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Evaluation of Colistin Susceptibility among Multidrug-resistant Gram-Negative Pathogens isolated from Blood Culture: Evidence from a Tertiary Care Setting in Delhi, India

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Abstract

Background: Antibiotic resistance is a grave threat to managing bloodstream infections, with multidrug-resistant (MDR) gram-negative pathogens like carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* escalating globally. The misuse of colistin, a last-resort antibiotic, has driven resistance, creating extensively drug-resistant (XDR) pathogens [1]. The surge of colistin and carbapenem resistance signals an urgent global health crisis demanding immediate action. This study sought to assess the colistin susceptibility profiles of multidrug-resistant gram-negative clinical isolates obtained from blood cultures within a high-burden tertiary care facility in Delhi.

***Methods*:** This cross-sectional study was conducted in the Department of Microbiology at UCMS & GTB Hospital, Delhi, from January 2023 to June 2024. A total of 80 multidrug-resistant gram-negative clinical isolates from blood cultures were included. Carbapenem resistance was confirmed using the Modified Carbapenem Inactivation Method (mCIM), while colistin resistance was determined through Broth Microdilution Testing (BMD).

***Results*:** A study conducted over one year tested 80 Enterobacterales isolates, with 74 (92.5%) identified as carbapenem-resistant (CRE) using the Kirby Bauer disc diffusion method and confirmed by mCIM testing. The majority of isolates (44%) were from the Neonatal Intensive Care Unit (NICU), with a male predominance (60%). *Enterobacter* species (40%) was the most prevalent, followed by *Klebsiella pneumoniae* (26.6%), *Citrobacter species* (20%), and *Escherichia coli* (13.4%). The study found high multidrug resistance, with 100% resistance to most antibiotics in *Enterobacter* and *Klebsiella* species. All 74 isolates showed intermediate colistin susceptibility, with 43.2% exhibiting an MIC of 0.031 µg/mL.

Conclusion: This study highlights the urgent need for colistin stewardship programs to address the rise of carbapenem-resistant *Enterobacterales* (CRE). The emergence of colistin resistance, especially in NICUs and ICUs, requires enhanced monitoring and rapid diagnostics. Focusing on vulnerable populations like neonates and critically ill patients is crucial to combat multidrug-resistant infections.

Keywords: Carbapenem-Resistant Enterobacterales (CRE), Broth Microdilution method, Modified Carbapenem Inactivation Method (mCIM).

Introduction

In the past two decades, antimicrobial resistance (AMR) has emerged as a formidable global health challenge, particularly among Gram-negative pathogens like those in the *Enterobacterales*. These bacteria have evolved a diverse array of resistance mechanisms, rendering several classes of antibiotics ineffective. As a result, there has been a surge in infections caused by multidrug-resistant (MDR) Gram-negative pathogens, with *Enterobacterales* at the forefront of this growing problem.

Multi-drug-resistant (MDR) gram-negative pathogens, including *Klebsiella Pneumoniae*, *Escherichia coli* and other *Enterobacterales* resistant to carbapenems, have emerged as a significant threat worldwide [2-4]. Colistin is regarded as the last resort against these organisms [1,2,5]. But due to the injudicious use of this antibiotic especially in intensive care settings, *GNRs* showing resistance to colistin are increasingly encountered due to selective antibiotic pressure and horizontal transmission [4,6]. Colistin resistance is often associated with carbapenem resistance, and such organisms are classified as extensively drug-resistant (XDR) [1]. Reports of colistin plus carbapenem-resistant cases have emerged from different parts of the world and because of limited therapeutic options available, is becoming a major global health concern.

For many years, carbapenems—a class of β-lactam antibiotics—have been the cornerstone in the treatment of Gram-negative bacterial infections, especially in intensive care settings, due to their broad-spectrum activity. However, the rise of Carbapenem-Resistant *Enterobacterales* (CRE)—which exhibit resistance to one or more carbapenems such as ertapenem, meropenem, imipenem, or Doripenem—has become a significant clinical hurdle. The mechanisms driving resistance in these bacteria include the production of carbapenemase, overexpression of efflux pumps, loss of membrane porins, and reduced binding of carbapenems to penicillin-binding proteins.

Colistin, a cationic polypeptide antibiotic from the polymyxin class, has emerged as a critical last-resort treatment for infections caused by CRE. It exerts its antimicrobial effects by disrupting the bacterial cell membrane through interaction with lipid A subunits of lipopolysaccharides, leading to cell death. However, increasing reports of colistin resistance have complicated its therapeutic role, further limiting treatment options. The concurrent emergence of resistance to both carbapenems and colistin is an alarming development that underscores the urgent need for effective surveillance, timely detection, and containment strategies.

In light of the increasing prevalence of colistin resistance, it is imperative to closely monitor the dissemination of these resistant strains. This study endeavours to assess the extent of colistin susceptibility among *Enterobacterale*s underscoring the critical need for prompt detection and the formulation of robust management strategies to combat these highly resistant pathogens.

Material and Methods

This was a cross-sectional study conducted in the Department of Microbiology, UCMS & GTB hospital, Delhi from January 2023 to June 2024. A total of 80 multidrug-resistant gram- negative clinical isolates isolated from blood culture were taken. Among 80 isolates, 74 (92.5%) exhibited carbapenem resistance, and confirmed by Modified Carbapenem Inactivation Method (mCIM) testing. Further, colistin susceptibility was ascertained using Broth Microdilution Susceptibility Testing (BMD).

Blood Sample Processing: Blood samples were inoculated into automated blood culture bottles and promptly transported to the bacteriology laboratory. Upon receipt, the bottles were immediately loaded into the BacT/ALERT automated system, which continuously monitors microbial growth through the colorimetric detection of carbon dioxide production. When flagged as positive, the samples were processed without delay, including Gram staining and inoculation onto agar plates for microbiological identification. Subsequent antibiotic susceptibility testing ensured accurate and timely diagnostic outcomes, supporting effective clinical management.

Antimicrobial Susceptibility Testing: The antibiotic susceptibility profiles of the isolated organisms were evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates, with results interpreted according to the CLSI guidelines. Not all antibiotics were tested for every microorganism. Control strains, such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853), were utilized to validate the accuracy of the Kirby-Bauer method. Carbapenem resistance was identified using standard phenotypic method in accordance with the most recent CLSI guidelines.

Phenotypic Detection of Carbapenem-Resistant Enterobacterales Using the Modified Carbapenem Inactivation Method (mCIM):

Under Clinical and Laboratory Standards Institute guidelines, an imipenem disc was used to perform the Modified Carbapenem Inactivation Method (mCIM) test. A suspension of bacterial colonies from an overnight blood agar bacterial culture was prepared in 2 ml of Tryptone Soya Broth (TSB), and then a 10µg imipenem disc was added to each broth. The suspension was incubated at 37°C for four hours. Following this, a suspension of 0.5 McFarland turbidity of *Escherichia coli* ATCC 25922 was prepared and spread evenly onto Mueller Hinton Agar (MHA). The imipenem disc from the Tryptone Soya Broth (TSB) suspension was carefully blotted to remove excess broth and then placed on the Mueller Hinton Agar plate, inoculated with an indicator strain of *Escherichia coli*. The plates were incubated for 18 to 24 hours at 37°C [7]. Positive (*Klebsiella pneumoniae* ATCC BAA-1705) and negative (*Escherichia coli* ATCC 25922) controls were used to ensure result accuracy, with interpretation conducted per Clinical and Laboratory Standards Institute guideline [8].

The Broth Microdilution (BMD) Method: was used to determine colistin susceptibility. Minimum inhibitory concentration (MIC) of the drug is measured by inoculation of drug in serial two-fold dilutions along with the isolate in micro quantities. Drug potency was determined before testing, and stocks were prepared and stored in cryovials. The stock solutions were diluted to a 4X concentration to prepare working solutions. Susceptibility testing was carried out at various concentrations using microtiter plates. Colistin dilutions (16–0.125 µg/ml) were prepared in CA-MHB broth and distributed into microtiter plate wells. Test strains and control strains (*Escherichia coli* ATCC 25922 and *E. coli* NCTC 13846) were prepared by adjusting bacterial suspensions to a 0.5 McFarland standard, further diluted, and inoculated into the wells. Plates were incubated at 35°C for 16–20 hours, sealed to prevent drying. MIC was defined as the lowest colistin concentration inhibiting visible growth compared to growth controls. Valid tests required proper growth in control wells and expected outcomes for positive and negative control strains.

Results

In the study during one year, 80 isolates of *Enterobacterales* were tested, with 74 (92.5%) identified as carbapenem-resistant using

the Kirby Bauer disc diffusion method and further confirmed by Modified Carbapenem Inactivation Method (mCIM) testing. These 74 Carbapenem-resistant *Enterobacterales* (CRE) isolates were derived from hospitalized patients across various wards. The study included isolates from patients aged newborn to 75 years, with the highest proportion (52%, n=42) from the newborn age group. Among these, 60% (n=25) were males and 40% (n=17) were females, indicating a male predominance (Figure 1).

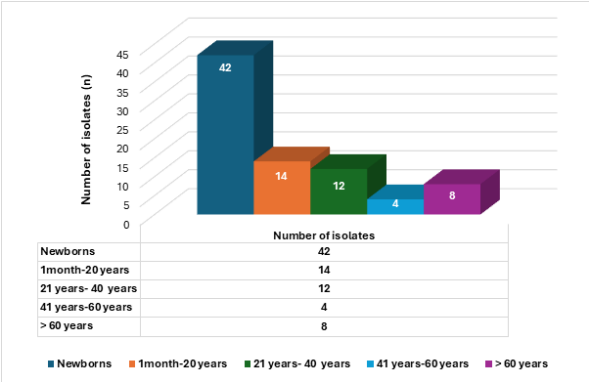

Figure 1: Trends of multidrug resistant gram-negative pathogen isolated from blood culture among various age-groups (n=80).

The study showed maximum number of Carbapenem-resistant *Enterobacterales* (CRE) isolates were from the Neonatal Intensive Care Unit (44%) (Figure 2).

PICU: Paediatric Intensive care unit, NICU: Neonatal Intensive care unit, MICU: Multidisciplinary Intensive care unit

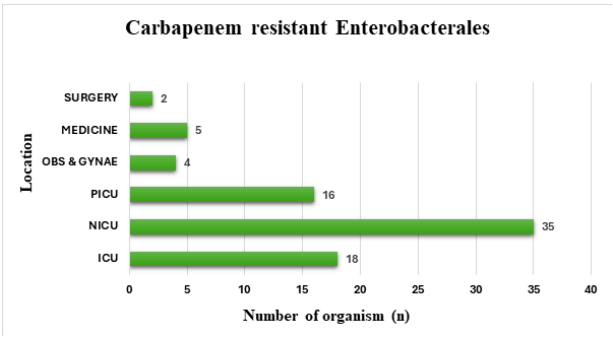

Figure 2: Location wise distribution of carbapenem-resistant *Enterobacterales* in the study population (n=74).

Out of 74 carbapenem resistant isolates, *Enterobacter species* (40%) was the most dominant followed by *Klebsiella pneumoniae* (26.6%), *Citrobacter species* (20%) and *Escherichia coli* (13.4%) (Table 1) (Figure 3).

Bacteria	Number (n)	Percentage (%)
Enterobacter species	30	40
Klebsiella species	20	26.6
Citrobacter species	15	20
Escherichia coli	9	13.4
TOTAL	**74**	**100**

Table 1: Distribution of Organisms Among Carbapenem-Resistant Enterobacterales.

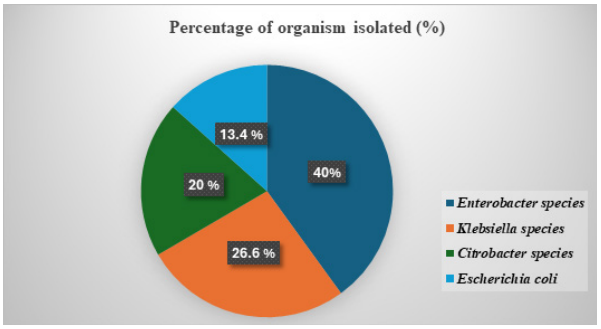

Figure 3: Percentage wise distribution of carbapenem-resistant *Enterobacterales* (n=74).

The antibiotic susceptibility trends among carbapenem-resistant Enterobacterales isolated from blood cultures indicate a critical level of multidrug resistance. *Enterobacter species* (n=30) and *Klebsiella pneumoniae* (n=20) showed resistance (100%) to all tested antibiotics, with the exception of cotrimoxazole, where resistance rates were 76.75% and 93.7%, respectively. Similarly, *Citrobacter species* (n=15) and *Escherichia coli* (n=9) displayed high resistance levels, with slightly reduced rates for imipenem (90.7% and 89.7%) and cotrimoxazole (91.5% and 88.6%).

The Broth Microdilution (BMD) test revealed that all 74 isolates (100%) exhibited intermediate susceptibility to colistin (MIC ≤2 µg/mL). Among these, the most frequently observed Minimum Inhibitory Concentration (MIC) was 0.031 µg/mL, detected in 32 isolates (43.2%) (Table 2).

MIC (µg/mL)	0.031	0.06	0.125	0.25	0.5	1	2	4	8	16
No. of isolates (n=74)	32	23	4	5	5	5	6	0	0	0

Table 2: Colistin Minimum Inhibitory Concentration Value for Carbapenem Resistant *Enterobacterales* by Broth Micro Dilution.

Discussion

This study underscores the substantial burden posed by Carbapenem-Resistant *Enterobacterales* (CRE) among hospitalized patients. The high prevalence of carbapenem resistance (92.5%) observed among *Enterobacterales* aligns with global trends of increasing resistance, which is a major public health concern due to associated morbidity and mortality [9,10]. Such resistance is particularly detrimental in healthcare settings where critically ill patients, including neonates, are disproportionately affected.

The study revealed that the majority of CRE isolates (52%) were obtained from neonates, with the highest proportion (44%) isolated from the Neonatal Intensive Care Unit (NICU). This finding reflects the vulnerability of neonates due to factors such as underdeveloped immunity, invasive procedures, and prolonged hospitalization. Similar trends have been reported by Logan et al. (2015), who highlighted the neonatal population as a significant risk group for multidrug-resistant infections [11]. Additionally, the male predominance (60%) observed in neonatal cases is consistent with earlier reports suggesting slower immune system maturation in male neonates contributes to a higher infection susceptibility [12].

Among the CRE isolates, *Enterobacter species* were the most prevalent (40%), followed by *Klebsiella pneumoniae* (26.6%), *Citrobacter species* (20%), and *Escherichia coli* (13.4%). These findings mirror those of Pitout et al. (2015), who observed a shift in CRE epidemiology, with *Enterobacter species* emerging as a leading cause of resistance due to Amp C β-lactamase production and porin loss [13]. *Klebsiella pneumoniae*, historically the dominant CRE species, continues to be a significant pathogen but with a declining relative frequency, possibly due to evolving antimicrobial selection pressures.

The antibiogram revealed near-universal resistance among CRE isolates to critical antibiotics such as imipenem, ciprofloxacin, ceftriaxone, and cotrimoxazole, ranging from 89.7% to 100%. This extensive resistance underscores the limited therapeutic options for treating CRE infections, consistent with findings from Nordmann et al. (2011), who emphasized the role of carbapenemase enzymes and efflux pumps in conferring high resistance levels [14].

Among colistin-intermediate isolates, the most common MIC value was 0.031 µg/mL, observed in 43.2% of cases. This heterogeneity in MIC values highlights the need for accurate susceptibility testing to guide therapy. The Broth Microdilution (BMD) method remains the gold standard for MIC determination, minimizing variability observed with other methods, as corroborated by Humphries et al. (2019) [15].

The distribution of CRE species and resistance patterns observed in this study aligns with findings by Kumar et al. (2020), who reported *Enterobacter species* as the predominant CRE isolate [16]. Similarly, the detection of colistin resistance in critical care unit mirrors results from Kaye et al. (2018), highlighting the ICU as a hotspot for multidrug-resistant organisms due to prolonged hospitalizations and intensive antimicrobial use [17].

Limitations

This study has certain limitations. Its single-centre design and relatively small sample size may limit the generalizability of the findings. Multicentre studies are required to provide a more comprehensive understanding of the regional and national epidemiology of carbapenem-resistant *Enterobacterales* (CRE). Additionally, the study did not include genotypic analysis of colistin resistance, which could have offered deeper insights into the molecular mechanisms underlying resistance.

Conclusion

This study emphasizes the critical need for colistin stewardship programs as part of broader antimicrobial strategies to combat the alarming rise of carbapenem-resistant *Enterobacterales* (CRE). The emergence of colistin resistance, particularly in high-risk settings like NICUs and MICU, demands strict monitoring, enhanced surveillance, and rapid diagnostic tools to guide effective interventions. Prioritizing vulnerable populations, such as neonates and critically ill patients, is essential to mitigate the devastating impact of multidrug-resistant pathogens. Robust infection control, innovative therapies, and unwavering surveillance are imperative to preserve treatment efficacy and prevent resistance escalation.

Abbreviation

CRE: Carbapenem-Resistant Enterobacterales

mCIM: Modified Carbapenem Inactivation Method

NICU: Neonatal Intensive Care Unit

MICU: Multidisciplinary Adult Intensive care unit.

BMD: Broth Microdilution

MIC: Minimum Inhibitory Concentration

ICUs: Intensive Care Units

XDR: Extensively Drug-Resistant

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