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Antibiotic Resistance Rate among Bacterial Pathogens Isolated from Bronchoalveolar Lavage Fluid at a Tertiary Care Center in Western Uttar Pradesh, India

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ABSTRACT

Introduction: Lower respiratory tract infections (LRTIs) are a common global health problem, and antibiotic resistance remains a significant concern for doctors. This study aimed to determine the prevalence of antibiotic resistance among bacterial pathogens isolated from bronchoalveolar lavage (BAL) fluid at a tertiary care center in Western Uttar Pradesh. **Methods:** A cross-sectional study was conducted from January 2021 to June 2022, in which BAL fluid samples were collected from patients attending the tertiary care center. The samples were processed for bacterial culture and antimicrobial susceptibility testing. **Results:** Out of 112 BAL samples cultured, 84 showed growths of bacterial pathogens, with 82 (97.6%) being Gram-negative bacteria and 29 (35%) of these being extended-spectrum beta-lactamase (ESBL) producers. The percentage of multiple drug-resistant (MDR) isolates was 77.38% (65/84). The Gram-negative isolates were most sensitive to imipenem, followed by ciprofloxacin, amikacin, and tetracycline. Cephalosporins and piperacillin-tazobactam showed a high resistance pattern to these bacteria. The Gram-positive isolates were susceptible to linezolid and vancomycin. **Conclusion:** The high prevalence of ESBL-producing and MDR isolates in BAL samples highlights the need for the prudent administration of antibiotics and the creation of local antibiograms to guide empirical therapy. This study provides valuable information on the antimicrobial susceptibility patterns of bacterial pathogens causing LRTIs, which can aid in developing effective treatment strategies.

INTRODUCTION

Lower respiratory tract infections (LRTI) are a group of respiratory system inflammations that affect the trachea, bronchi, bronchioles, and alveoli, leading to the multiplication of infectious agents [1, 2]. Acute lower respiratory tract infections (ALRI) are among the most prevalent infectious diseases worldwide, causing significant morbidity and mortality in patients of all ages [1, 3]. Gram-negative bacilli (GNB) such as *Escherichia coli*, *Klebsiella pneumoniae*, Acinetobacter species, Pseudomonas aeruginosa, and Gram-positive organisms like *Staphylococcus aureus* and *Streptococcus pneumoniae*, are common respiratory pathogens [2]. Multidrug-resistant (MDR) Gram-negative bacteria, including extended-spectrum beta-lactamase (ESBL) producers, have become a growing concern in healthcare settings. ESBLs are enzymes that confer resistance to beta-lactam antibiotics and can lead to treatment failure and poor outcomes [4, 5, 6, 7, 8, 9].

The emergence of antibiotic resistance in respiratory infections is a significant global concern, and the misuse of antibiotics is a major contributor to resistance [11]. Moreover, the failure to de-escalate therapy based on culture and sensitivity results can also contribute to antibiotic resistance. Therefore, monitoring the epidemiology of respiratory infections is crucial to combat the rise of multidrug-resistant pathogens [11].

Bronchoalveolar lavage (BAL) is a useful diagnostic tool for diagnosing lung diseases and detecting respiratory infections. The fluid recovered from BAL can be used to identify white blood cell profiles and recover pathogens from the epithelial surface of the lower respiratory tract [12]. The bacteriological profile of pulmonary infections can vary within the same country due to differences in antibiotic use, environmental factors, and ventilation in critically ill patients. Therefore, BAL is an ideal sample

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for recovering pathogens from the lower respiratory tract to guide empirical therapy [2, 12].

This study aimed to assess the prevalence of ESBL and MDR pathogens in BAL samples from a tertiary care center in Western Uttar Pradesh, India.

MATERIAL AND METHODS

Study design and setting. This cross-sectional study was conducted at the Microbiology Department, Sharda Hospital, Greater Noida, India, from January 2021 to June 2022. The study included all bronchoalveolar lavage (BAL) fluid samples from the Bacteriology Laboratory.

Sample processing and identification. The BAL fluid samples were processed according to standard bacteriological procedures. The bacterial isolates were identified by conventional biochemical assays, including catalase and oxidase, coagulase, urease, citrate, indole, MR-VP, Hugh-Leifson's oxidative fermentative test, triple sugar iron test, nitrate reduction, and amino acid decarboxylation tests.

Antimicrobial susceptibility testing. The Kirby Bauer disc diffusion method was used to determine antibiotic susceptibility according to the Clinical and Laboratory Standards Institute (CLSI) standards. Commercially available antibiotic disks (Himedia, Mumbai, India) were used for antimicrobial susceptibility testing, and the zone diameters were interpreted according to CLSI guidelines.

Screening for ESBL-producing isolates. Isolates resistant to third-generation cephalosporins were screened for ESBL production and subjected to phenotypic confirmation.

Phenotypic confirmation of ESBL production. ESBL production was confirmed phenotypically using the CLSI-recommended combination disk approach, which involved the antibiotics ceftazidime and cefotaxime alone and the inhibitor clavulanic acid (30 g). ESBL producers had a 5 mm increase in the zone of inhibition around the discs containing cephalosporin plus clavulanate over the discs containing cephalosporin alone. *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853 was used as a positive control for ESBL, while *E. coli* ATCC 25922 was used as the negative control.

MDR distribution. Multiple drug resistance (MDR) was defined as an organism's antimicrobial resistance to at least one antimicrobial drug from three or more antimicrobial categories. The distribution of MDR isolates was determined based on their antimicrobial susceptibility patterns.

RESULTS

Study population. Of the 112 BAL samples processed, 82 (73.21%) belonged to males and 30 (26.79%) to females. The age of the patients ranged from 16 to 85 years (Fig. 1), with the majority (n=51; 45.54%) being above 60 years of age. Of the 112 samples, 51 (45.54%) were obtained from the MICU ward, followed by 23 (20.54%) from the respiratory ward and 16 (14.29%) from the SICU ward (Fig 2).

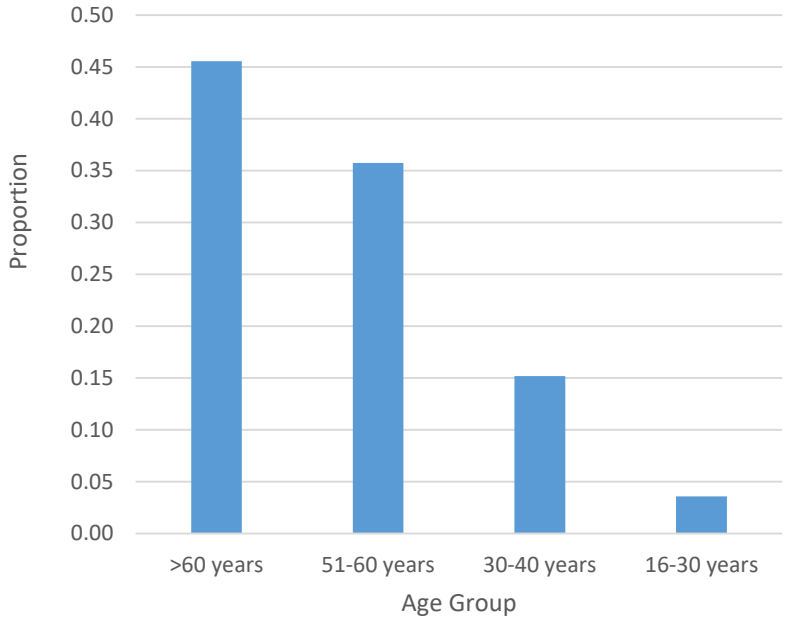

Fig. 1. Distribution of bacterial isolates by age group in the study population

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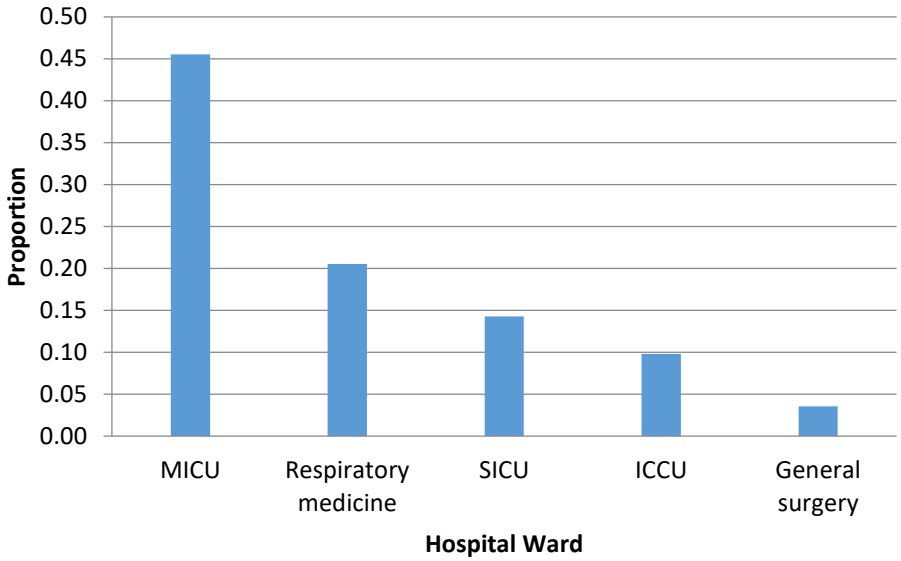

Fig. 2. Distribution of samples by ward in the study population

Bacterial pathogens. Of the 112 processed samples, 84 (75%) showed growth of bacterial pathogens, whereas 28 were negative. Of those 84 samples, 82 (97.6%) were Gram-negative, and 2 (2.4%) were Gram-positive. Among the Gram-negative bacteria (GNBs), *Acinetobacter* species (n=25; 29.76%) was the most frequently isolated organism, followed by *K. pneumoniae* (n=23; 27.38%), *P. aeruginosa* (n=17; 20.24%), and *E. coli* (n=14; 16.67%). *Citrobacter* and *Enterobacter* species accounted for 2 (2.4%) and 1 (1.19%) positive samples, respectively. Of the Gram-positive cocci, one was identified as Methicillin Resistant-Coagulase Negative Staphylococcus (1.19%) and the other as Beta hemolytic Streptococcus group A (1.19%) (Fig 3).

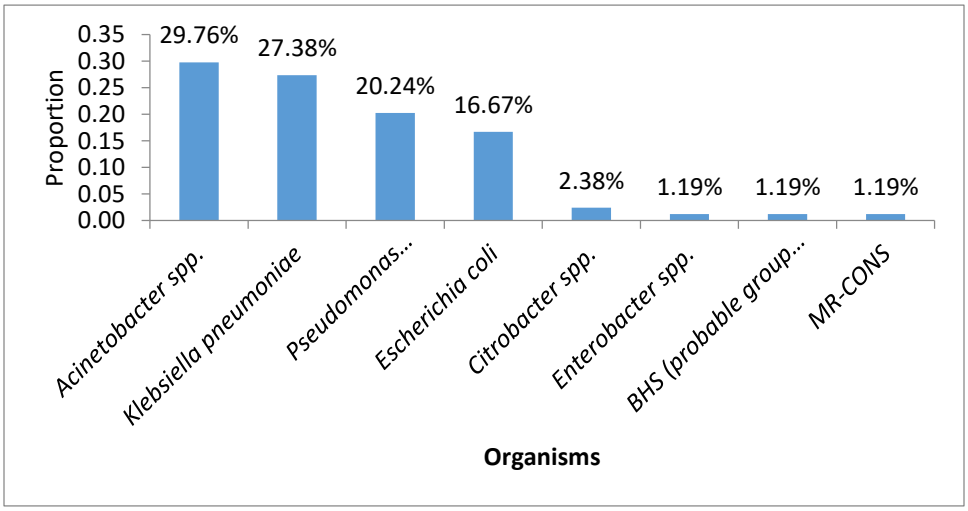

Fig. 3. Distribution of isolated bacterial pathogens

Antimicrobial susceptibility. A panel of 15 antibiotics was tested against the isolates. Imipenem was the most effective against all GNB isolates (43.9%), followed by amikacin (42.85%) and ciprofloxacin (39.28%). Ceftazidime was the least effective (4.87%), followed by cefotaxime (10.71%) and cefepime (13.09%). Gentamicin was 64.70% effective against *P. aeruginosa*. Vancomycin and linezolid were 100% effective against the Gram-positive isolates, whereas 100% resistance was shown to cotrimoxazole (Table 1).

Distribution of MDR isolates. Of the 84 isolates, 65 (77.38%) were MDR, whereas the remaining 19 (22.62%) were non-MDR. Among the 65 MDR isolates, 64 were GNBs, and one was a methicillin-resistant-coagulase-negative staphylococcus species. *Acinetobacter* spp. dominated the MDR count, accounting for 36.92%

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(24/65), followed by *K. pneumoniae* with 23.08% (15/65) (Fig. 4).

Table 1. Gram-negative isolate resistance patterns

Antibiotics	*Acinetobacter spp.* (n=25)	*Klebsiella pneumonia* (n=23)	*Pseudomonas aeruginosa* (n=17)	*E. coli* (n=14)	*Enterobacter spp.* (n=1)	*Citrobacter spp.* (n=2)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Amikacin	24 (96%)	15 (65.2%)	9 (52.9%)	7 (50%)	1 (100%)	1 (50%)
Piperacillin-tazobactam	24 (96%)	20 (86.95%)	9 (52.9%)	14 (100%)	1 (100%)	1 (50%)
Gentamicin	23 (92%)	16 (69.5%)	6 (35.3%)	5 (35.7%)	1 (100%)	1 (50%)
Tobramycin	23 (92%)	22 (95.65%)	7 (41.1%)	8 (57.1%)	1 (100%)	2 (100%)
Cefuroxime	25 (100%)	23 (100%)	16 (94.1%)	14 (100%)	1 (100%)	1 (50%)
Ceftriaxone	24 (96%)	22 (95.65%)	17 (100%)	12 (85.7%)	1 (100%)	1 (50%)
Cefepime	23 (92%)	22 (95.65%)	13 (76.4%)	11 (78.5%)	1 (100%)	1 (50%)
Ciprofloxacin	21 (84%)	15 (65.2%)	6 (35.3%)	11 (78.5%)	1 (100%)	1 (50%)
Tetracycline	19 (76%)	12 (52.17%)	17 (100%)	12 (85.7%)	0 (0%)	1 (50%)
Imipenem	23 (92%)	12 (52.17%)	6 (35.3%)	3 (21.4%)	1 (100%)	1 (50%)
Minocycline	**8 (32%)**	-	-	-	-	-

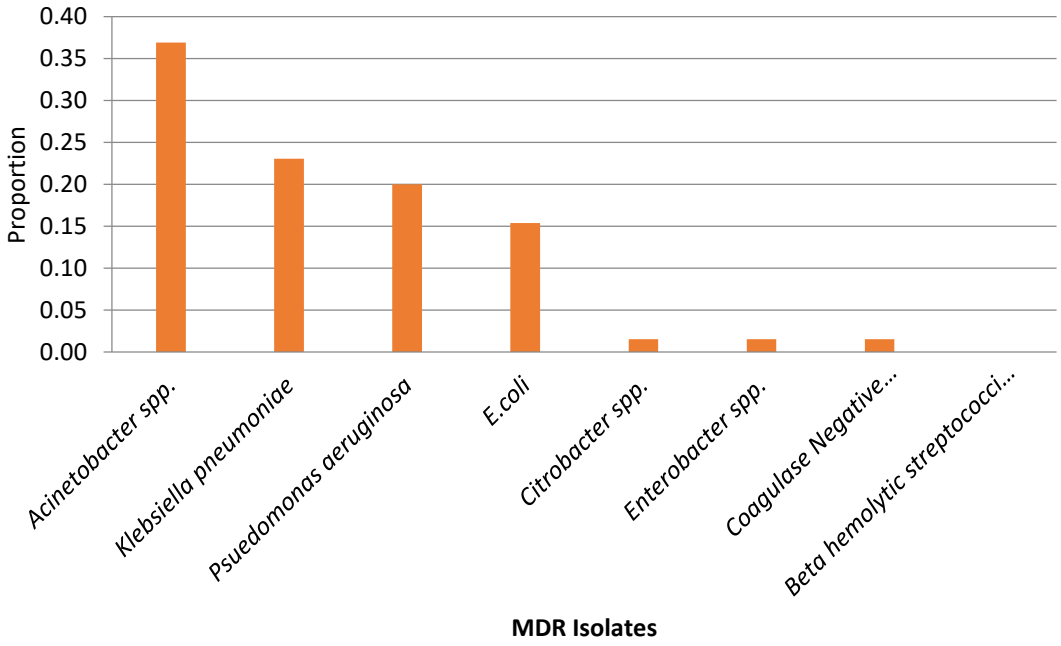

Fig. 4. Frequency of multi-drug resistant organisms (MDROs) among bacterial pathogens isolated from bronchoalveolar lavage fluid

ESBL-producing microorganisms. Of 82 isolates, 29 (35.36%) were ESBL producers. Among those, 19 belonged to the family *Enterobacteriaceae*. Nine (39.13%) isolates of *K. pneumoniae*, 9 (64.28%) of *E. coli*, and 1 (100%) isolate of *Enterobacter* spp. were found to be ESBL producers, whereas 8 (47.06%) isolates of *P. aeruginosa* and 2 (8%) of *Acinetobacter* spp. were ESBL producers (Fig. 5).

DISCUSSION

Lower respiratory tract infections pose a significant public health challenge worldwide due to their high prevalence and economic burden. Among nosocomial infections, ventilator-associated pneumonia (VAP) is the second most common cause, contributing to 15-20% of healthcare-associated infections (HAIs) [16, 17]. VAP is also the primary cause of mortality among HAIs [18]. Hence, effective prevention and management strategies for VAP are critical to reducing the burden of HAIs and improving patient outcomes.

This study aimed to investigate the frequency and antibacterial susceptibility profile of respiratory pathogens in bronchoalveolar lavage (BAL) samples obtained from different wards and intensive care units of a tertiary care hospital. To achieve this, 112 BAL samples were collected and processed at the Microbiology Laboratory to obtain data on respiratory pathogens' prevalence and antibiotic resistance patterns.

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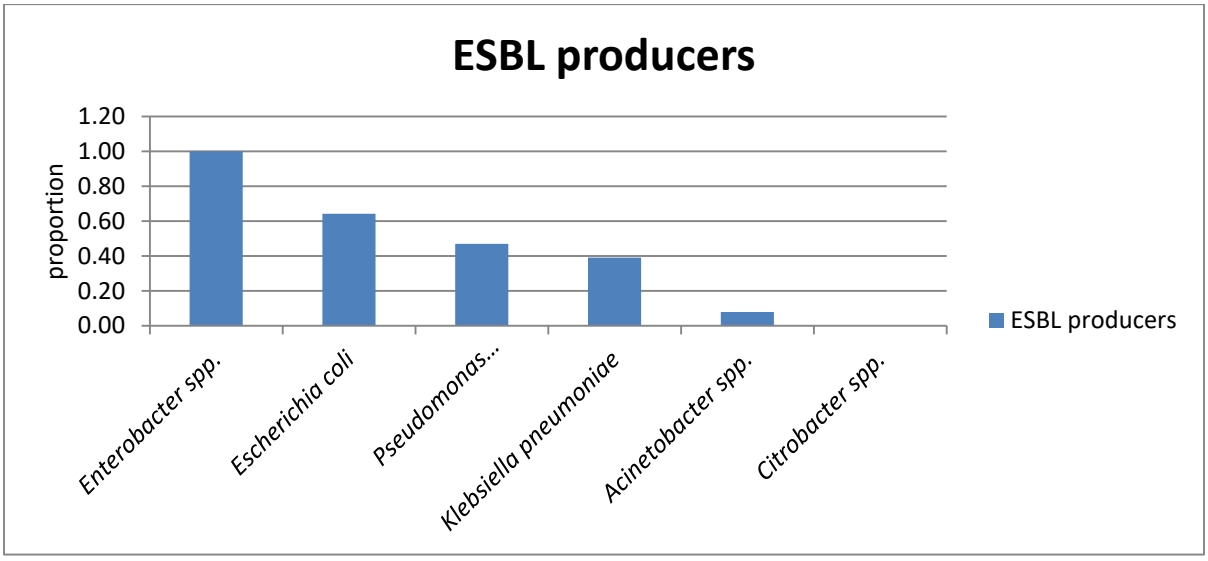

Fig. 5. Distribution of isolates based on ESBL production

The investigation included 112 bronchoalveolar lavage (BAL) samples submitted to the Microbiology Laboratory. Of these, 82 (73.2%) were obtained from male patients, whereas 30 (26.8%) were from female patients. This male predominance is consistent with findings reported in previous studies [19, 20]. Several factors could contribute to this gender disparity, including a higher prevalence of smoking, alcohol consumption, and occupational exposure to respiratory hazards among males, particularly in chronic obstructive pulmonary disease (COPD).

The highest percentage of respiratory pathogen isolates was observed in patients aged above 60 years, followed by those aged 51 to 60 years, while the lowest rate was found in the age group of 18 to 30 years. Thananki *et al*. (2018) reported that the most common age range of patients with respiratory infections was 41-60 years [19]. However, in investigations by Padmaja N *et al*. (2021) and Baishali *et al*. (2020), the majority of patients were in the 51-60 years age range [20, 21]. These discrepancies may be due to differences in study populations, such as variations in healthcare settings, geographic regions, and patient demographics.

Out of 84 growth-positive isolates in this study, 97.62% were Gram-negative, and 2.38% were Gram-positive. *Acinetobacter* spp. (30.49%) was the most frequently isolated Gram-negative pathogen, followed by *K. pneumoniae* (17.07%) and *P. aeruginosa* (14.63%). In contrast, another study reported *P. aeruginosa* (51.6%) as the predominant isolate [22]. These variations in pathogen distribution may reflect differences in patient populations, infection control practices, or geographic regions.

The most prevalent organism recovered in our study was *Acinetobacter* spp., which accounted for 29.76% of isolates. This finding is consistent with the results reported by Rajasekhar *et al*. (2006), who identified these bacteria in 30.59% of isolates [23]. In contrast, Mohammad H Afify *et al*. (2016) reported *K. pneumoniae* (50%) as the most common pathogen, followed by *Acinetobacter* spp. (42.5%) [24].

Klebsiella is a normal mouth flora and can be associated with pneumonia in hospitalized individuals and the elderly [25]. Therefore, its predominance in this study may be related to the predominantly older study population. In contrast, Swomya *et al*. (2014) reported *Pseudomonas* species (21.8%) as the most frequently isolated bacterium in their investigation [26].

Out of 82 Gram-negative bacterial (GNB) isolates in the present study, 64 were MDR, with 37.5% (24/64) belonging to the *Acinetobacter* spp. and 23.43% (15/64) to *K. pneumoniae*. Among the MDR-GNB, 27 were from the *Enterobacteriaceae* family. In comparison, Teklu *et al.* (2019) reported a total MDR prevalence of 68.3% among all *Enterobacteriaceae* isolates [27].

Antibiotic resistance is a significant concern in patients who have been hospitalized in intensive care units (ICUs). In this study, we observed high resistance rates to ceftriaxone among *P. aeruginosa*, *Acinetobacter* spp., and *Klebsiella* spp., with 100%, 96%, and 95.6%, respectively. These findings are consistent with those reported by other researchers, reporting resistance rates ranging from 96% to 100% for these pathogens [28, 29]. These high rates of resistance underscore the need for effective antibiotic stewardship programs and infection control measures to prevent the spread of resistant strains in ICU settings.

In this study, most Gram-negative isolates were sensitive to imipenem, ciprofloxacin, amikacin, and tetracycline. Gram-positive isolates showed 100% sensitivity to linezolid and vancomycin. These findings suggest that a combination of imipenem, ciprofloxacin, amikacin, and tetracycline may effectively treat infections caused by Gram-negative bacilli. These results are consistent with those reported by Olugbue *et al*. (2011)

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[30], who found similar sensitivity patterns among Gram-negative isolates.

In our study, the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* was 47.5%, higher than the rates reported by earlier Ethiopian studies. Siraj *et al*. (2015) reported a prevalence of 38.4% [31], Mulualem *et al*. (2012) found a prevalence of 36% [32], Seid *et al*. (2005) reported a prevalence of 33.3% [33], and Mulisa *et al*. (2016) reported a prevalence of 25% [34]. The increased prevalence of ESBL-producing isolates highlights the urgent need for effective infection control practices and antimicrobial stewardship programs to prevent the spread of resistant strains [35].

The main extended-spectrum beta-lactamase (ESBL)-producing isolates identified in this study were *E. coli* (64.2%) and *K. pneumoniae* (39.1%), which is consistent with the findings of several other studies. Mulisa *et al*. (2016) reported ESBL prevalence rates of 51.5% for *E. coli* and 11.5% for *K. pneumoniae* [34], while Ouedraogo *et al*. (2016) found rates of 67.5% for *E. coli* and 26% for *K. pneumoniae* [36]. Rao *et al*. (2014) reported rates of 61.4% for *E. coli* and 46.2% for *K. pneumoniae* [37], and Shashwati *et al*. (2014) reported rates of 50.14% for *E. coli* and 48.27% for *K. pneumoniae* [38]. These consistent findings highlight the importance of implementing effective infection control measures and antibiotic stewardship programs to prevent the spread of ESBL-producing strains.

Our study found that 47.05% of *P. aeruginosa* strains were positive for ESBL production, consistent with a survey by Goel *et al*. (2013) [39]. They reported a 42.30% prevalence for ESBL-producing *P. aeruginosa* isolates. These results emphasize the need for effective infection control measures and judicious use of antibiotics to prevent the spread of ESBL-producing strains.

The increasing prevalence of antibiotic-resistant bacterial strains is a significant public health concern primarily attributed to the overuse and misuse of antibiotics. In the case of respiratory illnesses, antibiotics are often prescribed unnecessarily and for prolonged periods, which can promote the growth of resistant pathogens. Combination therapy, commonly used to treat respiratory diseases, can also contribute to the emergence of resistant strains. Preventing nosocomial pneumonia requires implementing effective infection control measures, such as frequent hand hygiene, patient positioning in a semi-recumbent position, and minimizing sedation. These simple steps can be crucial in preventing the spread of resistant pathogens and improving patient outcomes.

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CONFLICT OF INTEREST

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

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