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Trends in the Microbial Profile of Bronchoalveolar Lavage Samples from Patients with Lower Respiratory Tract Infections

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ABSTRACT

Introduction: Lower respiratory tract infections (LRTIs) contribute substantially to global morbidity and mortality, with bacterial and fungal causative agents exhibiting regional and temporal variations. Evolving antimicrobial resistance patterns among bacterial pathogens pose challenges to empirical treatment strategies. This study aimed to identify the etiological agents of LRTIs and characterize their antimicrobial resistance profiles. **Methods**: Bronchoalveolar lavage (BAL) fluid was obtained from adult patients with suspected LRTIs undergoing bronchoscopy at a tertiary care center in India between August 2021 and December 2022, and processed using standard microbiological techniques for bacterial and fungal pathogen identification. Antimicrobial susceptibility testing (AST) was performed on isolated pathogens using the Kirby-Bauer disk diffusion method. Data were analyzed using descriptive statistics with Microsoft Excel. **Results**: Among 86 BAL samples, 33 (38.4%) yielded positive cultures, with 31 bacterial and 2 fungal isolates. Among the bacterial isolates, *Klebsiella pneumoniae* was the most frequent organism (36.4%), followed by *Acinetobacter* spp. (18.2%). The fungal isolates were identified as *C. albicans*. Among *K. pneumoniae* isolates, resistance to cephalosporins ranged from 66.7% to 100%, with the lowest resistance observed against piperacillin-tazobactam (25%). Among Gram-negative bacterial isolates, 60% of bacterial isolates were extended-spectrum β-lactamase (ESBL) producers, 36% were metallo-β-lactamase (MBL) producers, and 48% were carbapenemase producers. Both *C. albicans* isolates were susceptible to fluconazole and voriconazole, while one isolate exhibited resistance to itraconazole and the other to ketoconazole. **Conclusions**: This study found that Gram-negative bacteria were the predominant etiological agents of LRTIs, exhibiting high resistance to commonly used empirical antibiotics, such as cephalosporins and carbapenems. Notably, resistance to aminoglycosides was lower than to cephalosporins and carbapenems, which may warrant further investigation into local prescribing patterns. These findings highlight the variability of antimicrobial susceptibility and emphasize the critical need for accurate clinical and microbiological diagnosis, along with the development of evidence-based institutional antibiotic policies for the empirical management of LRTIs.

INTRODUCTION

Lower respiratory tract infections (LRTIs) are a common cause of morbidity and mortality worldwide, characterized by symptoms such as cough, sputum production, dyspnea, wheezing, and/or chest pain or discomfort, typically persisting for 1–3 weeks in acute cases [1]. LRTIs are the leading infectious cause of death in low-income countries and rank among the top ten overall causes of mortality in high-income economies [2]. A 2019 Global Burden of Disease study on the global burden of disease attributed approximately 2.49 million deaths to LRTIs, positioning them as the sixth leading cause of mortality worldwide and the leading cause of death among children under 5 years of age [3]. LRTIs are

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significant health concerns throughout the lifespan, frequently affecting young children and older adults [4].

Commonly identified pathogens in LRTIs include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, with their identification guiding the selection of appropriate empirical antimicrobial therapy [5]. Common LRTI pathogens, such as *S. pneumoniae* and *K. pneumoniae* utilize virulence factors, such as capsules for immune evasion, complicating treatment amid rising resistance [6]. For instance, encapsulated bacteria like *S. pneumoniae* and *K. pneumoniae* evade immune clearance, enhance adherence, and facilitate biofilm formation, promoting their persistence and proliferation. *S. aureus* produces toxins and enzymes that cause tissue damage, while *P. aeruginosa* exhibits rapid growth, toxin production, and flagella-mediated motility, facilitating dissemination.

These virulence factors, combined with rising antimicrobial resistance due to inappropriate antibiotic use, agricultural practices, and the spread of resistant organisms, pose a significant threat to public health [7]. This threat is exacerbated by inappropriate antibiotic use before culture results, necessitating updated pathogen and resistance data [8].

Despite regional variations, data from Indian tertiary centers are limited, necessitating this study to characterize local etiological agents and resistance patterns. Therefore, up-to-date knowledge of the causative pathogens and their antimicrobial susceptibility profiles is essential for guiding appropriate therapeutic decisions. This study aimed to identify the etiological agents of LRTIs and characterize their antimicrobial resistance patterns in adult patients with LRTI symptoms undergoing bronchoscopy with bronchoalveolar lavage at a tertiary care center in India.

MATERIAL AND METHODS

Study design and setting. This prospective cross-sectional study was conducted at the Department of Microbiology, in a tertiary care center in central India, from August 2021 to December 2022. BAL fluid specimens were collected from adult patients in the Department of Respiratory Medicine who showed no clinical response to ≥5 days of empirical antimicrobial therapy and required bronchoscopy for diagnostic evaluation.

Ethical considerations. The study was approved by the Institutional Ethics Committee at Government Medical College, Nagpur, India, on January 2, 2021.

Inclusion and exclusion criteria. Adult patients (≥18 years) with suspected LRTIs who showed no response to ≥5 days of empirical therapy and underwent bronchoscopy were included, while pediatric patients (<18 years), pregnant women, and those with active pulmonary tuberculosis or immunosuppressive conditions were excluded.

Sample collection. BAL fluid was collected from eligible adult patients admitted to the chest medicine ward after obtaining written informed consent. Patients were informed about the bronchoscopy procedure, its risks, benefits, and alternatives before providing consent.

Sample processing. A total of 86 BAL fluid specimens were processed in the microbiology laboratory following standard bronchoscopy collection by respiratory physicians. Upon receipt, BAL fluid specimens were examined for color, turbidity, mucopurulent appearance, blood, or pigmentation. Direct smears were examined using Gram stain for bacteria and yeast, Ziehl-Neelsen stain for acid-fast bacilli, and 10% potassium hydroxide (KOH) mount for fungal elements.

Culture and identification. BAL fluid specimens were cultured on blood agar, MacConkey agar, chocolate agar, and Sabouraud dextrose agar for bacterial and fungal identification. Blood and MacConkey agar plates were incubated aerobically at 37°C for 18–24 h, chocolate agar plates at 37°C in 5% CO_2 for 24–48 h, and Sabouraud dextrose agar at 25°C for up to 3 weeks. Bacterial isolates were identified to the species level based on colony morphology, Gram staining, and biochemical tests (indole, methyl red, Voges-Proskauer, triple sugar iron, citrate, and urea hydrolysis).

Antimicrobial susceptibility testing (AST). AST was performed on bacterial isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following CLSI M100 (2020) guidelines [9]. Antimicrobial panels, were selected based on organism identification. Interpretation of inhibition zone diameters was performed according to CLSI M100, 2020.

Organism growth and antimicrobial susceptibility data were collected and analyzed. Descriptive statistics, including percentages, were generated using Microsoft Excel.

RESULTS

Study population characteristics. Of the 86 patients with clinically diagnosed LRTIs, 52 (60.5%) were male and 34 (39.5%) were female. The majority (68.6%) of patients were aged 51–70 years. Positive cultures were observed in 33 (38.4%) of the 86 BAL samples. The distribution of microorganisms isolated from these positive cultures is summarized in Table 1.

Culture results. As shown in Table 1 and Figure 1, *K. pneumoniae* was the most frequently isolated organism, accounting for 12 (36.4%) of the 33 isolates, followed by *Acinetobacter* spp. (6 isolates, 18.2%).

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Table 1. Distribution of microorganisms isolated from bronchoalveolar lavage fluid (n=33)

Organism type	Number of isolates	Percentage (%)
Bacteria	31	93.9
Fungi	2	6.1
Total	**33**	**100**

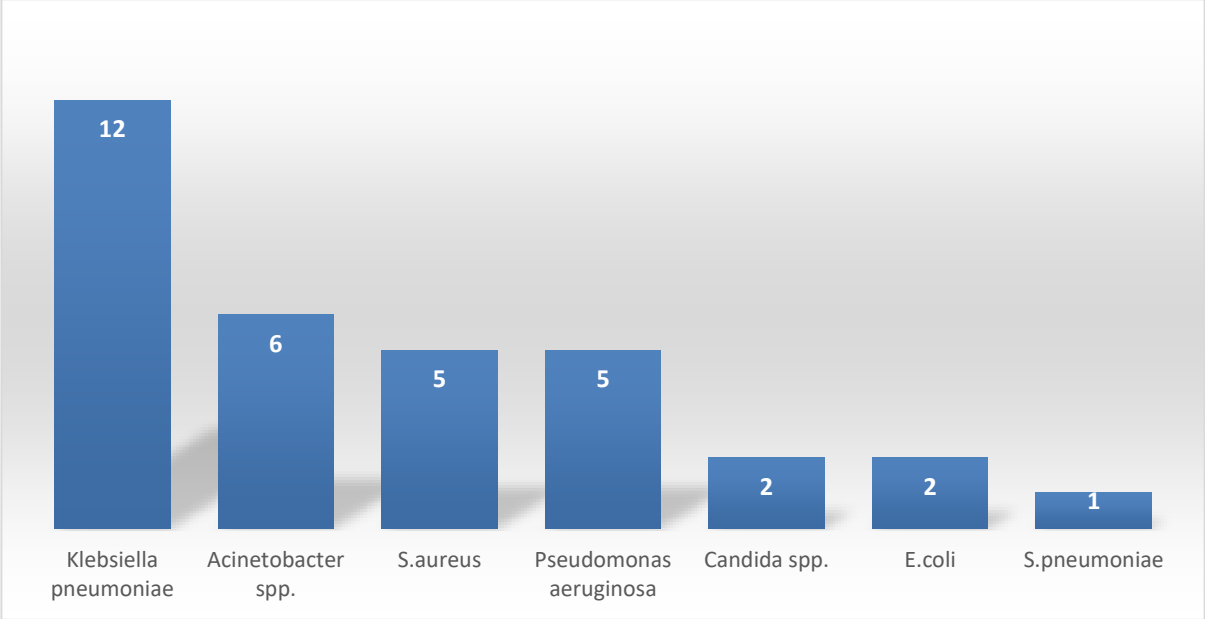

Fig. 1. Distribution of bacterial and fungal isolates from bronchoalveolar lavage fluid

Antimicrobial susceptibility. Antimicrobial susceptibility patterns of bacterial isolates are presented in Tables 2 and 3. As shown in Table 2, all *S. aureus* isolates were resistant to penicillin, and 60% were resistant to cefoxitin, indicating methicillin-resistant *S. aureus* (MRSA). A smaller proportion of *S. aureus* isolates showed resistance to gentamicin (20%), erythromycin (40%), and doxycycline (40%). All *S. aureus* isolates were susceptible to linezolid; vancomycin susceptibility was not tested (ND). In contrast, the single *S. pneumoniae* isolate was susceptible to penicillin, erythromycin, vancomycin, clindamycin, linezolid, levofloxacin, and trimethoprim/sulfamethoxazole (Table 2).

Table 2. Antimicrobial resistance patterns of *S. aureus* and *S. pneumoniae* isolates

Antimicrobial	*S. aureus* (n=5)	*S. pneumoniae* (n=1)
Penicillin (P)	5 (100%)	0 (0%)
Cefoxitin (CX)	3 (60%)	ND
Vancomycin (VA)	ND	0 (0%)
Gentamicin (GEN)	1 (20%)	ND
Doxycycline (DO)	2 (40%)	ND
Erythromycin (E)	2 (40%)	0 (0%)
Clindamycin (CD)	3 (60%)	0 (0%)
Linezolid (LZ)	0 (0%)	0 (0%)
Ciprofloxacin (CIP)	3 (60%)	ND
Levofloxacin (LE)	ND	0 (0%)
Trimethoprim/Sulfamethoxazole (COT)	ND	0 (0%)

ND: Not determined, as CLSI guidelines do not recommend routine testing for certain organism-antibiotic combinations.

As shown in Table 3, all *K. pneumoniae* isolates were resistant to ampicillin. The lowest resistance rate for *K. pneumoniae* (25%) was observed against piperacillin-tazobactam. *P. aeruginosa* isolates displayed high resistance rates to ceftazidime (80%) and cefepime (60%) but were fully susceptible to levofloxacin. Among *Acinetobacter* spp. isolates, 83.3% were resistant to ceftazidime and meropenem, while 66.7% were resistant to cefepime, piperacillin-tazobactam, and gentamicin. Notably, 83.3% of *Acinetobacter* spp. isolates were susceptible to minocycline. Among Gram-negative isolates, 60% were extended-spectrum β-lactamase (ESBL) producers, 36% were metallo-β-lactamase (MBL) producers, and 48% were carbapenemase producers, determined by CLSI-recommended phenotypic tests. Both *C. albicans* isolates were susceptible to fluconazole and voriconazole, while one exhibited resistance to itraconazole and the other to ketoconazole, determined by CLSI M44 disk diffusion testing.

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Table 3. Antimicrobial resistance patterns of Gram-negative bacterial isolates (n=25)

Antimicrobial	*K. pneumoniae* (n=12) (%)	*P. aeruginosa* (n=5) (%)	*Acinetobacter* spp. (n=6) (%)	*E. coli* (n=2) (%)
Ampicillin (AMP)	12 (100)	ND	ND	2 (100)
Cefazolin (CZ)	8 (66.7)	ND	ND	1 (50)
Cefuroxime (CXM)	9 (75)	ND	ND	2 (100)
Cefotaxime (CTX)	11 (91.7)	ND	ND	1 (50)
Ceftazidime (CAZ)	ND	4 (80)	5 (83.3)	ND
Cefepime (CPM)	10 (83.3)	3 (60)	4 (66.7)	0 (0)
Piperacillin/Tazobactam (PIT)	3 (25)	1 (20)	2 (33.3)	1 (50)
Amoxicillin/Clavulanate (AMC)	8 (66.7)	ND	ND	0 (0)
Ampicillin/Sulbactam (AMS)	ND	ND	4 (66.7)	ND
Meropenem (MRP)	8 (66.7)	2 (40)	5 (83.3)	0 (0)
Gentamicin (GEN)	7 (58.3)	2 (40)	3 (50)	0 (0)
Amikacin (AK)	6 (50)	1 (20)	3 (50)	0 (0)
Minocycline (MI)	ND	ND	1 (16.7)	ND
Ciprofloxacin (CIP)	7 (58.3)	ND	ND	2 (100)
Levofloxacin (LE)	ND	0 (0)	4 (66.7)	ND
Trimethoprim/Sulfamethoxazole (COT)	7 (58.3)	ND	2 (33.3)	0 (0)
Netilmicin (NET)	ND	1 (20)	ND	ND

ND: Not determined, as CLSI guidelines do not recommend routine testing for certain organism-antibiotic combinations.

DISCUSSION

The COVID-19 pandemic (2020–2022), overlapping with the study period, likely reduced BAL specimen collection due to minimized aerosol-generating procedures.

LRTIs contribute significantly to morbidity, mortality, and healthcare costs. This cross-sectional study examined the microbial profile of BAL fluid from adult patients with clinically diagnosed LRTIs at a tertiary care center in India. Our findings showed a male predominance (60.5%), consistent with studies by Panda *et al*. (2012, 63%), Vijay *et al*. (2016, 66%), and Ravichitra *et al*. (2019, 71.2%) [10-12]. Male predominance in LRTIs may be due to higher smoking, tobacco, and alcohol use among men. These factors impair respiratory immunity via mucociliary clearance dysfunction, mucus hypersecretion, airway obstruction, and comorbidities. However, further research is needed to clarify the interplay of gender-related biological, behavioral, and social factors in LRTI susceptibility.

Most patients (68.6%) were aged 51–70 years, reflecting increased LRTI susceptibility in older adults. Increased LRTI susceptibility in this age group is due to age-related declines in immune and pulmonary function. Chronic respiratory conditions (*e.g*., COPD, emphysema, bronchiectasis, post-tuberculosis sequelae) further predispose this population to Gram-negative infections. Cumulative antibiotic exposure in older adults may drive antibiotic-resistant pathogens, complicating LRTI management.

Of 86 BAL specimens, 38.4% yielded positive cultures, consistent with Padmaja *et al*. (2021, 38.52%), Dickson *et al*. (2014, 39.1%), and Kneidinger *et al*. (2013, 32.4%) [13-15]. Of the isolates, 93.9% were bacterial and 6.1% were fungal. These findings align with Ramana *et al*. (2013; 90.3% bacterial, 9.7% fungal) and Sarmah *et al*. (2016; 82.6% bacterial, 17.4% fungal) [16, 8]. Gram-negative bacteria predominated in LRTIs, consistent with Palewar *et al*. (2021), Gebre *et al*. (2021), and Padmaja *et al*. (2021) [13, 17, 18]. These studies reported 76–94% Gram-negative bacilli and 5.8–24% Gram-positive cocci.

K. pneumoniae was the most frequently isolated organism, followed by *Acinetobacter* spp. and *S. aureus*. These findings align with Padmaja *et al*. (2021) and Madhavi *et al*. (2012), who identified *K. pneumoniae* and *P. aeruginosa* as predominant LRTI pathogens [13, 19]. This predominance of Gram-negative pathogens informs empirical antimicrobial therapy selection, as highlighted by recent guidelines [20]. Clinicians must monitor these etiological patterns to ensure appropriate antibiotic use in LRTI management. Conventional culture-based methods used in this study may limit detection of fastidious or non-culturable organisms, highlighting the role of microbiome analysis [21].

Among *S. aureus* isolates, resistance was most frequently observed against penicillin, followed by cefoxitin and ciprofloxacin, and then clindamycin. Lower resistance to gentamicin (20%) was observed in *S. aureus* isolates. Notably, cefoxitin resistance, which is suggestive of methicillin resistance, was observed in 60% of *S. aureus* isolates. Hoban *et al*. (2003), Bajpai *et al*. (2013), and Rajkumar *et al*. (2016) reported MRSA rates of 43.7%, 55.6%, and 48.2%, respectively [22-24], indicating high MRSA prevalence. All *S. aureus* isolates were susceptible to linezolid, consistent with findings from Lee *et al*. (2018) and Bajpai *et al*. (2013) [21, 22]; vancomycin susceptibility was not tested in our isolates [23, 24]. Santella *et al*. (2021) reported that *S. aureus* isolates were 84% resistant to penicillin but fully susceptible to linezolid [25].

The antimicrobial susceptibility profile of *K. pneumoniae* isolates showed high resistance, consistent with Kumar *et al*. (2013) and Bajpai *et al*. (2013) [23, 26]. Kumar *et al*. (2013) and Bajpai *et al*. (2013) reported *K. pneumoniae* resistance rates of 7.3% and 28.9% for amikacin, 58.7% and 39.6% for gentamicin, and high

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resistance to β-lactam agents [23, 26]. A study by Khan *et al*. (2015) reported a 70% gentamicin resistance rate in *K. pneumoniae* [27]. High β-lactam resistance in *K. pneumoniae* raises concerns about the efficacy of empirical LRTI treatments, driven by molecular resistance mechanisms [28]. Lower gentamicin resistance (58.3%) in *K. pneumoniae* compared to Khan *et al*. (2015, 70%) may reflect reduced empirical use [27]. These resistance patterns highlight the need for ongoing surveillance and local susceptibility data to guide empirical antibiotic selection and reduce resistance.

High resistance in *Acinetobacter* spp. isolates aligns with Chung *et al*. (2011) and Shete *et al*. (2010) [29, 30]. Chung *et al*. (2011) reported resistance rates of 78.2% for ceftazidime, 75.9% for ampicillin-sulbactam, and 76.7% for piperacillin-tazobactam in *Acinetobacter* spp. [29]. Shete *et al*. (2010) reported 71.4% ceftazidime and 42.8% amikacin resistance, while Sohail *et al*. (2016) found 99.6% ampicillin-sulbactam, 98.3% cefepime, and 99.2% ceftazidime resistance in *Acinetobacter* spp. [31]. High resistance in *Acinetobacter* spp. is due to its ability to acquire and disseminate resistance genes and persist in hospital environments, consistent with recent multicenter data [32]. *P. aeruginosa* susceptibility patterns align with Tripathi *et al*. (2011), who reported higher ceftazidime and cefepime resistance and lower amikacin and meropenem resistance [33]. Ramana *et al*. (2013) reported higher cephalosporin resistance and lower aminoglycoside and carbapenem resistance in Gram-negative bacteria [16]. *P. aeruginosa*'s ability to acquire resistance mechanisms highlights the risks of injudicious antibiotic use, promoting resistant strain emergence.

Among Gram-negative isolates, 15/25 (60%) were ESBL producers, 36% were MBL producers, and 48% were carbapenemase producers, determined by CLSI-recommended phenotypic tests. These findings align with Gupta *et al*. (2017), who reported 54.5% ESBL and 22.1% MBL producers among Gram-negative isolates [34]. Similarly, Radhika *et al*. (2015) found that 43.5% of *K. pneumoniae* isolates produced either MBL or carbapenemase enzymes [35]. High ESBL, MBL, and carbapenemase prevalence in Gram-negative isolates underscores the need for strategies to combat antimicrobial resistance, as evidenced by recent surveillance data [36].

Both *C. albicans* isolates were susceptible to fluconazole and voriconazole, per CLSI M44 testing. However, one C. albicans isolate was resistant to itraconazole and another to ketoconazole, indicating potential antifungal resistance, consistent with recent Indian data [37].

In conclusion, Gram-negative bacilli, primarily *K. pneumoniae* and *Acinetobacter* spp., are the leading LRTI pathogens in our setting. We observed high resistance to empirical antibiotics, including third-generation cephalosporins and carbapenems. Aminoglycoside resistance was lower than cephalosporin and carbapenem resistance. ESBL, MBL, and carbapenemase production are major mechanisms of β-lactam resistance. Understanding these resistance mechanisms is crucial to avoid unnecessary β-lactam use and mitigate cross-resistance. Incorporating β-lactam/β-lactamase inhibitor combinations, such as piperacillin-tazobactam, may improve empirical LRTI treatment outcomes. Variable LRTI etiology and resistance patterns require tailored antimicrobial therapy. Collaboration between clinicians and microbiologists is essential for monitoring microbial trends and antibiotic susceptibility patterns. Regular research is crucial to update empirical treatment guidelines, optimizing patient care and reducing resistance.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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