly half of the isolates demonstrated biofilm-forming ability, particularly among *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp., which further contributes to persistent infections, treatment failure, and increased resistance. The coexistence of biofilm production and multidrug resistance poses a serious therapeutic challenge in managing lower respiratory tract infections.

Overall, this study reinforces the importance of BAL culture and antimicrobial susceptibility testing in guiding appropriate therapy. Regular surveillance of local pathogen profiles, resistance mechanisms and biofilm formation is essential to optimize empirical treatment, prevent the spread of resistant organisms, and support effective antibiotic stewardship practices in tertiary care settings.

## Conflict of Interest- Nil

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