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Original Research Article

Study of Gram-negative Organisms Isolated from Endotracheal Tube Secretions in ICU Patients with Special Reference to Carbapenemase Resistance

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Abstract

Objective: This study aims to identify Gram-negative organisms from endotracheal (ET) tube secretions in ICU patients, focusing on their antimicrobial resistance profiles, especially carbapenemase production.

Methods: A cross-sectional study was conducted over 20 months in the Department of Microbiology, JNMC, Sawangi. ET secretions from 104 patients were processed for microbiological culture and antimicrobial susceptibility testing. Special resistance mechanisms, including ESBL, AmpC, and carbapenemase production, were identified.

Results: The study showed a predominance of *Acinetobacter spp.* (42.3%) among isolates, followed by *Klebsiella pneumoniae* (22.1%) and *Pseudomonas aeruginosa* (18.3%). Resistance to imipenem was 100%, and carbapenemase production was noted in 30.7% of isolates. ESBL and AmpC production were seen in 19.2% and 11.5% of isolates, respectively.

Conclusions: This study emphasizes the high prevalence of MDR organisms in ICU patients and highlights the urgent need for antimicrobial stewardship and robust infection control practices.

Keywords: Gram-negative bacteria (GNB), Endotracheal tube secretions, Intensive care units (ICUs), Carbapenem resistance, antimicrobial resistance (AMR).

Introduction

Although many patients in intensive care units benefit from mechanical ventilation, it is linked to a significant risk of respiratory infections as well as a high rate of morbidity and mortality in patients who are very ill. The etiologic agents may vary according to the population of patients in ICU, type of ICU, pre-existing illness and prior antimicrobial therapy [1]. One of the main reasons why death rates in intensive care units (ICUs) are persistently high is lower respiratory tract infections in patients on mechanical ventilation [2]. About 25% of nosocomial infections are nosocomial pneumonia, and tracheal intubation raises the risk of infection by six to twenty times [3]. Although many lives have been saved by the use of invasive medications and treatment techniques, severe, chronic, and resistant infections have resulted in potentially fatal outcomes. Nosocomial infections can result from these intrusive therapeutic and diagnostic procedures, especially in ICUs and critical care units (CCUs). There is proof that hospital staff and the surrounding environment are the source of microorganisms, and that extended hospital stays and excessive use of antibiotics have caused these bacteria to become resistant to several drugs [4].

In order to administer antibiotics wisely, epidemiological studies of ventilated patients are desperately needed to understand the local microbial flora and their antibiotic profiles. In order to ascertain the frequency of harmful bacteria in ventilated patients' respiratory secretions as well as their patterns of antibiotic sensitivity, this study was conducted [5].

Aim

To study the etiological agents isolated from endotracheal tube from intubated patients along with their antibiotic resistance pattern with special references to Carbapenemase production.

Objectives

1. To isolate and identify the various bacterial pathogens from endotracheal tube.
2. To demonstrate the antimicrobial susceptibility pattern in different isolates.
3. To demonstrate the beta-lactamase production.
4. To demonstrate the carbapenmase production by different isolates.

Materials and Methods

Study Design and Setting: This cross-sectional study was conducted in the Department of Microbiology at JNMC, Sawangi, from December 2021 to August 2023. The study included 104 isolates obtained from endotracheal (ET) tube secretions of ICU patients.

Study Population and Sampling: Endotracheal secretions from mechanically ventilated ICU patients were collected under aseptic conditions. All samples were transported to the microbiology laboratory for immediate processing.

Microbiological Processing: The samples were screened using Gram staining to identify bacterial morphology. Cultures were prepared by inoculating the samples on blood agar, MacConkey agar, and nutrient agar plates, which were incubated at 37°C for 18–24 hours. Bacterial isolates were identified using standard microbiological techniques.

Antimicrobial Susceptibility Testing: Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following CLSI 2021 guidelines. Colistin resistance was assessed using the Epsilometer (E) test.

Detection of Resistance Mechanisms

1. Extended-Spectrum Beta-Lactamase (ESBL) Production

Screening Test: Performed on Mueller-Hinton agar (MHA) with a ceftazidime (30 µg) disk. A zone diameter ≤22 mm indicated potential ESBL production.

Phenotypic Confirmatory Test: MHA was inoculated with 0.5 McFarland suspension of the test isolate. Disks of ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) were placed. A ≥5 mm increase in the zone diameter for ceftazidime-clavulanic acid confirmed ESBL production.

2. AmpC Beta-Lactamase Production

Screening Test: Conducted using a cefoxitin (30 µg) disk. A zone diameter ≤18 mm indicated potential AmpC production.

Ceftazidime-Imipenem Antagonism Test: MHA was inoculated, and ceftazidime (30 µg) and imipenem (10 µg) disks were placed 20 mm apart. Blunting of the ceftazidime inhibition zone adjacent to imipenem confirmed inducible AmpC production.

3. Carbapenemase Production

Initial Screening Test: Conducted using an imipenem (10 µg) disk on MHA. A zone diameter between 16–21 mm suggested carbapenemase production.

Combined Disk Test (Disk Potentiation Test): This test used imipenem (10 µg) and imipenem-EDTA (10/750 µg) disks. A ≥7 mm difference in inhibition zone diameters confirmed metallo-beta-lactamase (MBL) production. A blank EDTA disk was included as a control.

Ethical Considerations: Ethical approval for the study was obtained from the institutional ethical committee, ensuring compliance with ethical guidelines for sample collection and patient confidentiality.

Data Analysis: Results were documented systematically, and resistance patterns were analysed. Statistical tools, including frequency and percentage calculations, were used for data interpretation.

Results

Gender Distribution: Out of 104 isolates obtained from endotracheal tube (ET) secretions, a significant male predominance was observed, with 85.6% (89 isolates) from male patients and 14.4% (15 isolates) from female patients. The chi-square test revealed a statistically significant difference in gender distribution ($\chi2=52.65$, $p < 0.001$), indicating that male patients were more affected than females. Table 1 summarizes the gender distribution.

Table 1: Gender-wise Distribution of Isolates

Gender	Number of Isolates	Percentage (%)	Chi-Square Statistic	p-Value
Male	89	85.6	52.65	<0.001
Female	15	14.4	52.65	<0.001

Age Distribution: The age-wise distribution of isolates showed the highest numbers from patients in the 0–10 years and ≥61 years groups, each contributing 19.2% (20

isolates). This was followed by patients aged 31–40 years and 51–60 years, each contributing 16.3% (17 isolates). The lowest numbers were recorded in the 21–30 years and 41–50 years age groups, each with 7.7% (8 isolates).

The chi-square test ($\chi2=10.56$, $p = 0.10$) indicated no statistically significant variation across age groups. Figure 1 shows the age-wise distribution of isolates.

Figure 1: Age-wise Distribution of Isolates

Microbial Distribution: Among the Gram-negative organisms isolated, *Acinetobacter spp.* was the most prevalent, accounting for 42.3% (44 isolates), followed by *Klebsiella pneumoniae* (22.1%) and *Pseudomonas aeruginosa* (18.3%). Other notable organisms included *Escherichia coli* (6.7%) and *Citrobacter freundii* (2.9%).

Rarely isolated organisms, such as *Burkholderia spp.*, *Citrobacter koseri*, *Proteus mirabilis*, and *Stenotrophomonas spp.*, each accounted for 1.9%. The chi-square test ($\chi 2=146.96$, $p < 0.001$) indicated a statistically significant difference in the prevalence of different organisms. Figure 2 illustrates the distribution of microbial isolates.

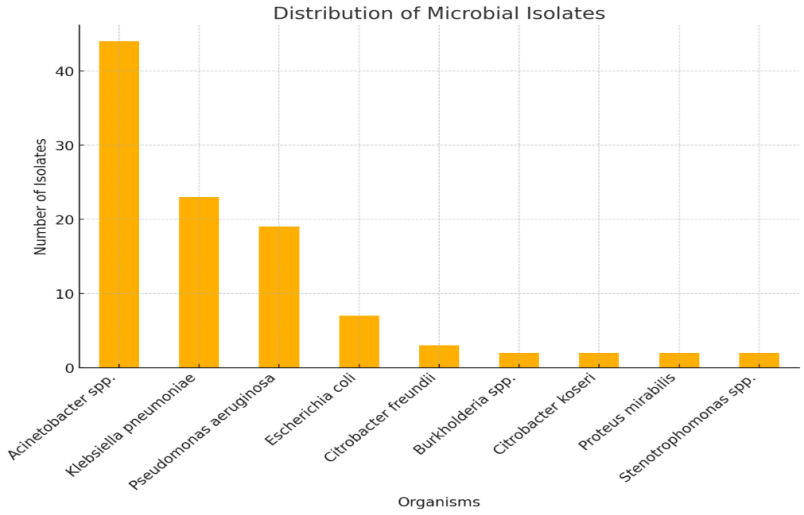

Figure 2: Microbial Distribution in ET Secretions

Antimicrobial Resistance Patterns: The antimicrobial resistance profiles of the Gram-negative isolates revealed a concerning prevalence of multidrug resistance (MDR). Resistance to imipenem was observed universally across all isolates (100%), underscoring the critical issue of carbapenem resistance in ICU settings. High resistance rates were also noted for other commonly used antibiotics, including tobramycin (86.5%), ciprofloxacin (85.5%), and cefepime (90.3%). These findings highlight the limited efficacy of traditional broad-spectrum antibiotics in treating infections caused by these pathogens. (Table 2)

Resistance to newer antibiotics, such as colistin and tigecycline, was notably low, with only 2.8% of isolates showing resistance to these agents. This indicates that these antibiotics remain effective treatment options, albeit as last-resort drugs. The overall resistance patterns emphasize the urgent need for antimicrobial stewardship programs to optimize antibiotic usage and mitigate the spread of resistant organisms

Table 2: Antimicrobial Resistance Patterns

Antibiotic	Number of Resistant Isolates	Percentage (%)
Imipenem (IMP)	104	100
Ceftazidime (CAZ)	103	99
Cefepime (CPM)	94	90.3
Aztreonam (AT)	92	88.4
Tobramycin (TOB)	90	86.5
Ciprofloxacin (CIP)	89	85.5
Amikacin (AK)	88	84.6
Amoxicillin-clavulanic acid (AMC)	87	83.7
Ampicillin (AMP)	86	82.7
Gentamicin (GEN)	80	76.9
Meropenem (MRP)	78	75
Cotrimoxazole (COT)	74	71.1
Colistin (CL)	3	2.8
Tigecycline (TGC)	3	2.8

Resistance Mechanisms: The study also investigated the underlying resistance mechanisms among the Gram-negative isolates, focusing on the production of carbapenemases, extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, and metallo-beta-lactamases (MBLs). Carbapenemase production was identified in 30.7% of isolates, representing a significant driver of carbapenem resistance. Additionally, 19.2% of isolates were positive for ESBL production, highlighting the role of this mechanism in mediating resistance to beta-lactam antibiotics.

AmpC beta-lactamase production was observed in 11.5% of isolates, and inducible AmpC producers were confirmed through ceftazidime-imipenem antagonism tests. MBL production was detected in 15.4% of isolates, further complicating treatment options as MBLs confer resistance to most beta-lactams, including carbapenems.

The chi-square test revealed statistically significant differences in the distribution of these resistance mechanisms ($\chi 2=11.2$, $p = 0.01$). These findings underscore the complexity of resistance patterns in ICU pathogens and highlight the importance of routine testing for specific resistance mechanisms to guide effective treatment strategies. Robust infection control measures are critical to prevent the dissemination of these highly resistant organisms. Figure 3 shows the distribution of resistance mechanisms among isolates.

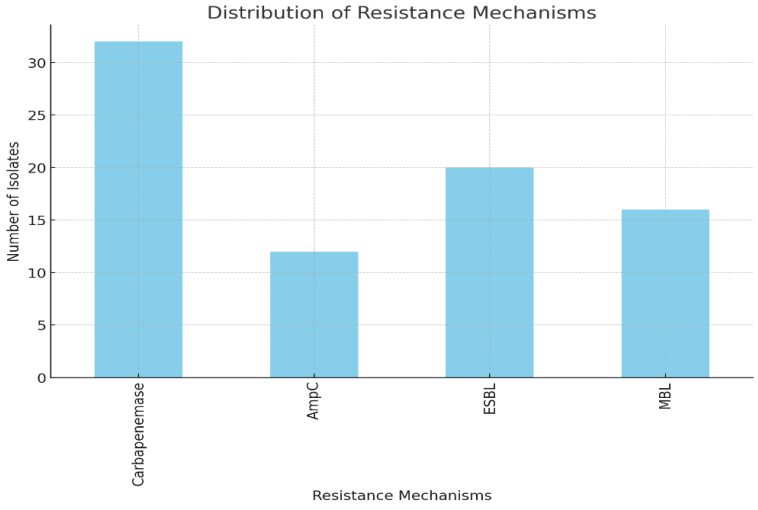

Figure 3: Resistance Mechanisms in Isolates

Discussion

This study highlights the prevalence of Gram-negative organisms isolated from endotracheal (ET) tube secretions in ICU patients and underscores the critical issue of antimicrobial resistance, with a particular focus on carbapenemase production.

The predominance of *Acinetobacter spp.* (42.3%), followed by *Klebsiella pneumoniae* (22.1%) and *Pseudomonas aeruginosa* (18.3%), is consistent with prior studies identifying these organisms as leading causes of ventilator-associated pneumonia (VAP) in ICU patients. Studies by McCann et al. (2018) (6) reported *Acinetobacter spp.* as the most

frequently isolated pathogen in ICU respiratory infections. The high prevalence of *Acinetobacter spp.* can be attributed to its ability to survive in the hospital environment and its propensity to acquire resistance mechanisms.

These findings align with global trends indicating a rise in carbapenem-resistant Gram-negative bacteria (CR-GNB) in ICU settings. A study conducted in a Kenyan ICU reported a significant prevalence of CR-GNB infections, identifying *Acinetobacter baumannii* as the most common carbapenem-resistant organism [7]. Similarly, research from Iran found that *Acinetobacter baumannii* was the most prevalent Gram-negative bacterium in ET secretions, with a 95.1% resistance rate to meropenem, another carbapenem antibiotic [8].

The high resistance rates observed in this study are consistent with reports from other regions. For instance, a study in a tertiary care hospital found that 93.3% of *Acinetobacter baumannii* isolates were resistant to carbapenems [9]. These patterns highlight the global challenge posed by carbapenem-resistant organisms in critical care environments.

The detection of carbapenemase production in 30.7% of isolates is particularly concerning, as these enzymes confer resistance to a broad spectrum of beta-lactam antibiotics, including carbapenems. This finding is in line with studies that have identified various carbapenemase genes, such as KPC, NDM, and OXA-48, in ICU settings [10]. The presence of these resistance mechanisms complicates treatment options and necessitates the use of last-resort antibiotics like colistin and tigecycline, which, fortunately, showed low resistance rates in this study.

The study also revealed a male predominance (85.6%) among patients with these infections, which is consistent with other research indicating higher rates of CR-GNB infections in male patients [11]. The age distribution showed the highest number of isolates in patients aged 0–10 years and those ≥61 years, suggesting that both pediatric and elderly populations are particularly vulnerable to these infections.

The findings of the research indicate the urgent need for strong antimicrobial stewardship initiatives, focused infection control tactics, and ongoing monitoring of resistance trends in intensive care units. Because carbapenem-resistant organisms are so common, precision diagnostics—like molecular assays—must be used to quickly identify resistance mechanisms. The transmission of multidrug-resistant pathogens must also be stopped by rigorous adherence to infection prevention measures, such as hand hygiene and environmental elimination.

However, limitations include the absence of molecular methods to identify specific resistance genes and the study's single-center design, which may limit generalizability.

Conclusion

The burden of multidrug-resistant Gram-negative organisms in intensive care unit patients and the serious problem of carbapenem resistance have been highlighted in this study. The results highlight the necessity of strict infection control protocols, prudent antibiotic usage, and the creation of quick diagnostic methods in order to successfully fight antimicrobial resistance. Future research should examine the molecular processes underlying resistance and assess how targeted therapies affect the infections' burden.

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