

Prevalence and Antimicrobial Resistance Patterns of ESKAPE Pathogens Isolated from Tracheal Aspirates of Patients with Ventilator-Associated Pneumonia in a Tertiary Care Hospital

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Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most common and serious healthcare-associated infections (nosocomial) in intensive care units (ICUs). It develops in patients receiving mechanical ventilation for at least 48 hours and is associated with increased morbidity, mortality, prolonged hospital stays, greater duration of mechanical ventilation, and increased healthcare costs. Among the causative organisms, the ESKAPE pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—are particularly important because of their ability to develop multidrug resistance and evade the effects of commonly used antimicrobials. Continuous surveillance of their prevalence and resistance patterns is essential to guide appropriate therapy and infection control measures.

Aim: To determine the prevalence and antimicrobial resistance patterns of ESKAPE pathogens isolated from tracheal aspirates of patients with ventilator-associated pneumonia in a tertiary care hospital.

Materials and Methods: A prospective observational study was conducted in the Department of Emergency Microbiology at Lok Nayak Hospital between January 2025 to December 2025. Tracheal aspirate specimen obtained from patients who developed infections after 48 hours of hospitalization were processed using standard microbiological methods. Isolates were identified and antimicrobial susceptibility testing was performed using automated system (VITEK2, bioMérieux, France) method according to the latest CLSI guidelines.. Data were analyzed using descriptive and inferential statistics.

Results: A total of 350 ESKAPE pathogen isolates were recovered. Klebsiella pneumoniae was the most common isolate, followed by Acinetobacter baumannii and Pseudomonas aeruginosa. Most infections occurred in intensive care unit patients. High resistance was observed to third-generation cephalosporins, fluoroquinolones, and carbapenems, while better susceptibility was noted to colistin, tigecycline, linezolid, and vancomycin, depending on the organism. More than half of the isolates were multidrug resistant.

Conclusion: ESKAPE pathogens remain major contributors to HAIs (nosocomial) and exhibit high levels of antimicrobial resistance. Strengthening antimicrobial stewardship, routine surveillance, infection prevention practices, and rational antibiotic use is essential to curb the spread of multidrug-resistant organisms.

Keywords: ESKAPE pathogens; nosocomial infection; hospital-acquired infection; antimicrobial resistance; multidrug resistance; antibiotic susceptibility; CLSI.

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Introduction

Hospital-acquired infections (HAIs), also known as nosocomial infections, remain a major public health challenge despite advances in medical technology, antimicrobial therapy, and infection prevention practices. According to the World Health Organization, an HAI is an infection that develops 48 hours or more after hospital admission

and was neither present nor incubating at the time of admission. Among HAIs, ventilator-associated pneumonia (VAP) is one of the most common and serious infections in intensive care units (ICUs), occurring in patients who have been mechanically ventilated for at least 48 hours. These infections are associated with increased morbidity and mortality,

prolonged hospital stay, higher healthcare costs, and significant pressure on healthcare systems. The burden is particularly high in low- and middle-income countries due to overcrowding, limited resources, inappropriate antibiotic use, and inadequate infection control measures. [1] Among the pathogens responsible for VAP, the ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—represent the greatest therapeutic challenge. These organisms are recognized for their ability to "escape" the action of multiple antimicrobial agents through diverse resistance mechanisms, including production of extended-spectrum β -lactamases (ESBLs), carbapenemases, altered penicillin-binding proteins, efflux pumps, porin mutations, and biofilm formation. As a result, infections caused by these pathogens are frequently associated with multidrug resistance (MDR), delayed initiation of effective therapy, prolonged intensive care unit (ICU) stay, increased healthcare expenditure, and higher mortality. [2] Each ESKAPE pathogen contributes significantly to healthcare-associated infections. *Enterococcus faecium* is an important cause of bloodstream and urinary tract infections, particularly with the emergence of vancomycin-resistant enterococci (VRE). Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a leading cause of pneumonia, surgical site infections, and bacteremia. *Klebsiella pneumoniae* is a major producer of ESBLs and carbapenemases, while *Acinetobacter baumannii* is notorious for environmental persistence and resistance to multiple antibiotic classes. *Pseudomonas aeruginosa* possesses intrinsic and acquired resistance mechanisms, and *Enterobacter* species commonly exhibit inducible AmpC β -lactamase production and increasing carbapenem resistance. [3] Several risk factors predispose hospitalized patients to infections caused by ESKAPE pathogens, including prolonged hospitalization, ICU admission, mechanical ventilation, urinary and central venous catheterization, major surgery, immunosuppression, diabetes mellitus, malignancy, advanced age, and indiscriminate use of broad-spectrum antibiotics. Poor hand hygiene, environmental contamination, and lapses in infection prevention practices further facilitate their transmission within healthcare facilities. [4] In India, surveillance studies have documented a rising prevalence of carbapenem-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, along with increasing rates of MRSA and VRE. These trends underscore the importance of continuous microbiological surveillance and antimicrobial stewardship. Institution-specific data on pathogen prevalence and susceptibility patterns are essential

for guiding empirical antibiotic therapy, detecting emerging resistance, strengthening infection control measures, and improving patient outcomes. [5] In view of the increasing burden of antimicrobial resistance and the limited local epidemiological data, the present study was undertaken to determine the prevalence and antimicrobial resistance pattern of ESKAPE pathogens isolated from patients with nosocomial infections in a tertiary care hospital. The findings are expected to support evidence-based antibiotic policies, reinforce antimicrobial stewardship programs, and enhance infection prevention strategies.

Aim: To determine the prevalence and antimicrobial resistance patterns of ESKAPE pathogens isolated from tracheal aspirates of patients with ventilator-associated pneumonia in a tertiary care hospital

Objectives

1. To determine the prevalence and distribution of ESKAPE pathogens isolated from tracheal aspirates of patients with ventilator-associated pneumonia in a tertiary care hospital.
2. To evaluate the antimicrobial susceptibility pattern and prevalence of multidrug-resistant (MDR) ESKAPE pathogens in accordance with the latest CLSI guidelines.
3. To compare the antimicrobial resistance patterns among different ESKAPE pathogens and generate local data to support empirical antibiotic policy, antimicrobial stewardship, and infection control practices.

Materials and Methods

A hospital-based retrospective observational study was conducted in the Department of emergency microbiology laboratory of a tertiary care teaching hospital over a One-year period from January 2025 to December 2025. The study aimed to determine the prevalence and antimicrobial resistance pattern of ESKAPE pathogens isolated from tracheal tube (endotracheal aspirate) samples obtained from patients with suspected hospital-acquired respiratory infections. All endotracheal aspirate samples received from hospitalized patients admitted for 48 hours or more were included in the study. Duplicate isolates from the same patient during the same infectious episode, contaminated specimens, and culture-negative samples were excluded. Demographic and clinical details, including age, sex, hospital department, ICU admission, and microbiological findings, were retrieved from laboratory records.

Samples were processed according to standard microbiological procedures. Isolates were identified to the species level using VITEK® 2 Compact system (bioMérieux, France). The study

focused on the ESKAPE pathogens, namely *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. Antimicrobial susceptibility testing was performed using the VITEK® 2 Compact automated system, and the results were interpreted according to the latest Clinical and Laboratory Standards Institute (CLSI) M100 guidelines. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriales*, carbapenem-resistant Gram-negative bacilli, and multidrug-resistant (MDR) isolates were identified using CLSI-recommended criteria. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes, as proposed by Magiorakos et al. Internal quality assurance was maintained using standard ATCC reference strains.

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean $\pm$ standard deviation, while categorical variables were summarized as frequency and percentage. Associations between categorical variables were

analyzed using the Chi-square test or Fisher's exact test, with a p-value <0.05 considered statistically significant.

Ethical Considerations: The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from patients or their legally authorized representatives wherever applicable. Patient confidentiality was maintained by anonymizing study data, and the study was conducted in accordance with the Declaration of Helsinki and institutional ethical guidelines

Results

During the one-year study period, a total of 350 ESKAPE pathogen isolates were recovered from tracheal aspirates of patients diagnosed with ventilator-associated pneumonia (VAP).

The majority of isolates were obtained from patients admitted to intensive care units (ICUs). Gram-negative organisms constituted the predominant group of ESKAPE pathogens, with *Klebsiella pneumoniae* being the most frequently isolated organism, followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Table 1: Demographic and Clinical Characteristics of Patients with Ventilator-Associated Pneumonia Caused by ESKAPE Pathogens (n = 350)

Variable	Number (n)	Percentage (%)
Age Group (Years)		
<20	24	6.9
21–40	82	23.4
41–60	146	41.7
>60	98	28.0
Gender		
Male	214	61.1
Female	136	38.9
Hospital Unit		
Medical ICU	164	46.9
Surgical ICU	67	19.1
OBGY ICU	18	5.1
Paediatrics ICU	22	6.3
Total	350	100

Table 1 summarizes the demographic and clinical characteristics of 350 patients with ventilator-associated pneumonia (VAP) included in the study. The majority of patients belonged to the 41–60 years age group (41.7%), followed by those aged >60 years (28.0%), while patients younger than 20 years constituted the smallest proportion (6.9%). Male patients were more frequently affected than females, accounting for 61.1% of the study population. Most cases were admitted to the Medical Intensive Care Unit (MICU) (46.9%), followed by the Surgical ICU (19.1%), whereas the OBGY ICU (5.1%) and Paediatrics ICU (6.3%) contributed fewer cases. Ventilator-associated pneumonia (VAP) was the predominant nosocomial infection, occurring in all 350 patients (100%), indicating that VAP was the exclusive infection type included in this study population.

Table 2: Distribution of ESKAPE Pathogens Isolated from Tracheal Aspirates of Patients with Ventilator-Associated Pneumonia (n = 350)

Organism	Total (%)
<i>Klebsiella pneumoniae</i>	110 (31.4%)
<i>Acinetobacter baumannii</i>	90 (25.7%)
<i>Pseudomonas aeruginosa</i>	75 (21.4%)
<i>Staphylococcus aureus</i>	40 (11.4%)
<i>Enterococcus faecium</i>	15 (4.3%)
<i>Enterobacter</i> spp.	20 (5.7%)
Total	350 (100)

A total of 350 ESKAPE pathogen isolates were recovered from tracheal aspirates of patients with ventilator-associated pneumonia (VAP). *Klebsiella pneumoniae* was the predominant pathogen, accounting for 110 (31.4%) isolates, followed by *Acinetobacter baumannii* 90 (25.7%), *Pseudomonas aeruginosa* 75 (21.4%), *Staphylococcus aureus* 40 (11.4%), *Enterobacter*

spp. 20 (5.7%), and *Enterococcus faecium* 15 (4.3%). Overall, Gram-negative ESKAPE pathogens predominated, with *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* together accounting for 78.5% of all isolates. This distribution highlights the predominance of multidrug-resistant Gram-negative pathogens in ventilator-associated pneumonia.

Table 3: Antimicrobial Resistance Patterns of Major ESKAPE Pathogens Causing Ventilator-Associated Pneumonia (VAP)

Antibiotic	<i>Klebsiella</i> (%)	<i>Acinetobacter</i> (%)	<i>Pseudomonas</i> (%)	<i>Staphylococcus</i> (%)	<i>Enterococcus</i> (%)
Ceftriaxone	88.3	NA	NA	NA	NA
Ceftazidime	82.5	86.7	78.7	NA	NA
Piperacillin–Tazobactam	42.5	68.9	54.7	NA	NA
Ciprofloxacin	71.7	76.7	69.3	58.3	64.0
Amikacin	39.2	54.4	42.7	NA	NA
Imipenem	44.2	71.1	41.3	NA	NA
Meropenem	47.5	74.4	45.3	NA	NA
Colistin	3.3	6.7	5.3	NA	NA
Tigecycline	9.2	11.1	NA	NA	NA
Cefoxitin (MRSA)	NA	NA	NA	46.7	NA
Vancomycin	NA	NA	NA	1.7	15.0
Linezolid	NA	NA	NA	0	0

NA = Not applicable

The antimicrobial susceptibility analysis demonstrated a high level of resistance among ESKAPE pathogens to commonly used antibiotics. *Klebsiella pneumoniae* exhibited very high resistance to ceftriaxone (88.3%) and ceftazidime (82.5%), with nearly half of the isolates also resistant to carbapenems (imipenem 44.2%, meropenem 47.5%).

Acinetobacter baumannii showed the highest resistance overall, particularly to meropenem (74.4%), imipenem (71.1%), ciprofloxacin (76.7%), and ceftazidime (86.7%). *Pseudomonas aeruginosa* demonstrated substantial resistance to ceftazidime (78.7%) and ciprofloxacin (69.3%), with moderate resistance to carbapenems. Among Gram-positive organisms, 46.7% of

Staphylococcus aureus isolates were methicillin-resistant (MRSA), while resistance to vancomycin (1.7%) was rare and no resistance to linezolid was observed. *Enterococcus faecium* exhibited 15.0% vancomycin resistance, whereas all isolates remained susceptible to linezolid.

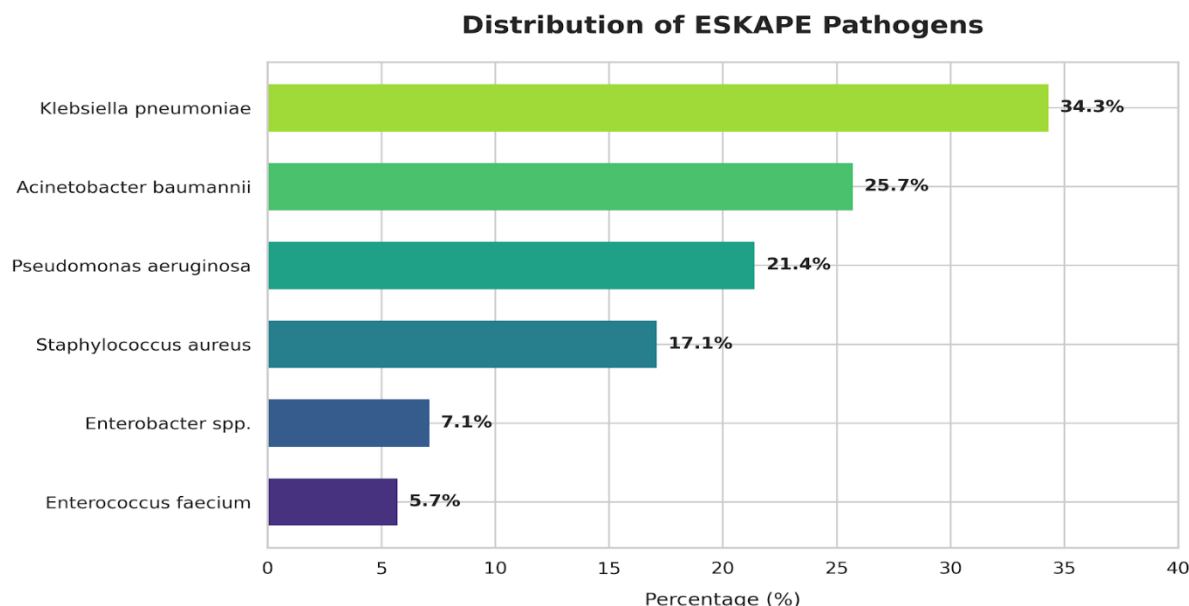
Notably, resistance to colistin (3.3–6.7%) and tigecycline (9.2–11.1%) among Gram-negative pathogens remained low, indicating that these reserve antibiotics retained good activity against multidrug-resistant ESKAPE isolates. Overall, the findings highlight widespread resistance to cephalosporins, fluoroquinolones, and carbapenems, emphasizing the growing challenge of treating ventilator-associated pneumonia (VAP) caused by ESKAPE pathogens.

Table 4: Prevalence of Important Resistance Phenotypes

Resistance Phenotype	Number	Percentage (%)
Multidrug-resistant (MDR) isolates	201	57.4
ESBL-producing Enterobacterales	98	28.0
Carbapenem-resistant Gram-negative bacilli	88	25.1
Methicillin-resistant <i>S. aureus</i> (MRSA)	28/60	46.7
Vancomycin-resistant <i>E. faecium</i> (VRE)	3/20	15.0
Colistin-resistant isolates	6	1.7

More than half of the ESKAPE pathogen isolates were multidrug resistant (MDR), with 201 (57.4%) isolates meeting the MDR criteria, highlighting the substantial burden of antimicrobial resistance in the study setting. Extended-spectrum β -lactamase (ESBL)-producing Enterobacterales accounted for 98 (28.0%) isolates, while carbapenem-resistant Gram-negative bacilli were identified in 88 (25.1%) isolates, indicating significant resistance to β -lactam antibiotics. Among Gram-positive pathogens, 28 of 60 (46.7%) *Staphylococcus aureus*

isolates were methicillin-resistant (MRSA), whereas 3 of 20 (15.0%) *Enterococcus faecium* isolates were vancomycin-resistant (VRE). Encouragingly, colistin resistance was observed in only 6 (1.7%) isolates, suggesting that this reserve antibiotic retained activity against most multidrug-resistant Gram-negative organisms. Overall, these findings underscore the high prevalence of resistant phenotypes among ESKAPE pathogens and emphasize the need for robust antimicrobial stewardship and infection control measures.

**Figure 1:**

Klebsiella pneumoniae accounted for the largest proportion of ESKAPE isolates, followed by *Acinetobacter baumannii*, indicating the predominance of Gram-negative pathogens in hospital-acquired infections.

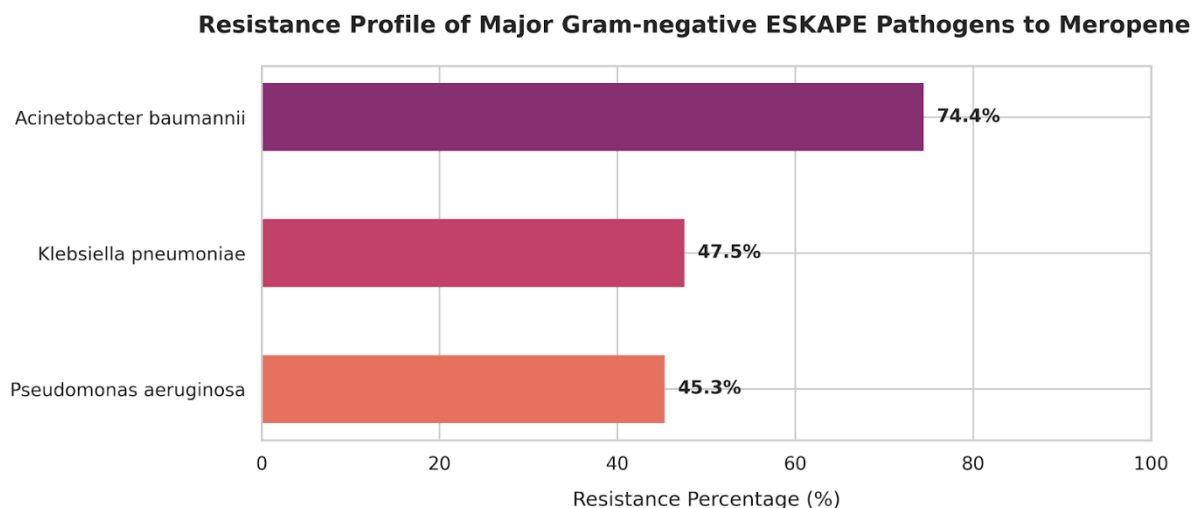


Figure 2: Resistance Profile of Major Gram-negative ESKAPE Pathogens to Meropenem

The highest meropenem resistance was observed in *Acinetobacter baumannii* (74.4%), highlighting its role as the most difficult-to-treat nosocomial pathogen. Carbapenem resistance was also substantial among *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Discussion

The present study evaluated the prevalence and antimicrobial resistance pattern of ESKAPE pathogens causing Ventilator associated Pneumonia (nosocomial infections) in a tertiary care hospital. The findings demonstrate a predominance of Gram-negative ESKAPE pathogens, a high burden of multidrug resistance, and substantial resistance to commonly used antibiotics, emphasizing the growing challenge of healthcare-associated infections.

With respect to the demographic profile, the highest proportion of patients belonged to the 41–60-year age group (41.7%), and males constituted 61.1% of cases. Nearly half of all infections (46.9%) occurred in Intensive Care Unit (ICU) patients, with ventilator-associated pneumonia (27.4%) being the most common nosocomial infection. These observations are consistent with the study by Bereanu et al. (2024) [6], who reported that multidrug-resistant hospital-acquired infections occur predominantly among critically ill ICU patients because of prolonged hospitalization, invasive procedures, mechanical ventilation, and broad-spectrum antibiotic exposure. Similarly, the ICMR Antimicrobial Resistance Surveillance Network (AMRSN) 2024 reported that ICU-acquired respiratory infections remain the major source of antimicrobial-resistant pathogens in Indian tertiary care hospitals. [7]

Among the isolated organisms, *Klebsiella pneumoniae* was the predominant pathogen

(34.3%), followed by *Acinetobacter baumannii* (25.7%) and *Pseudomonas aeruginosa* (21.4%). These findings closely parallel the ICMR AMRSN 2024 [7] report, which demonstrated that *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* together accounted for nearly 80% of pathogens causing ventilator-associated pneumonia in tertiary care hospitals. Likewise, De Prisco et al. (2024) [8] highlighted that ESKAPE pathogens remain the leading organisms responsible for serious hospital-acquired bloodstream and respiratory infections because of their multidrug-resistant nature and ability to persist in healthcare environments.

The antimicrobial susceptibility profile in the present study revealed very high resistance to third-generation cephalosporins, fluoroquinolones, and carbapenems among Gram-negative pathogens. *Klebsiella pneumoniae* exhibited 88.3% resistance to ceftriaxone, while *Acinetobacter baumannii* demonstrated carbapenem resistance exceeding 70%. These findings are comparable with the ICMR AMRSN 2024 [7] surveillance data, which documented persistently poor susceptibility of *Klebsiella pneumoniae* and *Acinetobacter baumannii* to cephalosporins and carbapenems across Indian tertiary care hospitals. The increasing resistance is attributed to the widespread production of extended-spectrum β -lactamases, carbapenemases, efflux pumps, porin mutations, and biofilm formation, all of which severely limit therapeutic options.

Among Gram-positive organisms, 46.7% of *Staphylococcus aureus* isolates were methicillin-resistant (MRSA), whereas 15.0% of *Enterococcus faecium* isolates were vancomycin-resistant (VRE). Encouragingly, resistance to linezolid was not observed, and vancomycin resistance among *S. aureus* remained negligible. Similar trends have

been documented in the WHO GLASS [9] report, which showed preserved susceptibility of Gram-positive isolates to linezolid and glycopeptides despite persistent MRSA and VRE prevalence. These findings support the continued effectiveness of these reserve agents for severe Gram-positive infections.

More than half of the isolates (57.4%) in the present study fulfilled the criteria for multidrug resistance (MDR), while ESBL-producing Enterobacterales and carbapenem-resistant Gram-negative bacilli accounted for 28.0% and 25.1% of isolates, respectively. These findings agree with recent surveillance studies from India and Europe demonstrating an increasing burden of MDR ESKAPE pathogens in healthcare settings. Bereanu et al. (2024) [6] also reported a high prevalence of MDR pathogens in ICU-acquired infections, emphasizing their association with prolonged hospitalization, invasive procedures, and increased mortality. The WHO [10] similarly reported widespread carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*, reinforcing the growing threat of antimicrobial resistance in Indian hospitals.

A noteworthy finding of the present study was the retained activity of reserve antibiotics. Resistance to colistin remained low (1.7%), while tigecycline, linezolid, and vancomycin also demonstrated good in vitro activity against most ESKAPE pathogens. Similar observations have been reported by the ICMR AMRSN 2024 [7], which identified polymyxins, tigecycline, and linezolid as among the few remaining effective therapeutic options for extensively drug-resistant organisms. However, increasing reliance on these last-line agents raises concerns regarding the future emergence of pan-drug-resistant strains.

Overall, the findings of the present study are consistent with recent national and international surveillance reports demonstrating the predominance of Gram-negative ESKAPE pathogens, increasing multidrug resistance, and declining susceptibility to commonly used antibiotics. These observations highlight the urgent need for continuous microbiological surveillance, development of institution-specific antibiograms, strict infection prevention measures, and robust antimicrobial stewardship programs to optimize empirical therapy and limit the further spread of antimicrobial resistance.

Conclusion

The present study demonstrates that ESKAPE pathogens are the predominant etiological agents of ventilator-associated pneumonia (VAP), one of the most common nosocomial infections in the tertiary care setting, with Gram-negative organisms,

particularly *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, accounting for the majority of isolates. A high prevalence of multidrug resistance, ESBL production, carbapenem resistance, and MRSA highlights the growing burden of antimicrobial resistance. Although reserve antibiotics such as colistin, tigecycline, linezolid, and vancomycin remained effective against most isolates, their judicious use is essential to preserve their efficacy. Continuous surveillance, institution-specific antibiograms, antimicrobial stewardship, and strict infection control practices are crucial to reduce healthcare-associated infections and improve patient outcomes.

Limitations

1. The study was conducted at a single tertiary care center, limiting the generalizability of the findings.
2. Molecular characterization of antimicrobial resistance genes was not performed.
3. Clinical outcomes, including mortality, length of hospital stay, and treatment costs, were not assessed.
4. Anaerobic, fungal, and viral healthcare-associated pathogens were not included.
5. Long-term patient follow-up after discharge was not undertaken.

Recommendations

1. Strengthen continuous surveillance of ESKAPE pathogens and antimicrobial resistance.
2. Regularly update institution-specific antibiograms to guide empirical antibiotic therapy.
3. Enhance antimicrobial stewardship programs to promote rational antibiotic use.
4. Strictly implement infection prevention measures, including hand hygiene and device-care bundles.
5. Encourage multicentric studies incorporating molecular epidemiology to monitor resistance trends and transmission patterns.

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