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Running Title (within 10 words)	Carbapenem resistance and carbepenemase-producing *Enterobacteriaceae*
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ABSTRACT

Introduction: Carbapenem-resistant Enterobacteriaceae (CRE) poses a significant challenge in treating infections, leading to increased mortality and healthcare burden. Early and accurate identification of CRE is crucial for appropriate antibiotic selection. This study aimed to evaluate the prevalence of CRE isolated from ICU patients and carbapenemase gene profiles among *Klebsiella pneumoniae* isolates. ***Methods:*** A cross-sectional study was conducted on Enterobacteriaceae isolates from 53 ICU patients from March to May 2023. Among 125 initial isolates, 89 non-duplicated Enterobacteriaceae strains were included. Carbapenem resistance was determined from Kirby-Bauer disk diffusion susceptibility testing results. Carbapenemase-producing phenotype and carbapenemase classification were classified from BD Phoenix M50 results using the NMIC-500 CPO panel. Carbapenemase-encoding gene were identified from amplification results of carbapenemase-encoding genes in *K. pneumoniae* strains using multiplex real-time PCR. ***Results:*** Of 89 isolates, 51 (57.3%) were carbapenem-resistant, predominantly *K. pneumoniae* (34/48, 70.8%) and *S. marcescens* (12/13, 92.3%). All isolates were carbapenem-resistant due to carbapenemase production. Class D carbapenemase was the most prevalent (39/51,

76.5%). Among *K. pneumoniae* isolates, 12/34 harboured single carbapenemase genes: $bla_{OXA-48-like}$ (17.7%), bla_{KPC} (14.7%), and bla_{NDM-1} (2.9%); Sixteen isolates carried two genes: bla_{NDM-1}+$bla_{OXA-48-like}$ and bla_{KPC}+$bla_{OXA-48-like}$; Three isolates possessed three genes: bla_{KPC}+bla_{NDM-1}+$bla_{OXA-48-like}$. Multiple gene carriage was significantly associated with increased antibiotic resistance ($p<0.01$). ***Conclusion:*** This study revealed a high prevalence of carbapenemase-producing, carbapenem-resistant Enterobacteriaceae. This significantly increased the risk of spreading multidrug-resistant pathogens. Therefore, stronger infection control measures are in demand. Additionally, automated susceptibility testing and carbapenemase class identification are crucial for guiding treatment decisions.

Keywords: Carbapenem-resistant Enterobacteriaceae, antimicrobial resistance, carbapenemase.

1. INTRODUCTION

The rising rate of antibiotic-resistant bacteria has emerged as a global crisis, creating significant challenges in the clinical management of infections. Carbapenems have long been regarded as a potent antibiotic for combating multidrug-resistant Gram-negative bacteria. However, the rise of carbapenem-resistant bacteria has complicated treatment options as effective and safe alternatives are limited. Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) are linked to considerable morbidity and mortality, making CRE a critical public health concern.

Recently, the prevalence of CRE has surged to alarming levels [2] that represents a critical global health threat. Domestic studies also reported the emergence of carbapenem-resistant Gram-negative bacteria, with resistance rates ranging from 15.2% to 38.0%, primarily in intensive care units [3, 4]. This resistance primarily occurs through carbapenemase production, classified into three main Ambler classes [5]. Class A carbapenemases, particularly KPC (*Klebsiella pneumoniae* carbapenemase), are predominant globally with prevalence reaching over 60% in endemic areas like Greece. Class B metallo-β-lactamases include NDM (endemic in Indian subcontinent, 20-30%), VIM (prevalent in Mediterranean region), and IMP (common in Japan and Taiwan), with mortality rates of 30-60% for bloodstream infections [7]. Class D carbapenemases, mainly OXA-48-type enzymes, are endemic in Turkey and the Middle East, showing treatment failure rates up to 50% [8].

While this resistance affects various Enterobacteriaceae species, the fact that *K. pneumoniae* has emerged as the predominant pathogen in carbapenem-resistant infections worldwide warrants

particular attention [9]. Several characteristics make *K. pneumoniae* a critical target for investigation, including: (1) it is frequently isolated from severe infections, especially in intensive care settings; (2) it demonstrates exceptional ability to acquire and maintain multiple resistance mechanisms simultaneously; and (3) it serves as a major reservoir for resistance gene transmission to other Enterobacteriaceae species through mobile genetic elements [10]. Additionally, successful clonal lineages of carbapenem-resistant *K. pneumoniae*, particularly ST258, have shown remarkable ability for hospital adaptation and global dissemination [11].

While carbapenem resistance can occur through multiple mechanisms, including porin loss and efflux pump overexpression, our study focused primarily on carbapenemase production for several reasons. First, carbapenemase genes are typically carried on mobile genetic elements, making them the most clinically significant mechanism due to their potential for rapid spread between bacteria [2]. Second, unlike other mechanisms that typically confer low-level resistance, carbapenemase production often results in high-level resistance and treatment failure [12]. Third, the presence of carbapenemase genes requires specific antimicrobial approaches, making their detection crucial for clinical decision-making [12]. Other resistance mechanisms, while important, generally play a supplementary role in enhancing resistance levels when combined with carbapenemase production. This focus aligns with current international surveillance priorities and clinical guidelines that emphasize the detection and monitoring of carbapenemase-producing organisms. Early and accurate identification of carbapenem-resistant bacterial strains allows clinicians to select suitable alternative antibiotics, which can lower mortality rates and ease the treatment-related burden for patients. Therefore, this study was conducted to evaluate the prevalence of carbapenem-resistant and carbapenemase-producing Enterobacteriaceae. It also aimed to investigate the gene types that encode carbapenemase in specific carbapenem-resistant *K. pneumoniae* strains.

2. METHODS

2.1. Study design and participants

A cross-sectional study was carried out at the Department of Microbiology, University Medical Center at Ho Chi Minh City from March 2023 to May 2023. The study included Enterobacteriaceae strains isolated from specimens of ICU patients with the inclusion criteria being patients aged 18 years and over; Enterobacteriaceae isolated from specimens were determined as the cause of infection in ICU patients during the period from March 2023 to May 2023. Regarding sputum samples, the specimen was considered eligible if the number of white blood cells was ≥ 25 cells

and epithelial cells were under 10 cells after microscopic examination (examination under a microscope at ×100 field). For urine samples, the specimen was considered eligible if the number of bacteria of the same species was ≥ 10^5 CFU/mL. The exclusion criteria were bacteria of the same species on the same specimen of the patient in subsequent isolations.

2.2. Sample size and sampling

Sample size was determined using the formula for estimating a population proportion with specified absolute precision:

$$n = Z_{1-\alpha/2}{}^2 * \frac{p(1-p)}{d^2}$$

where:

- $Z_{1-\alpha/2}$ = 1.96 (95% confidence level)
- P = 0.59 (expected proportion of CRE based on a prospective cohort study in the USA [13])
- d = 0.10 (absolute precision).

This yielded a minimum required sample size of 91 isolates. The study utilized a census sampling method. All strains of bacteria that satisfied the inclusion and exclusion criteria and were isolated during the sampling period were included in the study.

2.3. Variables

Main variables of the study included: (1) Specimen type (blood, sputum, urine, other fluids) were identified from specimen information; (2) Enterobacteriaceae species (*Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella* spp., *Enterobacter* spp., *Proteus* spp.) were identified from automated bacterial identification results; (3) Meropenem antibiotic resistance (susceptible, intermediate, resistant) were determined from Kirby-Bauer disk diffusion susceptibility testing results; (4) Carbapenemase-producing phenotype and carbapenemase classification (class A, class B, class D, undefined) were classified from BD Phoenix M50 results using the NMIC-500 CPO panel; (5) Carbapenemase-encoding gene (bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{IMP}, bla_{VIM}, not detected) were identified from amplification results of carbapenemase-encoding genes in *K. pneumoniae* strains using multiplex real-time PCR.

2.4. Data collection

Microbiological techniques performed in the study were carried out according to standard procedures at the Department of Microbiology - University Medical Center at Ho Chi Minh City, and the Ministry of Health's Guidelines for Specialized Microbiological Techniques [14].

Bacterial culture and isolation for identification of Enterobacteriaceae species: Gram staining was first performed to determine the type of pathogenic bacteria present. Specimens containing Gram-negative bacteria were then selected and cultured on selective media. The bacteria were cultured on both BA and MacConkey agar, and incubated at 37°C for 24-48 hours. Finally, the BD Phoenix M50 automated system was used to identify Enterobacteriaceae.

Determine the meropenem resistance in isolated enteric bacterial strains by the Kirby-Bauer method: Assessing the meropenem resistance in isolated enteric bacterial strains through the Kirby-Bauer method involved the following step: (1) inoculate pure bacterial colonies onto MHA agar plates and place meropenem antibiotic disks on the surface; (2) incubate the plates at 37°C for 24 hours; then (3) measure the diameter of the zone of inhibition. This antibiotic susceptibility testing was performed to assess the sensitivity of the isolated enteric bacterial strains to meropenem, following to CLSI M100 2023 standards [15]. Meropenem-resistant strains were classified according to CLSI 2023 criteria (zone of inhibition ≤ 19 mm).

[Insert Table 1]

Classification of carbapenemase types, and assessment of antibiotic resistance in meropenem-resistant bacteria using antimicrobial susceptibility testing and the BD Gram-Negative Carbapenemase Classification Panel (Panel NMIC-500 CPO) on the Phoenix M50 automated system: Automated testing could provide the following results: (1) Negative, (2) Class A carbapenemase producer, (3) Class B carbapenemase producer, (4) Class D carbapenemase producer, (5) Unclassified carbapenemase producer. Antimicrobial susceptibility testing was performed automatically on the Phoenix M50 system and results were interpreted as susceptible, intermediate, or resistant according to CLSI 2023 guidelines.

Carbapenemase-encoding gene survey of carbapenem-resistant K. pneumoniae strains using multiplex real-time PCR: Utilizing the MDR KPC/OXA Real-TM and MDR MBL (VIM, IMP, NDM) Real-TM test kits supplied by Sacace Biotechnologies (Italy). The multiplex real-time PCR was conducted using the LightCycler 480 System (Roche, Switzerland). Each 25-µL reaction contained: 12.5 µL of 2× QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany), 0.5 µM of each primer, 2 µL of template DNA, and RNase-free water. Primers targeting bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$ genes were used. PCR conditions were initial denaturation at 95°C for 15 min; 40 cycles of 94°C for 30s, 60°C for 90s, and 72°C for 60s; followed by a melting curve analysis (65-95°C, 0.5°C/s). Positive and negative controls were included in each run. The

specificity of amplification was confirmed by melting curve analysis and gel electrophoresis. The limit of detection was 10 copies/reaction, determined using serial dilutions of control plasmids.

2.5. Bias control

The study selected research subjects based on targeted sampling criteria. The microbiological techniques in the study followed the standard procedures of the Department of Microbiology - University Medical Center at Ho Chi Minh City and the Guidelines for Technical Procedures in Microbiology of the Ministry of Health [14]. The antibiotic susceptibility of the intestinal bacterial strains was determined based on the CLSI M100 2023 standard [15]. To ensure the validity of the microbiological results, ten bacterial strains were randomly selected from the sample list and ran through tests for a second time.

2.6. Statistical methods

Data were managed using Excel software, while data analysis was conducted with Stata16. No missing data were recorded for primary outcomes. For antibiotic susceptibility testing, any invalid results, for which there were two occurrences in the research, were repeated to ensure complete dataset.

Zone diameters were analysed as continuous variables and categorized according to CLSI breakpoints. MIC values were log2-transformed before analysis. Descriptive statistics were applied, utilizing frequency and percentage calculations to summarize qualitative variables. For normally distributed quantitative variables, the mean and standard deviation were calculated.

The association between the presence of carbapenemase-encoding genes and antibiotic resistance was assessed using the chi-squared test. For 2×2 tables where expected cell values were under 5 in ≥ 20% of cases, Fisher's exact test was used as an alternative. A p-value of under 0.05 indicated statistically significant difference. The strength of the association was measured using the prevalence ratio (PR) with a 95% confidence interval.

2.7. STROBE checklist adherence

This research report followed the STROBE reporting guidelines by the UK EQUATOR Center, last updated on March 6, 2023 [15]. This checklist was involved to ensure that the integrity of our report as a cross-sectional study with all the essential sections, including the article’s title and abstract, introduction, methods, results, and discussion, along with other relevant information (Supplementary Table S1).

2.8. Ethical consideration

Informed Consent Written was obtained from patient's next of kin in the intensive care unit before specimen collection and study enrolment. The consent process included detailed explanation of the study purpose, potential benefits for improving treatment strategies, and assurance of data confidentiality. For patients who were alert and oriented, additional consent was obtained directly from the patients. All patient data was anonymized before analysis to ensure privacy protection while maintaining the ability to track clinical outcomes. The research proposal received ethical approval for scientific research from the Biomedical Research Ethics Council No. 1017/HĐĐĐ-ĐHYD signed on December 5th, 2022.

3. RESULTS

3.1. Participants

In the period between March 2023 and May 2023, a total of 125 Enterobacteriaceae strains were identified as causative agents of infection from patient ICU specimens. In which, 36 bacterial strains were excluded from the study as they were the same species from the same patient specimen in subsequent isolations. Therefore, 89 Enterobacteriaceae strains were included in the study (Figure 1).

[Insert Figure 1]

3.2. Characteristics of participants and specimen sample

The study isolated 89 Enterobacteriaceae strains from the specimens of 53 patients. More than 50% of the bacterial strains were isolated from sputum specimens. Among the 89 bacterial strains isolated, we identified 6 species of Enterobacteriaceae, namely *K. pneumoniae*, *E. coli*, *S. marcescens*, *Proteus* spp., *Enterobacter* spp., and *Salmonella* spp. Among these, *K. pneumoniae*, *E. coli*, and *S. marcescens* were the most prevalent species (Table 2).

[Insert Table 2]

In the sputum specimen, we discovered five species of Enterobacteriaceae: *K. pneumoniae*, *S. marcescens*, *E. coli*, *Proteus* spp., and *Enterobacter* spp. Among these, *K. pneumoniae* and *S. marcescens* were the predominant strains. In the blood specimen, we identified the presence of 3 species: *K. pneumoniae*, *S. marcescens*, and *E. coli*. Among these, *K. pneumoniae* accounted for more than two-third of the bacteria. In the urine specimen, the study only identified 2 species of bacteria: *E. coli* and *K. pneumoniae* (Table 3).

[Insert Table 3]

3.3. Meropenem resistance, Carbapenemase-producing phenotype and Carbapenemase classification

Of the 89 bacterial strains that were examined, 35 were found to be susceptible to meropenem, 3 were only partially susceptible, and 51 were resistant. The study revealed a significant prevalence of meropenem-resistant Enterobacteriaceae, with a resistance rate of 57.3%. Among the resistant strains, *K. pneumoniae* and *S. marcescens* exhibited the highest levels of meropenem resistance (Table 4).

[Insert Table 4]

The study found that all meropenem-resistant bacterial strains exhibited a carbapenemase-producing phenotype. Among the 51 carbapenemase-producing strains, the majority (76.5%) were classified as class D, followed by class B. Notably, no strains producing class A carbapenemase were identified. (Table 5).

[Insert Table 5]

3.4. Carbapenemase-encoding gene in K. pneumoniae strains

Out of the 34 carbapenemase-producing *K. pneumoniae* isolates, 12 contained only one carbapenemase-encoding gene. Among these, the most prevalent genotype was $bla_{OXA\text{-}48\text{-}like}$, with none of the isolates harbouring the bla_{VIM} or bla_{IMP} genes. Additionally, 16 isolates were found to possess two carbapenemase-encoding genes, specifically $bla_{NDM\text{-}1}$+$bla_{OXA\text{-}48\text{-}like}$ and bla_{KPC}+$bla_{OXA\text{-}48\text{-}like}$, with $bla_{NDM\text{-}1}$+$bla_{OXA\text{-}48\text{-}like}$ being the most frequently observed combination. Three isolates had three carbapenemase-encoding genes: bla_{KPC}+$bla_{NDM\text{-}1}$+$bla_{OXA\text{-}48\text{-}like}$. Furthermore, three isolates did not carry any of these genes.

The resistance rate to amikacin among *K. pneumoniae* strains with a single carbapenemase gene was generally low. Results indicated that no strains with either the bla_{KPC} or $bla_{NDM\text{-}1}$ genes were resistant to amikacin, and only 1 out of 6 strains with the $bla_{OXA\text{-}48\text{-}like}$ gene exhibited resistance. *K. pneumoniae* strains with either the bla_{KPC} or $bla_{OXA\text{-}48\text{-}like}$ gene remained susceptible to ceftazidime-avibactam. Most strains harbouring multiple carbapenemase genes were resistant to ceftazidime-avibactam and co-trimoxazole. However, tigecycline remained effective against the majority of carbapenem-resistant *K. pneumoniae* strains in this study (Table 6).

[Insert Table 6]

The study indicated a correlation between *K. pneumoniae* strains lacking the bla_{NDM} gene and their resistance to the ceftazidime-avibactam antibiotic. Specifically, bacteria without the bla_{NDM} gene

exhibited a 0.22-time lower resistance rate than those with the gene. This difference was statistically significant, with a p-value of 0.01 (PR = 0.22, 95% CI 0.06 – 0.88). (Table 7).

[Insert Table 7]

4. DISCUSSION

The study examined 89 strains of Enterobacteriaceae isolated from 53 ICU patients. The majority of bacterial strains were obtained from sputum specimens, followed by blood and bronchial fluid specimens. This finding is consistent with studies conducted by Pham Thai Binh and Wang [16]. However, a study by Luong Hong Loan and Huynh Minh Tuan reported that the highest number of bacterial strains was isolated from urine specimens, followed by sputum and blood specimens [17]. Similarly, a research at Thai Nguyen Central Hospital found that urine specimens had the highest rate of bacterial isolation, followed by sputum and pus specimens [18]. These finding discrepancies suggested variations in the proportions of specimen type across studies, which may depend on the study population, clinician indications, and the clinical condition of the patients.

Our isolation results showed that *K. pneumoniae* and *E. coli* were the most frequently isolated bacteria, consistent with numerous domestic studies, including the research at Hue Central Hospital and the study by author Pham Thai Binh in 2020, which also reported *K. pneumoniae* and *E. coli* as predominant [19]. Other international studies, such as those conducted in the United States and Taiwan (2015) similarly identified *K. pneumoniae* as the predominant species [20, 21]. In contrast, the study by Luong Thi Hong Nhung showed *E. coli* to be the most frequently isolated bacterium [18]. This variability suggests that the distribution of bacterial strains in clinical samples may differ based on factors such as study population, departments, hospitals, and different geographical regions.

In sputum specimens, we identified *K. pneumoniae* and *S. marcescens* as the predominant strains. In blood specimens, *K. pneumoniae* constituted more than two-third of the bacterial species. In urine specimens, the study only found *E. coli* and *K. pneumoniae*. These results are consistent with the studies by Luong Hong Loan (2020), Phan Nu Dieu Hong (2021), and Nguyen Minh Tam (2022), who found that *K. pneumoniae* as the predominant strain in sputum specimens and *E. coli* in urine specimens [17]. However, the aforementioned authors reported *E. coli* as the predominant strain in blood specimens.

Our study demonstrated a high prevalence of meropenem-resistant Enterobacteriaceae, with a resistance rate of 57.3%. Among these, *K. pneumoniae* and *S. marcescens* strains exhibited a

higher rate of meropenem resistance than reported in other studies. Phan Nu Dieu Hong found that 25.2% of Enterobacteriaceae strains were resistant to imipenem and/or meropenem [22]. Another screening study of carbapenem-resistant gut bacteria in intensive care units indicated 35.11% of patients tested positive for CRE upon admission to intensive care units [23]. Notably, the carbapenem-resistant strains were mainly *K. pneumoniae* and *E. coli*. In our study, the rate of meropenem-resistant *K. pneumoniae* strains was 70.8%, lower than the 80% reported at the Intensive Care Unit of Bach Mai Hospital in 2020 [24]. A study on the prevalence of carbapenem-resistant Enterobacteriaceae in hospitalized patients in Vietnam found that 52% of patients were infected with CRE, most commonly *K. pneumoniae*, *E. coli*, and *Enterobacter* spp., with a carbapenem resistance rate similar to our findings [25]. Our study revealed a high prevalence of carbapenem-resistant Enterobacteriaceae in the ICU setting, with *K. pneumoniae* emerging as the predominant species. The observed resistance rate of 57.3% is notably higher than previously reported rates in Vietnam. This finding aligns with global trends showing increasing carbapenem resistance, particularly in intensive care settings.

All meropenem-resistant bacterial strains in our study exhibited a carbapenemase-producing phenotype. This rate is comparable to that reported by Luong Hong Loan, who found a 99.1% prevalence of carbapenemase-producing Gram-negative bacteria [17]. Phan Nu Dieu Hong identified class B carbapenemase using mCIM and eCIM methods, reporting similar results with 40 of 40 strains positive by mCIM and 37 of 40 strains positive by eCIM [22]. Another study by Haidar Ghady et al. found that 97% of the isolated Enterobacteriaceae strains had a carbapenemase-producing phenotype [26].

Among the 51 carbapenem-resistant bacterial strains, the carbapenemase phenotype was detected. The most prevalent strains belonged to class D, followed by class B, with no class A carbapenemase-producing strains identified. These findings are consistent with results from studies by Luong Hong Loan [17], Pham Thai Binh [19] and Juri Katchanov [27] in Germany, which also reported that *K. pneumoniae* strains carrying class D carbapenemase-encoding genes as the most prevalent. However, a study in Iran [28] found class B carbapenemase to be more prevalent. This variation in prevalence may reflect differences in the distribution of carbapenemase subclasses across geographical regions. Furthermore, our study indicated that five out of 51 strains could not be classified into carbapenemase classes using the NMIC-500 CPO panel, all of them were *K. pneumoniae* strains. This may be attributed to the presence of multiple carbapenemase-

encoding genes [29]. The molecular characterization of resistance mechanisms in our study provided important insights into the complexity of carbapenem resistance. This distinct pattern might reflect unique local selection pressures and warrants further investigation.

Among the 34 carbapenemase-producing *K. pneumoniae* strains, 12 strains carried single carbapenemase-encoding genes, namely $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}, and $bla_{NDM\text{-}1}$. Additionally, 16 strains harboured two types of carbapenemase-encoding genes, specifically $bla_{NDM\text{-}1}$+$bla_{OXA\text{-}48\text{-}like}$ and bla_{KPC}+$bla_{OXA\text{-}48\text{-}like}$. Furthermore, three strains did not carry any of these genes. H'Nuong Nie et al. [30] found that the prevalence of the bla_{KPC} gene type was the highest among *K. pneumoniae* strains, and did not report the presence of the bla_{VIM} or bla_{IMP} gene types. Another study by Pham Thai Binh [19] indicated that *K. pneumoniae* strains that carrying the $bla_{OXA\text{-}48\text{-}like}$ and $bla_{NDM\text{-}1}$ genes exhibited the highest prevalence. Studies in Turkey [31] and by Demir Yelda [32] found that carbapenem-resistant Enterobacteriaceae isolates, strains that carried the $bla_{OXA\text{-}48\text{-}like}$ gene were predominant. A critical discrepancy exists between Tables 5 and 6. Table 5 shows no Class A carbapenemase producers (0 isolates) among K. pneumoniae isolates based on phenotypic testing. However, Table 6 demonstrates that 11 K. pneumoniae isolates carried Class A carbapenemase genes (blaKPC): 5 isolates with blaKPC alone, 3 isolates with blaKPC + blaOXA-48-like, and 3 isolates with blaKPC + blaNDM-1 + blaOXA-48-like. This inconsistency suggests potential limitations in phenotypic testing methods using the BD Phoenix M50 system with NMIC-500 CPO panel for detecting Class A carbapenemases. Previous studies have reported that automated systems may sometimes fail to detect KPC producers, particularly when multiple carbapenemase genes are present [29, 33]. Therefore, molecular methods like PCR appear more reliable for accurate detection of carbapenemase types. This finding highlighted the importance of using both phenotypic and molecular methods for comprehensive carbapenemase classification.

In this study, we initially identified 89 carbapenem-resistant Enterobacteriaceae isolates, including K. pneumoniae (54%), E. coli (15.7%), S. marcescens (14.6%), and others. While phenotypic screening was performed on all isolates, detailed molecular analysis focused on K. pneumoniae for several essential reasons. First, K. pneumoniae showed significantly higher carbapenem resistance rates (70.8%) compared to other species (E. coli 14.3%, Proteus spp. 33.3%). Second, K. pneumoniae demonstrated the highest prevalence of multiple resistance gene combinations, with 19 isolates (55.9%) carrying two or more carbapenemase genes. Third, this species dominated in severe infections, representing 71.4% of blood isolates. These characteristics make K.

pneumoniae the most clinically urgent target for understanding resistance mechanisms. Additionally, since K. pneumoniae frequently shares mobile genetic elements with other Enterobacteriaceae species, understanding its resistance patterns could provide valuable insights for managing resistance across the family. The focus on K. pneumoniae thus represented a strategic approach to addressing the broader challenge of carbapenem resistance in Enterobacteriaceae.

Based on Tables 5, 6, and 7, the correlation analysis between carbapenemase genes and resistance patterns revealed several significant relationships. Our study results showed that single gene carriers generally showed lower resistance rates, with OXA-48-like alone (6 isolates) demonstrating low resistance to amikacin (16.7%) and ceftazidime-avibactam (16.7%), while KPC alone (5 isolates) showed no resistance to amikacin and only 20% resistance to ceftazidime-avibactam. The presence of two genes (16 isolates, Table 6) was associated with significantly higher resistance rates, particularly evident in the NDM-1 + OXA-48-like combination (13 isolates) which showed high resistance to ceftazidime-avibactam (84.6%) and trimethoprim-sulfamethoxazole (84.6%). Triple gene carriers (3 isolates, Table 6) demonstrated the highest resistance rates with 100% resistance to most antibiotics tested. Notably, Table 7 demonstrates a statistically significant correlation between NDM gene presence and ceftazidime-avibactam resistance ($p = 0.010$). Tigecycline maintained relatively low resistance rates across all gene combinations, suggesting its potential utility regardless of carbapenemase gene presence.

Identifying the types of carbapenemase-encoding genes in *K. pneumoniae* strains is crucial for selecting appropriate antibiotic therapy. To elaborate, ceftazidime-avibactam antibiotic is recommended for treating infections caused by KPC and OXA-48 enzyme-producing strains but is ineffective against class B carbapenemase-producing strains. In contrast, meropenem-vaborbactam is effective against class A carbapenemase-producing strains but not against class B and D carbapenemase-producing strains, while imipenem-relebactam is effective against KPC enzyme-producing and AmpC-producing strains [34, 35]. A study conducted by Nguyen Tuan Linh [36] in 2018 found that *K. pneumoniae* strains with single bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ genes were highly susceptible to CZA, amikacin, and tigecycline, while strains with multiple carbapenemase-encoding genes, including NDM, were resistant to CZA. H' Nuong Nie [30] discovered that carbapenemase-producing *K. pneumoniae* strains were resistant to most β-lactam antibiotics, such as piperacillin-tazobactam and cephalosporins of generations 2, 3, and 4, and were only susceptible to four antibiotics: amikacin, gentamicin, CZA, and fosfomycin. Strains carrying the bla_{KPC} gene

remained susceptible to amikacin, CZA, and fosfomycin in over 80% of cases, while strains carrying the $bla_{OXA48\text{-}like}$ gene were only susceptible to these four antibiotics in 55-70% of cases. Strains carrying $bla_{NDM\text{-}1}$ were only susceptible to amikacin and fosfomycin in about 60% of cases and to gentamicin in 40% of cases. Most strains carrying only this single gene were only susceptible to gentamicin in less than 50% of cases, whereas the susceptibility rate for strains carrying combined genes was 83.3%.

Several limitations of our study warrant discussion. First, the single-center design and three-month sampling period may not fully capture seasonal variations in bacterial ecology. Second, our focus on ICU patients might overestimate resistance rates compared to general hospital settings due to selection bias from increased antibiotic pressure and more severe illnesses. Third, while we controlled for several confounding variables, unmeasured factors such as infection control practices and antibiotic stewardship programs could influence our results.

The generalizability of our findings requires careful consideration. While our results may be applicable to similar tertiary care settings in Southeast Asia, they might not reflect patterns in community hospitals or different geographical regions. The high resistance rates we observed might be particularly relevant to settings with similar antibiotic usage patterns and patient populations. However, our molecular findings regarding resistance mechanisms are more likely to have broader applicability, as they reflect fundamental biological processes.

A significant methodological consideration is from potential detection bias, since automated systems for carbapenemase detection showed some discrepancies between phenotypic and molecular results, particularly for class A carbapenemases. Additionally, survivor bias might affect our findings, as patients with particularly severe infections might have died or been transferred before sample collection.

Our results should be interpreted within the context of existing evidence. While our overall resistance rates were higher than those reported in many international studies, our findings regarding the impact of multiple resistance genes aligned with global data. The predominance of specific carbapenemase types in our setting added to the growing body of evidence on regional variations of resistance mechanisms.

Notwithstanding these limitations and potential biases, our study provided valuable insights into the current state of carbapenem resistance in a high-prevalence setting. The comprehensive

molecular characterization of resistance mechanisms, combined with clinical correlation, offered important guidance for both local practice and future research directions.

Future studies should consider longer sampling periods, multiple centres, and more detailed analysis of transmission dynamics. Additionally, investigation of host factors and their interaction with resistance mechanisms can provide deeper understanding of risk factors for acquiring resistant organisms.

5. CONCLUSION

The Enterobacteriaceae strains isolated in this study exhibited a significant resistance to meropenem. Given the current prevalence of carbapenem-resistant and carbapenemase-producing gut bacteria, there is an urgent need for enhanced infection control measures to curb the spread of both pathogenic and antibiotic-resistant bacteria. Implementing automated antibiotic susceptibility testing and carbapenemase classification can assist clinicians in making informed decisions about empirical antibiotic therapy, ultimately reducing the waiting time for microbiological results, the duration of antibiotic treatment, hospital stays, and patient mortality rates. Considering the challenges posed by multidrug-resistant Enterobacteriaceae, it is necessary for further research to assess the therapeutic effectiveness of existing combination antibiotics, providing a clearer understanding of the current antibiotic resistance landscape for these microorganisms.

Supplementary Materials

Supplementary materials are only available online from:
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COMPETING INTERESTS

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AUTHOR'S CONTRIBUTION

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Investigation: HQN Le, LML Dang, NTT Pham, CV Le, TK Lam, LH Luong, TM Huynh

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AVAILABILITY OF DATA AND MATERIAL

Upon reasonable request, the datasets of this study can be available from the corresponding author.

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Table 1. Zone Diameter and MIC Breakpoints for Enterobacteriaceae

Interpretive Category and Zone diameter (mm)			Interpretive Category and MIC Breakpoints (µg/mL)		
Susceptible	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
≥ 23	20 – 22	≤ 19	≤ 1	2	≥ 4

Table 2. Characteristics of specimen samples

Characteristic	Frequency	Proportion (%)
Specimen type (n = 89)		
Sputum	55	61.8
Blood	14	15.7
Urine	4	4.5
Other fluids	16	18.0
Bacterial species (n = 89)		
Klebsiella pneumoniae	48	54.0
Escherichia coli	14	15.7
Serratia marcescens	13	14.6
Proteus spp.	9	10.1
Enterobacter spp.	4	4.5
Salmonella spp.	1	1.1

Table 3. Prevalence of bacterial species isolated by type of specimen

Specimen type	Bacterial species	Frequency	Proportion (%)
Sputum (n = 55)	*Klebsiella pneumoniae*	29	52.7
	Serratia marcescens	11	20.0
	Escherichia coli	6	10.9
	Proteus spp.	5	9.1
	Enterobacter spp.	4	7.3
Blood (n = 14)	*Klebsiella pneumoniae*	10	71.4
	Escherichia coli	3	21.4
	Serratia marcescens	1	7.2
Urine (n = 4)	*Escherichia coli*	3	75.0
	Klebsiella pneumoniae	1	25.0
Other fluids (n = 16)	*Klebsiella pneumoniae*	8	50.0
	Proteus spp.	4	25.0
	Escherichia coli	2	12.5
	Serratia marcescens	1	6.25
	Salmonella spp.	1	6.25

Table 4. Susceptibility of isolated bacterial strains to meropenem

Bacterial species	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Klebsiella pneumoniae (n = 48)	12 (25.0)	2 (4.2)	34 (70.8)
Escherichia coli (n = 14)	12 (85.7)	0 (0)	2 (14.3)
Serratia marcescens (n = 13)	0 (0)	1 (7.7)	12 (92.3)
Proteus spp. (n = 9)	6 (66.7)	0 (0)	3 (33.3)
Enterobacter spp. (n = 4)	4 (100)	0 (0)	0 (0)
Salmonella spp. (n = 1)	1 (100)	0 (0)	0 (0)
Total	35 (39.3)	3 (3.4)	51 (57.3)

Table 5. Carbapenemase-producing phenotype and carbapenemase classification of isolated bacterial strains

Bacterial species	Carbapenemase producer (%)	Class A	Class B	Class D	Undefined
Klebsiella pneumoniae (n = 34)	34 (100)	0 (0)	4 (11.8)	25 (73.5)	5 (14.7)
Escherichia coli (n = 2)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
Serratia marcescens (n = 12)	12 (100)	0 (0)	0 (0)	12 (100)	0 (0)
Proteus spp. (n = 3)	3 (100)	0 (0)	1 (33.3)	2 (66.7)	0 (0)
Total	51 (100)	0	7 (13.7)	39 (76.5)	5 (9.8)

Table 6. Carbapenemase-encoding gene and antibiotic resistance in carbapenem-resistant *Klebsiella pneumoniae* strains

Number of gene	Carbapenemase-encoding gene	Frequency (%)	Antibiotic resistance (%)				
			GEN	AMK	CZA	TGC	SXT
1 gene	$bla_{OXA-48-like}$	6 (17.7)	4 (66.7)	1 (16.7)	1 (16.7)	0 (0)	4 (66.7)
	bla_{KPC}	5 (14.7)	1 (20.0)	0 (0)	1 (20.0)	0 (0)	2 (40.0)
	bla_{NDM-1}	1 (2.9)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)
2 gene	$bla_{NDM-1-OXA-48-like}$	13 (38.3)	7 (53.8)	8 (61.5)	11 (84.6)	1 (7.7)	11 (84.6)
	$bla_{KPC-OXA-48-like}$	3 (8.8)	3 (100)	2 (66.7)	3 (100)	2 (66.7)	3 (100)
3 gene	$bla_{KPC-NDM-1-OXA-48-like}$	3 (8.8)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)
Not detected		3 (8.8)	1 (33.3)	1 (33.3)	3 (100)	1 (33.3)	3 (100)

Table 7. Association between NDM *K. pneumoniae* that not carried bla_{NDM} gene and ceftazidime-avibactam resistance

Genotype	Ceftazidime-avibactam resistance		p*	PR (KTC 95%)
	Resistant (%)	Non-resistant (%)		
Not carried bla_{NDM} gene	8 (47.1)	9 (52.9)	**0.010**	**0.22 (0.06 – 0.88)**
Carried bla_{NDM} gene	15 (88.2)	2 (11.8)		**1**

*Fisher's exact test

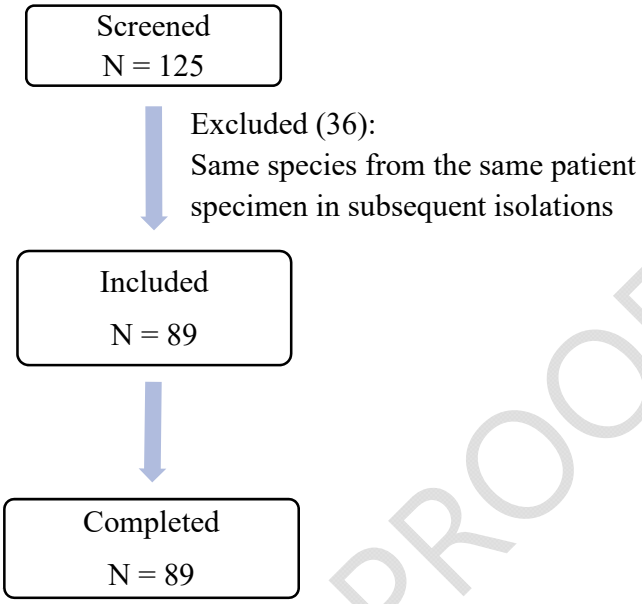

Figure 1: Flow diagram