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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF CARBAPENEM RESISTANCE *KLEBSIELLA PNEUMONIAE*, *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI*: FROM A TERTIARY CARE HOSPITAL IN DHAKA, BANGLADESH

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ABSTRACT

Background: Gram-negative bacteria, particularly *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, have become increasingly resistant to β-lactam antibiotics, particularly carbapenems, posing a significant global health risk. Resistance is largely driven by carbapenemases, but many strains exhibit resistance via overproduction of cephalosporinases, extended-spectrum β-lactamases (ESBLs), reduced outer membrane permeability, and enhanced efflux pump activity, complicating treatment options. **Objectives:** This study aims to characterize carbapenem-resistant Gram-negative bacteria phenotypically and molecularly to identify the mechanisms driving resistance, guiding appropriate antibiotic use, and informing drug development strategies. **Materials and Methods:** A total of 75 carbapenem-resistant isolates were collected from various clinical specimens. Identification was performed using MacConkey and chromogenic agars, standard biochemical testing, and the Vitek-2 compact (Biomerieux France). Carbapenemase production was screened using the mCIM and eCIM per CLSI guidelines, and PCR was employed to detect carbapenemase and efflux pump genes. **Results:** Of the 75 isolates, 34 (45.33%) were phenotypically carbapenemase producers. Molecular analysis identified resistance genes in 67 (89.33%) isolates, with 28 (37.33%) carrying carbapenemase genes, 20 (26.66%) carrying efflux pump genes, and 19 (25.33%) carrying both. KPC and OXA-48 were the predominant carbapenemases, while MexB and AcrAB were the most common efflux pump genes. A significant association was also determined between efflux pump genes and carbapenem resistance ($p < 0.001$). **Conclusion:** The co-existence of carbapenemase and efflux pump genes reflects the complexity of resistance mechanisms, emphasizing the need for ongoing surveillance and novel antimicrobial strategies.

KEYWORDS: Carbapenemase, KPC, OXA-48, Efflux pump, Bangladesh, Vitek 2.

INTRODUCTION

Carbapenem resistance has emerged as a critical challenge in healthcare, primarily due to the multifaceted mechanisms underlying resistance, such as the production of carbapenemases and the enhanced activity of efflux pumps. These multidrug resistance mechanisms complicate treatment and contribute to the global spread of resistant strains.[1,2] At present it becomes a critical threat for global public health because of their limited treatment options, increased mortality rates, and high transmission potential in healthcare environments. Among these, carbapenem-resistant *Klebsiella pneumoniae* (CRKP), *Escherichia coli* (CREC), and *Pseudomonas aeruginosa* (CRPA) are the most prominent multidrug-resistant pathogens, widely disseminated across hospitals and healthcare systems.[3-8] In China, nationwide antimicrobial surveillance by CHINET revealed a concerning increase in the prevalence of carbapenem-resistant Enterobacteriaceae (CRE), rising from 3.1% in 2005 to 10.4% by 2022. The sharpest increase occurred in CRKP, whose prevalence grew from 3.0% to 24.2% over the same period.[9] Together, CRKP and CREC now account for over 90% of all CRE cases, reflecting a significant shift in the

epidemiology of antibiotic resistance.[10] These organisms contribute substantially to nosocomial infections and are often associated with delayed effective treatment, elevated healthcare costs, and increased mortality.[11,12] Carbapenem resistance in Enterobacteriaceae primarily arises from the production of carbapenemase enzymes. The most prevalent resistance genes include bla-KPC, bla-NDM, bla-VIM, bla-IMP, and bla-OXA-48, many of which are plasmid-mediated, facilitating horizontal gene transfer. A multicenter study involving 65 hospitals across 25 provinces in China demonstrated that CRKP isolates are most frequently associated with the bla-KPC gene, while other CRE strains are more commonly linked to bla-NDM.[12,13] The varying distribution of resistance genes across regions underscores the importance of ongoing molecular surveillance to guide local treatment protocols and inform public health strategies.[14]

Pseudomonas aeruginosa, while not part of the Enterobacteriaceae family, has also emerged as a major concern due to its remarkable adaptability and ability to develop resistance. CRPA exhibits both intrinsic and acquired resistance mechanisms, including reduced outer membrane permeability, overexpression of efflux pumps, and production of β-lactamases.[15] Unlike CRE, carbapenem resistance in *P. aeruginosa* is frequently non-enzymatic and often results from the loss or downregulation of the OprD porin, which is responsible for the uptake of imipenem.[16] Additionally, efflux pumps—particularly those of the resistance-nodulation-division (RND) family—play a central role in multidrug resistance in *P. aeruginosa*. The MexAB-OprM efflux system is constitutively expressed in nearly all clinical isolates and actively exports a broad range of antibiotics, including fluoroquinolones, tetracyclines, chloramphenicol, and β-lactams such as piperacillin, ceftazidime, and Cefepime.[17–19] Although imipenem is not typically a substrate for MexAB-OprM, meropenem can be extruded via this pump due to its hydrophobic structure, reducing its efficacy.[20,21]

Multiple-antibiotic-resistance (Mar) mutants of *Escherichia coli* exhibit enhanced resistance to a wide array of structurally unrelated antimicrobial agents. This resistance is primarily mediated through the activation of the MarA regulon, a global regulatory network that modulates the expression of numerous genes involved in antibiotic resistance, stress responses, and membrane transport. MarA, a transcriptional activator encoded by the *marRAB* operon, upregulates genes responsible for reducing intracellular antibiotic concentrations.[22,23,24] Notably, it induces the overexpression of the AcrAB-TolC efflux pump system, also a member of the resistance-nodulation-division (RND) family, which actively expels a broad range of antibiotics including fluoroquinolones, β-lactams, chloramphenicol, tetracyclines, and dyes such as ethidium bromide. In addition to efflux activation, MarA downregulates outer membrane porins such as OmpF, thereby reducing antibiotic influx. Together, these mechanisms significantly decrease the intracellular accumulation of antimicrobial agents.[25] The AcrAB efflux pump is a well-characterized multidrug resistance system that shares structural and functional homology with other bacterial efflux mechanisms. Its expression is partially controlled by the transcriptional repressor AcrR, which modulates the system's activity in response to environmental signals. AcrB, a component of RND family, functions as the inner membrane transporter responsible for substrate recognition and energy-dependent export. AcrA, a periplasmic membrane fusion protein, facilitates the connection between AcrB and the outer membrane channel, forming a continuous conduit for drug efflux. This tripartite assembly allows for the direct extrusion of toxic compounds from the cytoplasm to the external environment, effectively bypassing the periplasmic space and outer membrane, and thereby contributing significantly to bacterial antibiotic resistance.[26,27,28] The primary role of the AcrAB efflux system in *Escherichia coli* is the active extrusion of antibiotics, a function underscored by the limited expression of its homolog AcrEF in the *E. coli* K-12 strain.[29] This functional significance is further supported by observations in *Pseudomonas aeruginosa*, where MexAB, the closest homolog to AcrAB, is a key determinant of the bacterium's intrinsic resistance to a broad spectrum of antibiotics, with the notable exception of aminoglycosides. These parallels highlight the conserved and critical role of RND-type efflux pumps in multidrug resistance across different Gram-negative species.[30,31] Studies have demonstrated that the enhanced β-lactam resistance observed in *Enterobacterales* Mar mutants is primarily attributable to upregulated efflux activity, which acts in synergy with reduced expression of the OmpF porin, thereby limiting drug influx. In *Pseudomonas aeruginosa*, homologous efflux systems related to AcrAB have similarly been shown to effectively expel β-lactam antibiotics, further reinforcing the role of efflux mechanisms in mediating multidrug resistance among Gram-negative pathogens.[32]

Multidrug resistance (MDR) in Gram-negative pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* is a serious clinical challenge, largely driven by the presence of carbapenemase genes and the overexpression of efflux pumps. The carbapenemase genes, particularly *bla*KPC (Klebsiella pneumoniae carbapenemase) and *bla*OXA (oxacillinase-type enzymes), encode β-lactamases that hydrolyse carbapenem antibiotics, rendering them ineffective. These genes are often plasmid-borne, allowing for horizontal gene transfer between species and contributing to the rapid spread of resistance.[33] In parallel, efflux pumps—especially those of the resistance-nodulation-division (RND) family—play a crucial role in expelling a wide range of antibiotics from the bacterial cell. In *E. coli* and *K. pneumoniae*, the AcrAB-TolC efflux system is a key mediator of resistance, while *P. aeruginosa* relies on the MexAB-

OprM system to eliminate carbapenems, fluoroquinolones, and other antimicrobials.[34] The combined action of carbapenemase production and increased efflux leads to significantly elevated resistance levels. Recent studies highlight the synergistic effect of these two mechanisms. For example, efflux activity can lower intracellular antibiotic concentrations, enhancing the efficacy of carbapenemase enzymes in inactivating the drug. This co-resistance mechanism is particularly problematic in clinical settings, as it narrows therapeutic options and contributes to persistent infections.[35] Understanding these mechanisms is essential for developing effective strategies to combat MDR bacteria.

The increasing prevalence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), *Escherichia coli* (CREC), and *Pseudomonas aeruginosa* (CRPA) poses a critical challenge to infection control and effective clinical treatment. These pathogens exhibit high levels of resistance due to the combined action of carbapenemase production (e.g., *bla*KPC, *bla*OXA) and efflux pump overexpression, which together significantly reduce antibiotic efficacy. This study aimed to identify both phenotypic and genotypic markers of carbapenemase and efflux pump genes in clinical isolates of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. Understanding the mechanisms of resistance and the local epidemiology of these organisms is vital for guiding appropriate empirical antibiotic therapy, enhancing antimicrobial stewardship, and preventing further nosocomial spread. Timely molecular surveillance and targeted intervention strategies are essential to mitigate the growing threat posed by these multidrug-resistant pathogens in healthcare settings.

MATERIALS AND METHODS

A cross-sectional observational study was conducted over a 12-month period, from March 2024 to February 2025, at a leading academic medical center in Dhaka, Bangladesh. The study protocol received ethical approval from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU) (IRB Registration No. 5069). The primary objective was to investigate the mechanisms underlying carbapenem resistance in carbapenem-resistant Gram-negative organisms (CR-GNOs) isolated from both hospitalized patients and community-acquired cases. This study aimed to enhance understanding of the molecular and phenotypic characteristics contributing to resistance, as well as the epidemiological trends and distribution patterns of CR-GNOs. Findings from this study are intended to support evidence-based interventions for infection control, antimicrobial stewardship, and public health planning in response to the growing burden of multidrug-resistant infections.

Patients of all age groups diagnosed with infections caused by carbapenem-resistant Gram-negative organisms (CR-GNOs) were enrolled in the study. A total of 75 clinical specimens were obtained from patients who met the inclusion criteria. These specimens included a range of sample types such as urine, blood, sputum, and other relevant clinical materials, as per physician's request. All samples were processed in the clinical microbiology laboratory of the medical center using standard protocols. Carbapenem resistance was assessed according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). Isolates exhibiting reduced susceptibility or resistance to carbapenems were classified as carbapenem-resistant, and were subjected to further phenotypic and genotypic analysis to investigate resistance mechanisms.

All carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates were subjected to the modified carbapenem inactivation method (mCIM) and the EDTA-modified carbapenem inactivation method (eCIM) for phenotypic identification of carbapenemase production and Metallo-β-lactamase (MBL) activity, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Finally, polymerase chain reaction (PCR) was performed to detect specific resistance genes. Conventional PCR was used to identify the presence of carbapenemase genes – *bla*KPC[36], *bla*OXA-48[37] and efflux pump-related genes- *acrA*[38], *acrB*[38], *MexB*[39] from the cultured isolates. The procedure was carried out in the Molecular Laboratory of the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU) using standard protocols.

Descriptive analysis of all relevant variable were done by using frequency and percentage. Collected data were checked, edited and analyzed with SPSS software package version-27 (Strata corporation, College station, Taxes). Chi-square test was used to determine whether there is a significant association between categorical variable. The result of chi-square test was interpreted using the p-value, which indicates the probability of observing the data if the null hypothesis (no association between the variable). In this study the low p-value ≤ 0.001 suggest that the observed differences were statistically significant.

The prepared culture medium was properly packed to avoid moisture loss and microbiological contamination, and they were kept at 2-8°C. Sterility and performance control of culture medium were done batch to batch. All reagents and prepared medium were labeled with an expiration date. After data collection, it was examined for insufficiency, irrelevancy, and consistency. Irrelevant and inconsistent data were discard.

RESULT

A one-year observational study was conducted to evaluate the resistance mechanisms associated with carbapenem resistant *Klebsiella pneumoniae* (CRKP), *Escherichia coli* (CREC), and *Pseudomonas aeruginosa* (CRPA) from patients in tertiary care hospitals. Out of an initial pool of 205 clinical specimens screened, 75

isolates were confirmed to be carbapenem-resistant Gram-negative bacteria (CR-GNB) and met the inclusion criteria for further analysis, following the exclusion of non-eligible samples.

Of the 75 carbapenem-resistant Gram-negative bacterial isolates, 34 (45.33%) tested positive using the modified Carbapenem Inactivation Method (mCIM). Among these mCIM-positive isolates, 19 (55.88%) also tested positive using the EDTA-modified Carbapenem Inactivation Method (eCIM), indicating the presence of metallo-β-lactamase (MBL) enzymes. The remaining 15 (44.11%) were positive only by mCIM, suggesting the production of serine carbapenemases (Table 1).

Specifically, among 40 carbapenem-resistant *Klebsiella pneumoniae* isolates, 9 (22.5%) were identified as MBL producers (positive for both mCIM and eCIM), while 8 (20%) were confirmed as serine carbapenemase producers (positive for mCIM only). In the case of *Escherichia coli*, 4 (44.44%) isolates showed phenotypic evidence of MBL production, and 2 (22.22%) were positive only by mCIM, indicating serine carbapenemase activity. Additionally, among 26 *Pseudomonas aeruginosa* isolates, 6 (23.07%) were phenotypically identified as MBL producers while 5 (19.23%) as serine carbapenemase producers.

Table 1: mCIM and eCIM for phenotypic detection of carbapenemase producing *Klebsiella pneumoniae, Escherichia coli* and *pseudomonas aeruginosa*.

Tests	*Klebsiella pneumoniae* n (%)	*Escherichia coli* n (%)	*Pseudomonas aeruginosa* n (%)	Total n (%)
Total mCIM positive	17 (42.5%)	6 (66.66%)	11 (42.30%)	34 (45.33%)
Both mCIM and eCIM positive	9 (22.5%)	4 (44.44%)	6 (23.07%)	19 (25.33%)
mCIM positive but eCIM negative	8 (20%)	2 (22.22)	5 (19.23%)	15 (20%)

The study also evaluated the distribution of carbapenemase and efflux pump genes among the 75 carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates. Of these, 67 isolates (89.3%) were found to harbor at least one of the targeted resistance genes, whereas no target genes were detected in 8 isolates (10.66%) (Table 2).

Among the 67 gene-positive isolates, 21 (52.3%) *Klebsiella pneumoniae*, 2 (22.22%) *Escherichia coli*, and 5 (19.23%) *Pseudomonas aeruginosa* carried only carbapenemase-encoding genes. In contrast, exclusive presence of efflux pump genes was observed in 5 (12.5%) *Klebsiella pneumoniae*, 5 (55.55%) *E. coli*, and 10 (38.46%) *P. aeruginosa* isolates. Co-harboring of both carbapenemase and efflux pump genes was identified in 10 (25%) *Klebsiella pneumoniae*, 1 (11.11%) *E. coli*, and 8 (30.76%) *P. aeruginosa* isolates. Furthermore, 4 (10%) *Klebsiella pneumoniae*, 1 (11.11%) *E. coli*, and 3 (11.53%) *P. aeruginosa* did not test positive for any of the screened genes.

In summary, among the 75 CR-GNB isolates, 47 (62.93%) were positive for carbapenemase genes and 39 (52%) for efflux pump genes. This overlap is explained by the presence of 19 isolates that concurrently harbored both carbapenemase and efflux resistance genes.

Table 2: Distribution of carbapenemases gene, Efflux gene and both gene among CR- *Klebsiella pneumoniae, Escherichia coli* and *pseudomonas aeruginosa.*

Isolates (n)	Types of detected studied genes			
	Carbapenemase genes n(%)	Efflux pump genes n(%)	Both genes n(%)	Total n(%)
***Klebsiella pneumoniae* (n=40)**	21 (52.5)	5 (12.5)	10 (25)	36 (90)
***E. coli* (n=9)**	2 (22.22)	5 (55.55)	1 (11.11)	8 (88.99)
***Pseudomonas aeruginosa* (n=26)**	5 (19.23)	10 (38.46)	8 (30.76)	23 (88.46)
Total (n=75)	28 (37.33)	20 (26.66)	19 (25.33)	67 (89.33)

Class A carbapenemase-producing *blaKPC* was detected in 8 (22.22%) *Klebsiella pneumoniae*, 2 (25%) *Escherichia coli*, and 3 (13.04%) *Pseudomonas aeruginosa* isolates out of 67 total isolates. Among the Class D carbapenemase-producing genes, *blaOXA-48* was predominantly found in *Klebsiella pneumoniae* (63.88%), followed by *Pseudomonas aeruginosa* (8.34%), and *E. coli* (12.5%). Regarding efflux pump-mediated carbapenem resistance, the *acrA* gene was present in 2 (5.56%) *Klebsiella pneumoniae*, 6 (75%) *E.*

coli. For the *acrB* gene, 4 (11.11%) *Klebsiella pneumoniae* isolates and 6 (75%) *E. coli* isolates tested positive. None of the *Pseudomonas aeruginosa* harboring AcrAB gene. The *MexB* efflux pump gene was most frequently detected in *Pseudomonas aeruginosa* (18, 78.26%), followed by *Klebsiella pneumoniae* (4, 11.11%), and a single *E. coli* isolate (1, 12.5%) (Table 3).

Among the isolates harboring resistance genes, the most prevalent carbapenemase gene was *blaOXA-48* (32, 47.46%), followed by *blaKPC* (13, 19.40%). The efflux pump gene *MexB* was the most common (23, 34.32%), followed by *acrB* (10, 14.92%) and *acrA* (8, 11.94%). Notably, no studied gene was detected in 8 (10.66%) isolates. Statistical analysis revealed that the occurrence of efflux genes was significantly greater than that of carbapenemase genes ($p < 0.001$).

Table 3: Distribution of carbapenem resistant genes among isolated *Klebsiella pneumoniae, Escherichia coli* and *pseudomonas aeruginosa* in accordance with resistance mechanism (n=67).

Carbapenem resistant mechanism		*Klebsiella pneumoniae*	*E. coli.*	*Pseudomonas aeruginosa*	Total n (%)	P value
Class A carbapenemase	KPC	8 (22.22)	2 (25)	3 (13.04)	13 (19.40)	0.625
Class D carbapenemase	OXA-48	23 (63.88)	1(12.5))	8 (34.78)	32 (47.46)	0.009
Efflux pump genes	acr A	2 (5.56)	6 (75)	0	8 (11.94)	<0.001*
	acr B	4 (11.11)	6 (75)	0	10 (14.92)	<0.001*
	Mex B	4 (11.11)	1 (12.5)	18 (78.26)	23 (34.32)	<0.001*

*p value <0.001 is statistically significant.

DISCUSSION

The findings of this one-year prospective study underscore the alarming prevalence of carbapenem-resistant *K. pneumoniae*, *P. aeruginosa*, and *E. coli* in a tertiary care hospital setting in Dhaka. The predominance of resistance among these clinically significant pathogens reflects a growing AMR burden in Bangladesh and aligns with global trends observed in other low- and middle-income countries (LMICs). Notably, the co-existence of multiple resistance genes, including bla-KPC, bla-OXA-48, and efflux gene, highlights the genetic complexity and the potential for horizontal gene transfer among these organisms. Phenotypic testing revealed extensive resistance to carbapenems, with imipenem and meropenem showing markedly reduced efficacy. In line with this, molecular analysis identified a high frequency of carbapenemase-encoding genes, particularly bla-OXA-48, which has been previously reported as endemic in the Indian subcontinent.[40] The detection of bla-OXA-48 in *K. pneumoniae* and *E. coli* suggests the dissemination of plasmid-mediated resistance elements that are capable of rapid spread within healthcare facilities. Meanwhile, *P. aeruginosa* exhibited significant levels of multidrug resistance, often attributed to a combination of intrinsic resistance mechanisms (e.g., efflux pumps, porin loss) and acquired carbapenemases.

The phenotypic detection of carbapenemase-producing organisms in this study provides critical insight into the underlying resistance mechanisms among *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* isolated in a tertiary care hospital in Dhaka. Of the 75 carbapenem-resistant isolates, 34 (45.33%) tested positive using the modified carbapenem inactivation method (mCIM), confirming the presence of carbapenemase-producing organisms. This proportion is comparable to previous studies conducted in South and Southeast Asia, where mCIM positivity among CR-GNB ranged between 40–60%.[40,41] The subsequent use of the EDTA-modified carbapenem inactivation method (eCIM) allowed differentiation between metallo-β-lactamase (MBL) and serine carbapenemase producers. Of the 34 mCIM-positive isolates, 19 (55.88%) were also eCIM-positive, indicating MBL production, while the remaining 15 (44.11%) were mCIM-positive but eCIM-negative, suggesting the production of serine carbapenemases. These findings align with global trends showing the co-circulation of both MBL and serine carbapenemases in healthcare settings.[42,43]

Among the *K. pneumoniae* isolates (n=40), 9 (22.5%) demonstrated MBL activity while 8 (20%) were identified as serine carbapenemase producers. *K. pneumoniae* is a well-documented reservoir for various carbapenemases, including NDM, VIM, and OXA-48-like enzymes.[44] The predominance of MBL production in a subset of these isolates is consistent with the high prevalence of bla-NDM reported in similar regional studies.[45,46] Furthermore, the detection of serine carbapenemases is suggestive of bla-OXA-48 or bla-KPC genes, which have been increasingly detected in South Asian healthcare institutions.[47] In the case of *E. coli*, a relatively lower number of isolates (n=9) were tested, among which 4 (44.44%) showed MBL activity and 2 (22.22%) were mCIM-positive only, suggesting serine carbapenemase production. Although *E. coli* is not as frequently associated with carbapenem resistance as *K. pneumoniae*, the presence of MBL and serine carbapenemases in this species is increasingly recognized as a significant concern, particularly in regions with high antibiotic pressure and poor infection control.[48] This further underscores the ability of resistance genes to disseminate across species via plasmids and other mobile genetic elements.[49] For *P. aeruginosa*, 6 (23.07%) isolates were phenotypically confirmed as MBL producers, while 5 (19.23%) were identified as serine carbapenemase producers. This

distribution reflects the complex resistance mechanisms employed by *P. aeruginosa*, which include both acquired carbapenemase genes and intrinsic factors such as efflux pumps and porin modifications.[50] MBL production, particularly via bla-VIM and bla-IMP, remains a dominant mechanism of resistance in *P. aeruginosa* globally and regionally.[51] The use of mCIM and eCIM in combination has proven to be a valuable tool for routine laboratory screening of carbapenemase activity, offering reasonable sensitivity and specificity in differentiating MBL and serine carbapenemase producers.[52] Accurate differentiation is not only important for epidemiological purposes but also critical for guiding appropriate antimicrobial therapy. For example, infections caused by MBL producers may require treatment with agents such as aztreonam–avibactam combinations, while serine carbapenemase-producing organisms may remain susceptible to newer β-lactam/β-lactamase inhibitor combinations.[53]

The present study highlights the genetic basis of carbapenem resistance among *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* isolates, focusing on carbapenemase and efflux pump gene detection. Among 75 carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates, 67 (89.3%) carried at least one resistance gene, while 8 (10.66%) were negative for the targeted genes. These findings align with previous regional reports indicating a high prevalence of acquired resistance determinants among clinical CR-GNB isolates.[45] Among gene-positive isolates, 47 (62.93%) harbored carbapenemase genes, supporting the widespread dissemination of major enzymes such as bla-NDM, bla-KPC, and bla-OXA-48-like in clinical settings.[54] *K. pneumoniae* was the most frequent carbapenemase carrier, consistent with its known role as a major reservoir for such genes.[55] Conversely, 39 isolates (52%) possessed efflux pump genes, with *P. aeruginosa* and *E. coli* showing a higher reliance on efflux mechanisms, possibly due to their intrinsic resistance profiles.[56]

Co-harboring of carbapenemase and efflux genes in 19 isolates illustrates the multifactorial nature of resistance, compounding treatment difficulties and infection control challenges. Notably, a subset of isolates (10.66%) lacked detectable genes, implying alternative resistance mechanisms such as porin mutations or uncharacterized genes—underscoring the need for advanced molecular diagnostics like whole-genome sequencing.[57]

This study further delineates the molecular landscape of carbapenem resistance by characterizing the distribution of specific carbapenemase and efflux pump genes. Here a comprehensive overview of the molecular mechanisms contributing to carbapenem resistance in *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* by targeting key carbapenemase and efflux pump genes. Among the 67 gene-positive CR-GNB isolates, the most prevalent carbapenemase gene was bla-OXA-48 (47.46%), particularly dominant in *K. pneumoniae* (63.88%). This finding aligns with reports from the Indian subcontinent and Middle East where bla-OXA-48 has become endemic, primarily due to its plasmid-mediated dissemination and limited detection by standard assays.[58,59]

Class A carbapenemase gene bla-KPC, although less common overall (19.40%), was detected in 22.22% of *K. pneumoniae*, 25% of *E. coli*, and 13.04% of *P. aeruginosa* isolates. The emergence of bla-KPC in *E. coli* and *P. aeruginosa*—traditionally associated with *K. pneumoniae*—suggests horizontal gene transfer events that may lead to broader dissemination within hospital environments.[60] These findings underscore the clinical importance of monitoring for both KPC and OXA-type enzymes to guide therapeutic strategies effectively.

Efflux pump gene profiling revealed notable species-specific trends. *acrA* and *acrB* were predominantly detected in *E. coli* (75% each), supporting the role of AcrAB-TolC in mediating multidrug resistance, especially in the absence or low expression of carbapenemases.[61] In contrast, *P. aeruginosa* showed high prevalence of *MexB* (78.26%), consistent with its intrinsic resistance phenotype driven by the MexAB-OprM system, known to contribute to both β-lactam and non-β-lactam antibiotic resistance.[62]

Statistical analysis revealed that efflux pump genes were significantly more prevalent than carbapenemase genes ($p < 0.001$), particularly in *Escherichia coli* and *Pseudomonas aeruginosa*. This underscores the increasing relevance of efflux-mediated resistance, which reduces intracellular antibiotic accumulation and contributes to multidrug resistance even without enzymatic degradation.[63] Prominent systems such as AcrAB-TolC in *E. coli* and MexAB-OprM in *P. aeruginosa* are key contributors.[64]

All these findings reinforce the complex interplay of enzymatic and non-enzymatic mechanisms contributing to carbapenem resistance across *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The species-specific prevalence of resistance genes, coupled with the detection of isolates lacking known genetic markers, highlights the need for broader molecular investigations. Collectively, these findings underscore the multifactorial and species-specific mechanisms underpinning carbapenem resistance among Gram-negative pathogens. The integration of advanced molecular diagnostics with routine phenotypic assays is imperative for comprehensive surveillance, guiding targeted infection prevention strategies and informing antimicrobial stewardship efforts, particularly in high-burden tertiary healthcare settings.

CONCLUSION

This study provides key insights into the molecular and phenotypic profiles of carbapenem-resistant *Klebsiella*

pneumoniae, *Escherichia coli*, and *Pseudomonas aeruginosa* isolated from a tertiary care hospital in Dhaka. The high prevalence of bla-OXA-48 carbapenemase gene, along with the frequent detection of efflux pump genes such as MexB, highlights the complex mechanisms driving resistance. Efflux-mediated resistance was particularly prevalent in *E. coli* and *P. aeruginosa*, suggesting a need for broader diagnostic strategies. Additionally, gene-negative but phenotypically resistant isolates indicate alternative resistance mechanisms, emphasizing the need for integrated molecular and phenotypic surveillance.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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