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Microbiological and Antibiotic Profile of Tracheal Aspirate in Mechanically Ventilated Patients in a Tertiary Hospital

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Abstract

Introduction: Ventilator-associated pneumonia (VAP) is a prevalent hospital-acquired infection in mechanically ventilated patients, leading to increased morbidity, mortality, and antimicrobial resistance. Early identification and understanding of microbiological patterns and antibiotic susceptibility are crucial to optimizing treatment strategies.

Methods: A cross-sectional study was conducted in the ICU of Manipal College of Medical Sciences, Pokhara, from March 5, 2024, to March 6, 2025. Patients on mechanical ventilation for >72 hours exhibiting signs of infection were included. Endotracheal aspirates were obtained and cultured. Pathogens were identified, and antibiotic susceptibility testing was performed. Data were analyzed using SPSS to determine the prevalence, microbiological profile, and antibiotic resistance patterns.

Results: Of 107 patients, 75 (70.1%) had culture-positive tracheal aspirates. The predominant organisms were Gram-negative bacteria (62.5%), notably *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. *A. baumannii* showed complete resistance to first-line antibiotics and 92.6% resistance to carbapenems, while all isolates remained sensitive to polymyxin B, colistimethate, and tigecycline. *K. pneumoniae* demonstrated 60–93.3% resistance to cephalosporins and aminoglycosides. *P. aeruginosa* was moderately resistant to beta-lactams but susceptible to carbapenems and aminoglycosides. Fungal isolates (*Candida* spp.) accounted for 4.7%, and *Staphylococcus aureus* for 2.8%.

Conclusions: This study underscores a high burden of VAP in ICU patients, predominantly caused by multidrug-resistant Gram-negative pathogens. The alarming resistance to first-line antibiotics necessitates robust infection control measures and prudent antibiotic use. Targeted empirical therapy guided by local microbiological trends is vital to improving patient outcomes in mechanically ventilated populations.

Keywords: *Acinetobacter baumannii*; *Antimicrobial resistance*; *Tracheal aspirate*; *Ventilator-associated pneumonia*.

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is a type of hospital-acquired pneumonia that occurs 48–72 hours after endotracheal intubation, presenting with new infiltrates, fever, altered white blood cell counts, and sputum changes.[1] As reported it affects 9–27% of mechanically ventilated patients, with early-onset VAP (≤4 days) caused by antibiotic-sensitive pathogens and late-onset VAP (>4 days) by multidrug-resistant bacteria.[2] VAP increases ICU stays, healthcare costs, and antibiotic resistance, making early diagnosis and treatment critical.[3]

VAP arises due to impaired secretion clearance in ventilated patients, allowing colonization of pathogens from oral flora, stomach contents, or equipment.[4] Risk factors include prolonged intubation, underlying illnesses, and interventions like H2 blockers. Mechanical ventilation, while life-saving, heightens VAP risk, especially within the first 5 days. Despite advances, diagnostic challenges and antibiotic resistance complicate VAP management, necessitating improved prevention and treatment strategies.[5]

This study aims to analyze the microbiological profile and antibiotic susceptibility pattern of tracheal aspirates in mechanically ventilated patients diagnosed with ventilator-associated pneumonia (VAP) in a tertiary care hospital.

METHODS

This cross-sectional study was conducted in intensive care unit in Manipal college of medical sciences teaching hospital, Pokhara between 5th March 2024 to 6th March 2025. Approval was obtained from the Institutional Review Committee (Reference number ID MCOMS/IRC/597/GA. Written informed consent was taken from the first-degree relatives. Patient requiring Mechanical Ventilator for more than 72 hours, purulent tracheal secretions, leukopenia or leukocytosis, new onset radiographic features were included in the study. Patient presenting with pneumonia at time of admission, patient discharged against medical advice within 48 hours of mechanical ventilation, patient who expired with in day of ventilator placement were excluded from the study.

The prevalence of culture positive was 42 % according to previous study.[6]

Sample size was calculated using the following formula:

$n = z^2PQ/E^2$

E = allowable error (up to 10%)

n = sample size

z = reliability coefficient (1.96% taken at 95% confidence interval)

Where P= proportion, P = 42

Q= 100 - P

E= 10 %

n= 4* 42*658/100 = 97

As 10% non-response =97+10 = 107.

Thus, a sample size of 107 was taken.

Patient's demographic data and primary diagnosis, co-morbidities, date of admission in hospital and days in ventilator in ICU was noted. The study patients were monitored at every third day for development of VAP using clinical and microbiological criteria until discharge or death. The relevant data were recorded from medical records, bedside flow sheets, radiographic reports and reports of microbiological studies of the patients.

Modified Clinical Pulmonary Infection Score (CPIS) criteria were used for the diagnosis of VAP.CPIS at baseline was assessed based on the first five variables i.e., temperature, blood

leukocyte count, tracheal secretions, oxygenation and character of pulmonary infiltrate. CPIS at 72 hours was calculated based on all seven variables and was taken into consideration the progression of the infiltrate and culture results of the tracheal aspirate. A score of greater than 6 at baseline or at 72hours was considered suggestive of VAP.

The endotracheal aspirate (ETA) specimen was collected via a sputum suction trap. Tracheal aspirates were categorized as absent, non - purulent or purulent who are involved in daily care of patients. The sample were sent quickly to the lab and directly used for staining and culturing to detect microorganisms present. Various types of agar plates were used for culturing, including blood, chocolate, and McConkey agar. After incubation for 24 hours, any organism present was transferred to Mueller Hilton agar for antibiotic susceptibility testing. Results from both the culture and antibiotic testing were examined after 48 hours. Additional testing was conducted to identify the specific type of organism using biochemical methods. The amount of growth observed was noted, categorized as light, moderate, or heavy, as determined by the microbiologist.

All the included patients had their endotracheal tube aspirated and sent to microbiology department for culture and sensitivity. The reports were collected after 72 hours of inoculation and recorded in Microsoft excel, which was later imported to SPSS and the data was calculated for prevalence and pattern of microbial grown during culture and antimicrobial sensitivity. Descriptive statistics were applied to find the frequencies and percentages. Chi square test was used to calculate p-value for age and gender statistics. p-value less than 0.05 was considered as statistically significant.

RESULTS

Out of 107 clinical samples analyzed, 75 (70.1%) yielded positive culture. (Figure 1) Among these, Gram-negative organisms predominated, accounting for 62.5% (67) of isolates, whereas Gram-positive bacteria comprised only 2.8% (3). Candida species were identified in 4.7% (5) of cases. There were predominantly higher number of males 63(58.9%) as compared to 44 females (41.1%) in our study. In our study there were 69 individuals (64.5%) population from age group 51-80 years of age. (Figure 2) There was no significant difference between males and females among patients showing blood culture growths (p-value=0.429). (Table:1)

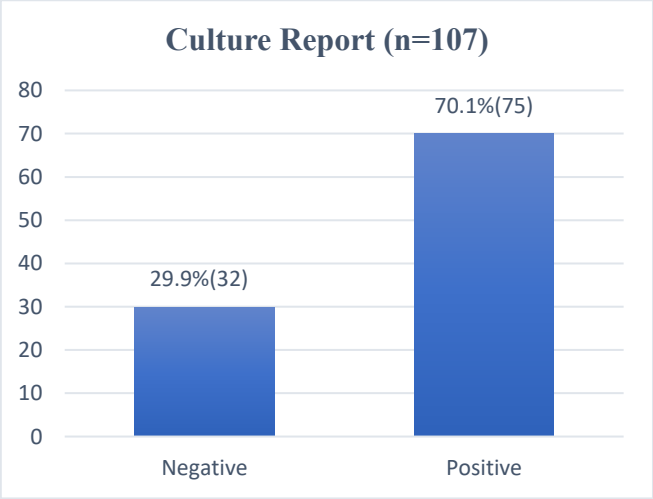

Figure 1: Culture positivity among patients (n=107)

Table 1: Association between Gender and Culture Positivity in Mechanically Ventilated Patients

		Culture		Total	
		Negative	Positive		p-value
Gender	Female	15	29	44	0.429
	Male	17	46	63	
Total		32	75	107	

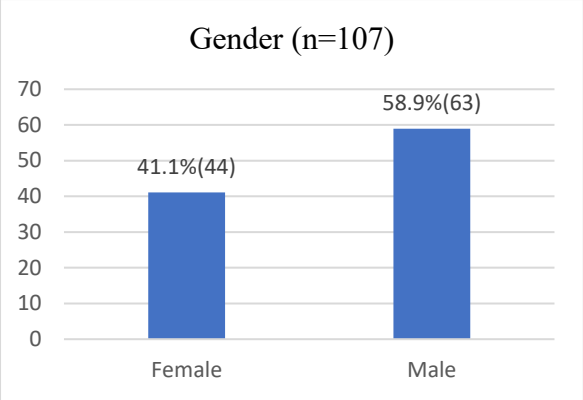

Figure 2: Demographic Characteristics of Study Population (n=107)

The most frequently isolated Gram-negative organisms during culture and Gram staining were *Acinetobacter baumannii* (27 isolates), followed by *Klebsiella pneumoniae* (15 isolates), and *Pseudomonas aeruginosa* (13 isolates). Additional Gram-negative isolates included *Escherichia coli* and *Citrobacter* species. Among Gram-positive bacteria, *Staphylococcus aureus*, including methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains, were also identified. (Figure 3)

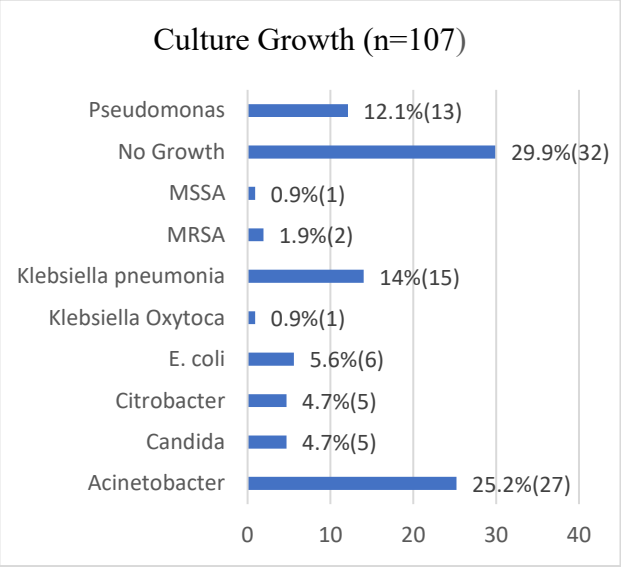

Figure 3: Distribution of microorganisms isolated (n=107)

Acinetobacter baumannii isolates demonstrated complete resistance (100%) to first-line antibiotics such as cefepime, cefotaxime/ceftazidime, and cefoperazone-sulbactam. Additionally, 92.6% of these isolates were resistant to meropenem, tazobactam-piperacillin, amikacin, and gentamicin. However, all *A. baumannii* isolates were susceptible to second-line agents including polymyxin B, colistimethate, and tigecycline.

Klebsiella pneumoniae showed high resistance to first-line antibiotics, with 60% of isolates resistant to Meropenem,tazobactam-piperacillin, amikacin, gentamicin, cefotaxime, and cefoperazone-sulbactam. Resistance to cefepime was even more pronounced, observed in 93.3% of cases. Conversely, all isolates remained sensitive to second-line agents such as polymyxin B, colistimethate, and tigecycline.

Pseudomonas aeruginosa demonstrated resistance rates of 53.8% to cefepime, 61.5% to cefotaxime/ceftazidime, and 38.5% to cefoperazone-sulbactam. Nonetheless, all isolates were sensitive to meropenem, tazobactam-piperacillin, amikacin, and gentamicin. Similar to other Gram-negative organisms, second-line agents were effective across all samples. (Figure 4)

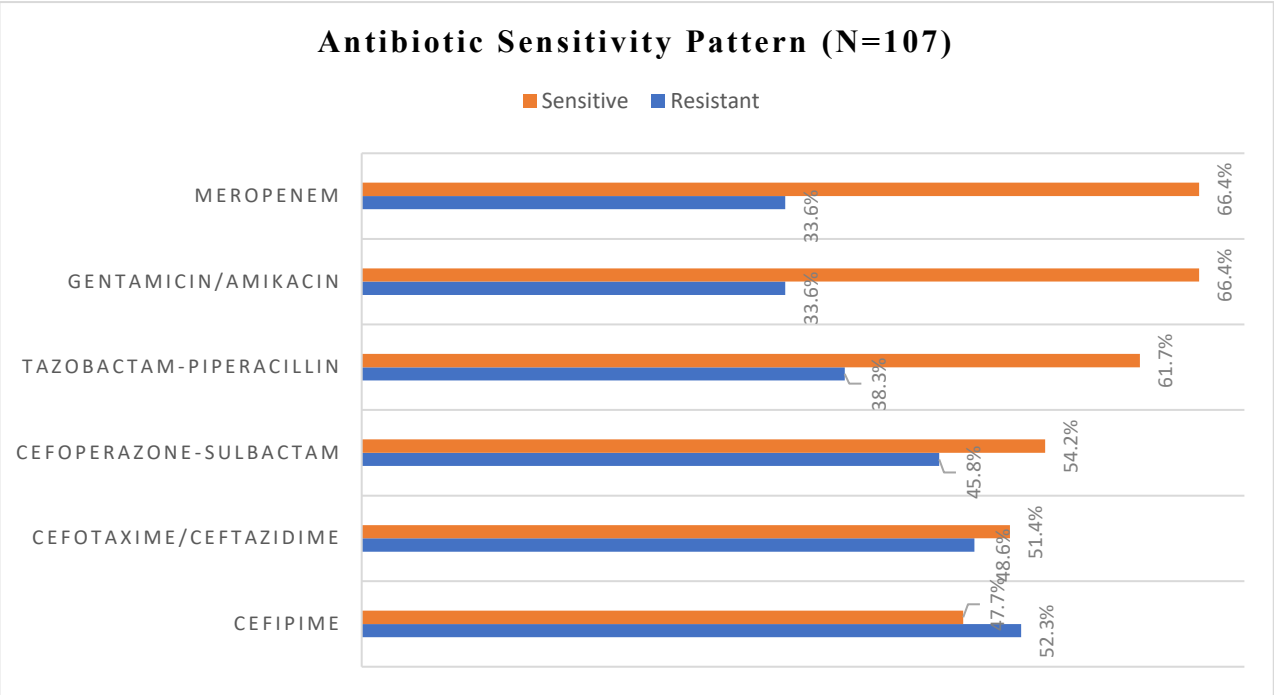

Figure 4: Antibiotic sensitivity profile of isolates (n=107)

DISCUSSION

Ventilator-associated pneumonia (VAP) remains one of the most challenging nosocomial infections in intensive care units (ICUs), contributing significantly to morbidity, mortality, and healthcare costs.[7] This study aimed to assess the microbiological profile and antibiotic susceptibility patterns of tracheal aspirates in mechanically ventilated patients, offering critical insights to guide empirical therapy and enhance infection control practices.

Our results revealed a high incidence of VAP, with 70.1% (75 out of 107) of tracheal aspirate samples showing positive culture growth. This prevalence, though higher than that reported in some previous studies, aligns with data from other tertiary centers in similar contexts. Factors such as prolonged ventilation, prior antibiotic exposure, limited infection control measures, and colonization by hospital flora likely contributed to this elevated rate.[8] The majority of patients were male (58.9%), and the most affected age group was 51–80 years (64.5%). This gender distribution may reflect broader ICU admission trends; wherein critically ill males often require mechanical ventilation. Our findings align with several other studies, and others who reported a higher prevalence of VAP in older male patients—a population more susceptible due to diminished immunity and multiple comorbidities.[9]

The bacteriological profile demonstrated a predominance of Gram-negative organisms (62.5%), consistent with several other studies. The most frequently isolated pathogen was *Acinetobacter baumannii*, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Similar results were reported, where they noted *Acinetobacter* as the leading cause of VAP, followed by *Pseudomonas*. The ability of *Pseudomonas* to form biofilms, especially in endotracheal tubes, could explain its frequent isolation.[10–12] George et al. also reported *Acinetobacter* (37.5%) as the predominant organism, followed by *Pseudomonas* (21.8%) and *Klebsiella* (15.6%).Gram-positive cocci, primarily *Staphylococcus aureus*, were less commonly detected (2.8%), while *Candida*

species were identified in 4.7% of cases.[11] These findings reflect global trends, underscoring the dominance of Gram-negative bacilli in ICU-acquired infections, particularly in resource-limited settings. *Acinetobacter* has emerged as a particularly concerning pathogen due to its environmental resilience, biofilm formation, and robust resistance mechanisms.

Alarmingly, *Acinetobacter baumannii* displayed 100% resistance to first-line antibiotics, including cefepime, cefotaxime, ceftazidime, and cefoperazone-sulbactam. Resistance to carbapenems, often reserved as last-line agents, was extremely high (92.6%), indicating the emergence of extensively drug-resistant (XDR) strains. These results mirror global concerns about rising antimicrobial resistance in ICU pathogens.

Klebsiella pneumoniae also exhibited significant resistance, with 60–93.3% of isolates resistant to cephalosporins, aminoglycosides, and piperacillin-tazobactam. Resistance to cefepime was particularly notable, reflecting declining efficacy of third and fourth-generation cephalosporins against Enterobacteriaceae. Nevertheless, these isolates retained sensitivity to polymyxin B, colistimethate sodium, and tigecycline—antibiotics typically reserved for multidrug-resistant infections. This narrow therapeutic window highlights the urgent need for antimicrobial stewardship and novel drug development.

Interestingly, *Pseudomonas aeruginosa*, although resistant to several beta-lactams, remained susceptible to carbapenems and aminoglycosides, offering some therapeutic hope. However, its potential for rapid resistance development necessitates cautious, targeted treatment. The detection of MRSA and MSSA in a limited number of samples is in line with trends showing a decline in Gram-positive VAP pathogens, possibly due to better infection control targeting MRSA and increased prophylactic use of vancomycin. Meanwhile, the isolation of *Candida* spp., although infrequent, suggests fungal superinfections in patients on prolonged antibiotics and ventilation.[11,13]

Diagnosing VAP remains complex due to overlapping features with other pulmonary conditions in the ICU, such as ARDS, pulmonary edema, or atelectasis. In this study, the Modified Clinical Pulmonary Infection Score (CPIS) was employed to enhance diagnostic accuracy by incorporating clinical, radiographic, and microbiological parameters.[14] While CPIS has its limitations, it proved to be a practical tool in our resource-constrained setting, showing good correlation with culture results. Routine surveillance of tracheal aspirates, as practiced here, serves a dual purpose—supporting early diagnosis and guiding empirical antibiotic therapy. Although more specific methods like bronchoalveolar lavage are available, they may not be feasible in all ICUs due to patient instability and resource limitations, making endotracheal aspirate cultures a practical alternative.

The high prevalence of multidrug-resistant organisms highlights the urgent need to strengthen infection prevention protocols. These should include strict hand hygiene, minimizing unnecessary intubation, daily sedation breaks with spontaneous breathing trials, elevating the head of the bed, and careful use of stress ulcer prophylaxis. Additionally, regular disinfection of respiratory equipment, use of closed suction systems, and subglottic secretion drainage are effective strategies to prevent VAP.

This study has several limitations. First, it was conducted in a single tertiary care center, which may limit the generalizability of the findings to other healthcare settings with different patient

populations and infection control practices. Second, the use of tracheal aspirates, while practical in our setting, is less specific than invasive techniques like bronchoalveolar lavage, potentially affecting the accuracy of pathogen identification. Additionally, we did not evaluate the clinical outcomes associated with specific pathogens or resistance patterns, which would have provided more insight into the implications for patient management. Finally, molecular testing to detect resistance genes was not performed due to resource constraints, limiting our ability to characterize the mechanisms underlying antimicrobial resistance.

CONCLUSIONS

In conclusion, our study reveals a high burden of VAP in mechanically ventilated patients, predominantly caused by multidrug-resistant Gram-negative bacteria, especially Acinetobacter baumannii and Klebsiella pneumoniae. The high resistance rates to first-line antibiotics and retained susceptibility to polymyxins and tigecycline emphasize the need for vigilant antibiotic stewardship and infection control practices.

CONFLICT OF INTEREST

None

SOURCES OF FUNDING

None

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