

Laboratory-based surveillance of carbapenem resistance in clinical enterobacterales strains in a Nigerian tertiary hospital: Detection of bla_{NDM} and bla_{VIM} genes

Laborbasierte Überwachung der Carbapenem-Resistenz bei klinischen Enterobacterales-Stämmen in einem nigerianischen Tertiärkrankenhaus: Nachweis der bla_{NDM}- und bla_{VIM}-Gene

Abstract

Background: Carbapenem-resistant enterobacterales (CRE) are a critical priority that require continuous surveillance to track their dissemination and to guide antimicrobial chemotherapy and infection prevention and control measures. This study determines the species diversity and the prevalence of carbapenem resistance among enterobacterales from patients attending the Federal Teaching Hospital Gombe (FTHG) in Northeastern Nigeria.

Methods: In this cross-sectional laboratory-based surveillance conducted from 1 June to 1 October 2024, 223 non-replicative Gram-negative bacteria (GNB) were recovered from various clinical samples of patients attending FTHG. These isolates were identified using the API 20E system. Meropenem-resistant strains were subjected to the Modified Hodge Test to detect carbapenemase activity phenotypically. Other antimicrobial resistance phenotypes were determined by the disk diffusion method, and the presence of carbapenem resistance genes was assessed by multiplex PCR. Multivariate regression tests were used to assess the association between carbapenemase production and enterobacterales species.

Results: Of the GNB, 87 (39%) were enterobacterales, with 39% being *Escherichia coli*, 20% *Klebsiella (K.) oxytoca*, and 18% *K. pneumoniae*. About 19.5% of enterobacterales (17/87) were phenotypically resistant to meropenem, of which 10 (58.8%) were carbapenemase producers. The carbapenemase producers had the highest rates of resistance to amoxicillin-clavulanate (70%), gentamicin (50%), ceftazidime (40%), and ciprofloxacin (40%), and 50% (5/10) exhibited multidrug resistance (MDR). Of the MDR carbapenemase-producers, bla_{NDM} and bla_{VIM} genes were detected in *Serratia (S.) odorifera*, *Enterobacter cloacae*, *S. marcescens* from urine samples and *Proteus mirabilis* and *K. oxytoca* from blood culture.

Conclusion: A high frequency of CRE was identified, with some simultaneously resistant to third-generation cephalosporins. Remarkably, the bla_{NDM}- and bla_{VIM}-genes were found in non-conventional enterobacterales. These indicate their evolving roles as reservoirs of carbapenem-resistance genes, underscoring the need for a broader surveillance scope and improved antimicrobial stewardship in the hospital.

Keywords: carbapenem resistance, multidrug resistance, enterobacterales, metallo-beta-lactamase, bla_{NDM} genes, bla_{VIM} genes

Zusammenfassung

Hintergrund: Carbapenem-resistente Enterobacterales (CRE) stellen ein vorrangiges Problem dar, das eine kontinuierliche Überwachung erfordert, um ihre Ausbreitung zu verfolgen und Schlussfolgerungen für

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die antimikrobielle Chemotherapie und für Maßnahmen zur Infektionsprävention und -kontrolle abzuleiten. Die Studie sollte die Artenvielfalt und die Prävalenz der CRE von Patienten, die das Federal Teaching Hospital Gombe (FTHG) im Nordosten Nigerias aufsuchen, ermitteln.

Methoden: Im Rahmen der vom 1. Juni bis zum 1. Oktober 2024 durchgeführten laborbasierten Surveillance wurden 223 nicht-replikative Gram-negative Bakterien (GNB) aus verschiedenen klinischen Proben von Patienten des FTHG isoliert. Die Isolate wurden mithilfe des API-20E-Systems identifiziert. Meropenem-resistente Stämme wurden dem modifizierten Hodge-Test unterzogen, um die Carbapenemase-Aktivität phänotypisch nachzuweisen. Andere Phänotypen der Antibiotikaresistenz wurden mittels Plattendiffusionstest bestimmt, und das Vorhandensein von Carbapenem-Resistenzgenen wurde mittels Multiplex-PCR untersucht. Zur Beurteilung des Zusammenhangs zwischen der Carbapenemase-Produktion und Enterobacterales-Arten wurden multivariate Regressionstests durchgeführt.

Ergebnisse: Von den GNB gehörten 87 (39%) zur Ordnung der Enterobacterales, wobei 39% *Escherichia coli*, 20% *Klebsiella (K.) oxytoca* und 18% *K. pneumoniae* waren. Etwa 19,5% der Enterobacterales (17/87) waren phänotypisch resistent gegen Meropenem, davon waren 10 (58,8%) Carbapenemase-Produzenten. Die Carbapenemase-Produzenten wiesen die höchsten Resistenzraten gegenüber Amoxicillin-Clavulanat (70%), Gentamicin (50%), Ceftazidim (40%) und Ciprofloxacin (40%) auf, und 50% (5/10) waren multiresistent (MDR). Bei den MDR-Carbapenemase-Produzenten wurden *bla*_{NDM}- und *bla*_{VIM}-Gene in *Serratia (S.) odorifera*, *Enterobacter cloacae* und *S. marcescens* aus Urinproben sowie in *Proteus mirabilis* und *K. oxytoca* aus Blutkulturen nachgewiesen.

Schlussfolgerung: Es wurde eine hohe Häufigkeit von CRE festgestellt, von denen einige gleichzeitig gegen Cephalosporine der dritten Generation resistent waren. Bemerkenswerterweise wurden die *bla*_{NDM}- und *bla*_{VIM}-Gene in nicht-konventionellen Enterobacterales nachgewiesen. Das deutet auf ihre zunehmende Bedeutung als Reservoir für Carbapenem-Resistenzgene hin und unterstreicht die Notwendigkeit einer umfassenderen Überwachung sowie eines verbesserten Antibiotika-Managements im Krankenhaus.

Schlüsselwörter: Carbapenenresistenz, Multiresistenz, Enterobacterales, Metallo-beta-lactamase, bla_{NDM}-Gene, bla_{VIM}-Gene

Introduction

Carbapenem-resistant Enterobacterales (CRE) are a group of Gram-negative bacteria that have developed resistance to carbapenems, broad-spectrum antibiotics considered the last line of defence against severe bacterial infections [1]. CRE infections are associated with high mortality rates, ranging from 40% to 60%, often linked to health-care-associated infections, particularly in intensive care units [2]. The detection of CRE is crucial in clinical settings because these bacteria are highly resistant to many antibiotics, including carbapenems, which are often a last resort for treating severe infections [3]. Their presence poses significant challenges for patient care and public health due to the risk of untreatable infections and rapid dissemination of resistance genes [4]. They can lead to increased healthcare costs, prolonged hospital stays, and increased risk of transmission to others. Furthermore, the emergence of CRE has limited the therapeutic options

for treating infections, making it essential to detect and report CRE cases accurately [5].

The evolution and transmission of CRE in hospitals is driven by a complex interplay of clonal expansion and horizontal gene transfer, where resistance determinants jump across species boundaries [6]. Hospitals function as unique evolutionary incubators for CRE due to selective pressure from heavy antimicrobial use and the prolonged length of stay of critically ill patients [6]. CRE dissemination does not rely solely on patient-to-patient contact; it is highly heterogeneous, heavily influenced by the environment, and multi-scale [6], [7]. The incidence of CRE—most notably *Klebsiella (K.) pneumoniae* (CRKP) has demonstrated a concerning upward trajectory over the last decade [8]. Studies in intensive care units show steady increases in CRE isolation rates over time [9], [10]. Specifically, the COVID-19 pandemic caused temporary spikes in CRE prevalence [10]. During surges in critical care admissions, hospitals observed a marked increase

in multidrug-resistant (MDR) organism isolation rates, largely correlated with a near doubling in broad-spectrum antibiotic consumption and overwhelmed infection prevention and control (IPC) resources [10].

Carbapenems are widely regarded as one of the most effective and last-resort antibiotics for treating infections caused by MDR Gram-negative bacteria. The major types of carbapenem antibiotics in clinical use are imipenem, meropenem, ertapenem, and doripenem. Other types available in specific countries include panipenem and biapenem [11]. However, with the emergence of carbapenem resistance, it is clear that quality healthcare delivery through antimicrobial chemotherapy would be impaired without proper measures [12]. The Clinical and Laboratory Standards Institute (CLSI) recommends using meropenem as the primary indicator antimicrobial because it offers the best balance of sensitivity and specificity for the direct detection of CRE [13].

According to the World Health Organisation, CREs are considered a critical-priority target for antibiotic development and surveillance [4]. The prevalence of CRE depends on the hosts (human, animal, or environmental), geographical location, antibiotic use policy in the region, and the extent of surveillance. A recent meta-analysis of 116,743 participants found that the prevalence of CRE colonization worldwide was 14%, with Vietnam (43%) and Iran (39%) having the highest rates and the United States (5%) the lowest [14]. Moreover, a meta-analysis in Nigeria reported pooled prevalence estimates of imipenem and meropenem resistance among CRE of 11.2% and 13.5%, respectively [15]. These data highlight the need for effective surveillance and control measures. Despite growing global concern about CRE, there is a significant research gap regarding the prevalence and molecular characteristics in developing countries. It is important to remark that the primary research gap in developing countries is the over-reliance of most hospitals on agar disk diffusion or automated systems for detecting CRE. These approaches cannot distinguish specific CRE. Moreover, without knowing the exact genetic mechanism of carbapenem resistance, epidemiological tracing and effective antimicrobial stewardship are severely compromised. In north-eastern Nigeria, there is a data scarcity (local research gap) regarding CRE. This hampers tracking of resistance patterns, ultimately leading to higher rates of empirical treatment failure. Furthermore, inadequate IPC measures compound the risks of nosocomial transmission.

The accurate detection of CRE is crucial for effective IPC and antibiotic stewardship. However, phenotypic detection methods, such as disk diffusion and minimum inhibitory concentration tests, have limitations in the identification and diagnosis of CRE [16]. Nucleic acid amplification tests, such as polymerase chain reaction (PCR) and whole-genome sequencing (WGS), offer a more sensitive and specific approach to detecting CRE [17]. These methods can identify resistance mechanisms, such as genes that mediate carbapenemase production in bacteria, and provide valuable information for epidemiological tracking and outbreak investigations [18].

Resistance mechanisms may be mediated by transferable genes conferring carbapenem resistance [19]. Moreover, chromosomal point mutations in non-carbapenemase genes have been linked to carbapenem resistance [20]. Identifying carbapenem resistance genes is crucial for guiding effective antibiotic therapy, understanding the molecular epidemiology, and implementing IPC measures to prevent the spread of CRE. Therefore, this study aims to determine the prevalence of carbapenem-resistant phenotypes and genotypes in enterobacterales from patients attending the Federal Teaching Hospital, Gombe (FTHG), Northeastern Nigeria.

Materials and methods

Study area

The study was carried out at the Department of Medical Microbiology of the Federal Teaching Hospital (FTH), Gombe, North-eastern Nigeria. The hospital is located at 10° 17' 59" north and 11° 8' 12" east within the Sahel Savannah belt. This hospital was chosen because it is one of the largest and referral hospitals in Gombe State, Northeastern Nigeria. FTH was established in 1966 and has a 500-bed capacity. There are 33 central wards in the hospital, including the amenity ward.

Study design

This was a cross-sectional laboratory-based surveillance.

Sample size

The sample size was calculated using a previous study in Nigeria. Based on the 5.6% (17/305) prevalence of carbapenem resistance among clinical enterobacterales reported by Shitta et al. [21], the minimum sample size for this cross-sectional surveillance study was calculated after substituting the previous prevalence value and other parameters in the equation below:

$$\text{Number of participants} = \frac{Z \times Z (1 - P) p}{e \times e}$$

where (n) is the sample size, (Z) is the statistic (1.96) corresponding to the 95% confidence level, p is the expected prevalence, and (d) is the margin of error.

$$n = \frac{1.96 \times 1.96 (1 - 0.056) 0.056}{0.05 \times 0.05} = 81$$

The minimum sample size of 81 was calculated; however, 87 were collected to improve the statistical credibility.

Ethical consideration

Ethical approval was obtained from the Ethics Review Committee of FTHG (approval number NHREC/25/10/2013). All generated data were handled with the utmost confidentiality and analysed anonymously. The collected data were stored on a password-protected computer, accessible only to the principal investigator.

Sampling method

A probability-based random method was used to ensure that every enterobacterales isolate in the target bacterial collections had an equal chance of being selected within the study time frame. We assigned unique numbers to each isolate, and a computer-generated random number table was used to select those included in this study.

Source of isolates

This was a laboratory-based study that focused on bacterial isolates rather than patients. Enterobacterales isolates were obtained from the medical microbiology laboratory of FTH Gombe (Nigeria) within four months (1 June to 1 October 2024). The isolates were collected from various clinical samples, including urine, stool, sputum, wound swabs, and blood culture. The isolates were sub-cultured on MacConkey agar plates and incubated at 37 °C for 24 hours. For this study, 87 pure enterobacterales isolates were randomly selected from 223 Gram-negative bacteria.

Identification of the isolates

Pure colonies of the enterobacterales were identified using the Analytical Profile Index (API) system. A 0.5 McFarland standard suspension of each isolate from a 24-hour culture was prepared in sterile saline. The bacterial suspension was used to rehydrate each well on the API strip, and the strips were incubated for 24 hours. Colour changes were observed after incubation. The positive and negative test results were compiled to obtain a profile number, after which the profile number for each isolate was interpreted using the BioMérieux online package to identify the isolate.

Screening of isolates for carbapenem resistance

A confirmed enterobacterales isolate prepared to 0.5 McFarland standard was screened for carbapenem resistance using meropenem by the Kirby-Bauer disk diffusion method, following the Clinical Laboratory Standards Institute [22]. Using a sterile swab stick, a standardized suspension of each isolate was inoculated on Mueller-Hinton Agar (MHA) plates and incubated at 37 °C for 24 hours. The susceptibility of the isolates to the antibiotic used was interpreted in accordance with the 2025 CLSI

guidelines [22]. To confirm carbapenem resistance, two meropenem antibiotic discs were used. One disc was modified by soaking in an ethylenediaminetetraacetic acid (EDTA) containing tube, and incubated for 30 minutes, while the other disc was not modified. Both meropenem disks (EDTA-soaked and unsoaked) were then placed on an MHA plate containing the test bacterium. The discs were placed 15 mm apart, and thereafter the MHA plates were incubated at 37 °C for 24 hours. The zones of inhibition were measured, and a distance of ≥ 5 mm between the disks indicated carbapenemase production, commonly known as metallo-beta-lactamases (MBLs) production, such as New Delhi metallo-beta-lactamase (NDM) and Verona Integron-encoded metallo-beta-lactamase (VIM).

Antimicrobial susceptibility profile of the confirmed carbapenemase-producing isolates

The confirmed carbapenem-resistant isolates were subjected to antimicrobial susceptibility testing using other antibiotic discs on MHA by the Kirby-Bauer disc diffusion method. Six Oxoid® (Thermo Fisher Scientific, UK) antibiotics, gentamicin (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), nitrofurantoin (300 µg), meropenem (10 µg) and amoxicillin-clavulanate (30 µg) were tested against the isolates. After incubation at 37 °C for 24 hours, the inhibition zones were measured and interpreted according to the breakpoint provided by the 2025 CLSI [22].

Molecular detection of carbapenem resistance genes

The QIAamp extraction kit (QIAGEN) was used to extract genomic DNA following the manufacturer's instructions. PCR was used to detect carbapenem resistance genes (*bla_{KPC}*, *bla_{NDM}* and *bla_{VIM}*) in a 20 µl reaction mixture made up of 2 µl genomic DNA, 1 µl of each primer (Table 1), 4 µl of FIREPOL (BIOLIS) PCR master mix (2X), and 12 µl sterile distilled water. The conditions for amplification were as follows: 5 minutes of initial denaturation at 95 °C, followed by 45 seconds of annealing. Elongation at 60 °C for 45 seconds and final extension at 72 °C for 2 minutes for 35 cycles [18]. An aliquot of 5 µl of the PCR products was loaded onto a 1% agarose gel, and electrophoresis was run at 80 V and 200 mA for 40 minutes. Thereafter, the gel was viewed using a Flou-link UV transilluminator. A 1kb DNA ladder was used to determine the size of the gene fragments in gel electrophoresis without experimental controls.

Statistical analysis

Categorical and continuous variables were expressed as frequencies. Multivariate regression analyses were used to estimate adjusted odds ratios (aORs) to assess the association between carbapenemase production and

Table 1: Oligonucleotide primers used for the amplification of carbapenem resistance genes [23]

Target gene	Primers	Sequences	Amplicon size
<i>bla_{KPC}</i>	KPC-F	5'-ATGTCACGTATCGCCGTCT-3' 5'-TTACTGCCCGTTGACGCCC-3'	785
<i>bla_{NDM}</i>	NDM-F NDM-R	5'-GGTTTGGCGATCTGGTTTTC-3' 5'-CGGAATGGCTCATCACGATC-3'	550
<i>bla_{VIM}</i>	VIM-F VIM-R	5'-GTTTGGTTCGCATATCGCAAC-3' 5'-AATGCGCAGCACCAGGATAG-3'	382

enterobacterales species, using MedCalc Version 23.0.2 (Ostend, Belgium). All analyses with p-values <0.05 at a 95% confidence interval (CI) were considered statistically significant.

Results

Identification of clinical enterobacterales isolates

The biochemical reactions of isolates, as determined by the API 20E System (Table 2), showed that *E. coli* was the predominant (37%), followed by *K. oxytoca* 17%, *K. pneumoniae* 15%, *Providencia* spp. (10%) *Citrobacter* spp. (4%) and *P. mirabilis* (4%) (Table 2). Based on sample type, urine samples had the highest recovery rate of enterobacterales (42%), followed by sputum (16%), wound swabs (12%), stool (12%) and blood cultures (5%) (Table 3).

Frequency of CRE

The prevalence of carbapenem resistance among the enterobacterales isolates was 19.5% (17/87) (Table 3). The distribution of isolates among species shows that *E. coli* accounts for the highest proportion (29%), followed by *K. oxytoca* (17%), *K. pneumoniae*, and *S. odorifera* (12%) (Table 3). The remaining species, including *Providencia* spp., *E. cloacae*, *Citrobacter* spp., *P. mirabilis* and *S. marcescens*, each account for a smaller proportion (≤12%). Using the EDTA-modified Hodge test, the prevalence of CRE was 11.5%, and *S. odorifera* showed the highest carbapenem resistance (p=0.04; Table 4).

Antimicrobial susceptibility pattern of CRE

The pattern of resistance among carbapenemase producers to non-carbapenem agents in descending order of frequency: amoxicillin-clavulanate (70%), gentamicin (50%), ceftazidime (40%), ciprofloxacin (40%), and nitrofurantoin (20%) (Table 5). Of the MDR carbapenemase producers, *bla_{NDM}* and *bla_{VIM}* were detected in *Serratia odorifera*, *E. cloacae*, *S. marcescens* from urine samples and *P. mirabilis* and *K. oxytoca* from blood culture (Figure 1).

Discussion

Enterobacterales are a major component of the human gut; they are dynamic and play significant ecological roles in health and disease [23]. The prominent role of enterobacterales is their contribution to the spread of the AMR pandemic. The gut acts as a significant reservoir for these resistomes, such as carbapenemases, facilitating their transfer to other organs and tissues, leading to colonisation and subsequent infections (urinary tract infections, bloodstream infections) that are difficult to treat. Reporting CRE from human samples is critically important for guiding patient treatment, preventing the spread of these “superbugs” within healthcare facilities and the community, and informing public health policy on AMR. It provides essential data to guide clinical decisions and prevent widespread transmission.

From the current study, *E. coli* (36.8%) was the predominant etiological agent of clinical infections. This corroborates numerous previous studies worldwide, such as the 35.1% reported in Nigeria (35.1%) [21], China (33.9%) [24], and Zambia (37.8%) [25]. However, higher prevalence values in Romania (41.5%) [26] and the Democratic Republic of Congo (58.9%) [27]. We reported a higher frequency of *E. coli* clinical isolates than those identified in Palestine (30.3%) and Ethiopia (14.2%) [28], [29]. These variances could be attributed to geographical disparities, technical differences, the procedures utilised, and the experience of the laboratory personnel who performed the investigations. Other important factors that could influence the recovery rate of *E. coli* include the specific sample type and patient population (e.g., healthy individuals vs. hospitalised patients). In this regard, urinary tract infections are the most common source, as corroborated by our findings.

Among enterobacterales species, carbapenem-resistant *E. coli* (CREc) was the most common (29.4%, 5/17), higher than the 7% global pooled prevalence of CREc [30]. Subregional comparison shows that CREc prevalence is higher than in high-income countries, such as Portugal, whereas CREc was as low as 0.5% among invasive isolates. It appears that CREc is endemic in Nigeria, as reflected by a recent study in Lagos, which reported a prevalence of 46% [31]. Moreover, the previous meta-analysis suggests that CRE is generally endemic in Nigeria [15]. Higher prevalence of CREc in human patients is primarily driven by healthcare-associated factors, including extensive antibiotic use, prolonged hospital stays, and the presence of invasive medical devices.

Table 2: Biochemical reactions profile specific for each enterobacterales

Organism	O	A	L	Or	CI	H	U	T	I	VP	G	GL	M	In	So	R	S	Me	Am	Ar
<i>E. coli</i>	+	–	–	+	–	–	–	–	+	+	–	+	+	–	–	+	–	–	–	+
<i>K. oxytoca</i>	+	–	–	+	+	–	+	–	–	+	–	+	+	–	+	+	+	+	–	+
<i>K. pneumoniae</i>	+	–	–	+	+	–	–	–	–	+	–	+	+	–	–	+	+	+	–	+
<i>E. cloacae</i>	+	+	+	+	+	–	–	+	–	+	–	+	+	+	+	+	+	+	+	+
<i>S. marcescens</i>	+	+	+	+	+	–	–	+	–	+	+	+	+	–	–	+	+	–	–	+
<i>S. odorifera</i>	+	+	+	+	+	–	–	+	–	+	+	+	+	–	–	+	+	–	–	+
<i>Citrobacter</i> spp.	+	–	–	+	+	+	–	–	–	+	–	+	+	–	–	+	+	+	–	+
<i>P. mirabilis</i>	+	+	+	+	–	+	+	+	+	–	+	+	+	–	–	+	–	–	–	+
<i>Providentia</i> spp.	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	–	–	+

KEY: O=o-nitrophenyl-β-galactopyrsonide, A=argininedihydrolase, L=lysinedecarboxylase, Or=ornithine decarboxylase, Ci=citrate, H=hydrogensulfide, U=urea, T=tryptophandeaminase, I=indole, Vp=voges-proskauer, G=gelatin liquefaction, Gl=Glucose, M=mannitol, In=inositol, So=sorbitol, R=rhamnose, S=saccharose, Me=melibiose, Am=amygdalin, Ar=arabinose, +=positive, –=negative

Table 3: Distribution and frequency of enterobacterales based on sample type

Organisms	Urine	Stool	Blood culture	Sputum	Wound swab	Frequency	Percentage
<i>E. coli</i>	14	5	3	2	8	32	37
<i>K. oxytoca</i>	7	7	–	–	–	14	16
<i>K. pneumoniae</i>	2	–	–	13	–	15	17
<i>E. cloacae</i>	3	–	2	–	–	5	6
<i>S. marcescens</i>	–	–	–	1	–	1	1
<i>S. odorifera</i>	2	–	–	–	–	2	2
<i>Citrobacter</i> spp.	3	–	–	–	1	4	5
<i>P. mirabilis</i>	3	–	–	–	1	4	5
<i>Providencia</i> spp.	8	–	–	–	2	10	11
Total	42	12	5	16	12	87	100

Table 4: Prevalence of confirmed carbapenem-resistant strains by species of enterobacterales

Organisms	Nº of iso-lates	Nº of CRE	aOR* (95% confidence interval)	p value	Nº of carbapene mase-producers	aOR (95% CI)	p value
<i>E. coli</i>	32	5	Referent	Referent	1 (3.1)	Referent	Referent
<i>K. oxytoca</i>	14	3	1.47 (0.29 to 7.25)	0.6340	1 (7.1)	2.38 (0.13 to 41.08)	0.5496
<i>K. pneumoniae</i>	15	2	0.83 (0.14 to 4.87)	0.8372	1 (6.6)	4.76 (0.39 to 57.31)	0.2181
<i>E. cloaca</i>	5	1	1.35 (0.12 to 14.73)	0.8056	1 (20)	7.75 (0.4 to 149.71)	0.1753
<i>S. odorifera</i>	2	2	25 (1.05 to 596.04)	0.0467	2 (100)	105 (3.33 to 3307.71)	0.0082*
<i>S. marcescens</i>	2	1	5.4 (0.29 to 101.28)	0.2595	1 (50)	31 (1.02 to 941.06)	0.0486
<i>Providencia</i> spp	10	1	0.6 (0.06 to 5.84)	0.6600	1 (10)	3.44 (0.19 to 60.72)	0.3982
<i>Citrobacter</i> spp	4	1	1.8 (0.15 to 20.98)	0.6390	1 (25)	10.33 (0.51 to 210.59)	0.1289
<i>P. mirabilis</i>	4	1	1.8 (0.15 to 20.99)	0.6390	1 (25)	10.33 (0.51 to 210.59)	0.1289
Total	87	17			10 (11.5)		

- Adjusted odds ratio ** significant association determined by the multivariate regression test

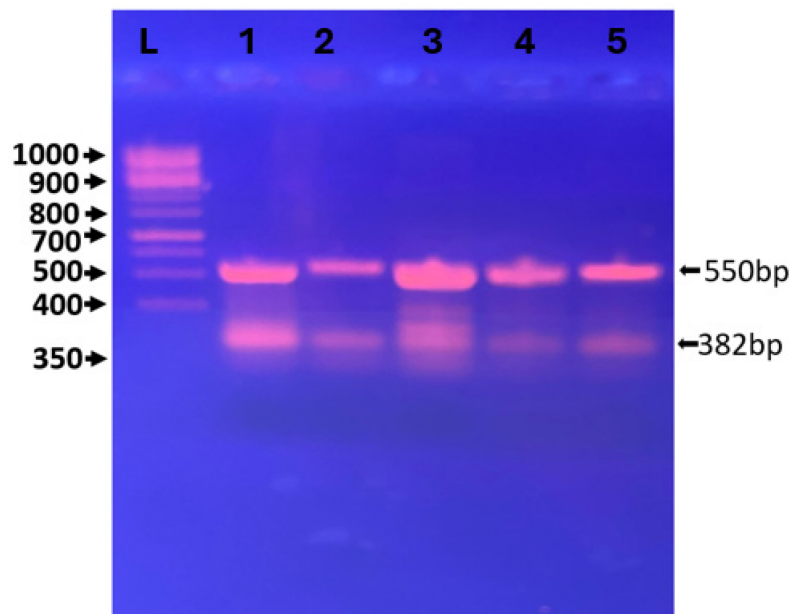
The 19.5% prevalence of CRE observed in this study was higher than the 14% reported in a global meta-analysis of CRE colonization [14]. The difference could be likened to the fact that enterobacterales from clinical infections have a greater capacity to acquire carbapenem resistance than in colonisation states due to antibiotic pressure [3].

It is important to note that some meropenem-resistant CRE were negative on the modified Hodge test, indicating they are non-carbapenemase producers. The key mechanisms responsible for carbapenem resistance in non-carbapenemase producers involve both chromosomal factors (such as point mutations in OprD protein) and

Table 5: Antimicrobial resistance pattern of carbapenemase-producing enterobacterales

Antibiotics	<i>K. oxytoca</i> (n=3)	<i>E. cloacae</i> (n=1)	<i>S. marcescens</i> (n=2)	<i>S. odorifera</i> (n=2)	<i>P. mirabilis</i> (n=2)	All CRE (n=10)
CAZ	0	1 (100)	0	2 (100)	1 (50)	4 (40)
AUG	2 (66.7)	0	2 (100)	1 (50)	2 (100)	7 (70)
CPR	1 (33.3)	1 (100)	0 (100)	2 (100)	0	4 (40)
GEN	1 (33.3)	1 (100)	2 (100)	0	1 (50)	5 (50)
NIT	0	0	2 (100)	0	0	2 (20)
MDR	1 (33.3)	1 (100)	1 (50)	1 (50)	1 (50)	5 (50)

KEY: AUG= augmentin, CAZ= ceftazidime, CPR=ciprofloxacin, MDR= Multidrug resistant NIT=nitrofurantoin



The *bla*_{NDM} (550 bp) and *bla*_{VIM} (382 bp) genes
 Lane L = 1Kb DNA Ladder (Thermo Scientific), Lane 1: *S. odorifera* (urine),
 Lane 2: *P. mirabilis* (blood culture); Lane 3: *E. cloacae* (urine);
 Lane 4: *K. oxytoca* (blood culture); Lane 5: *S. marcescens* (urine).

Figure 1: PCR Product of carbapenemase-encoding genes detected from enterobacterales on 1% (w/v) agarose gel.

non-chromosomal factors, including the production of non-carbapenemase β -lactamases [32], [33]. Worryingly, 40% of the MDR-CRE were simultaneously resistant to both carbapenems (CRE) and ceftazidime (a third-generation cephalosporin). This will ultimately result in limited treatment options, higher rates of treatment failure, increased patient mortality, longer hospital stays, and higher healthcare costs [34]. The *bla*_{NDM} and *bla*_{VIM} genes we detected in our CRE strains are among the major ones encoding enzymes that hydrolyze carbapenems. Remarkably, these CR genes are found in non-conventional (less common genera) enterobacterales, such as *S. marcescens* and *Citrobacter* spp., in clinical samples. Similar findings have been reported in recent studies globally [35], [36], [37]. These bacterial genera are predominantly environmentally adapted, highlighting the hospital environment as a vector and reservoir of carbapenem resistance. Recent studies globally have consistently highlighted the significant role of the hospital environment, particularly surfaces and plumbing systems

(e.g., sink traps/drains), as key reservoirs and vectors for the transmission of carbapenem-resistant *S. marcescens* and *Citrobacter* spp. in clinical settings [38], [39]. Many antibiotic-resistant bacterial infections cause diseases, including pneumonia, which have been shown to contribute substantially to mortality [40], [41]. According to projections, there could be 1.91 million and 8.22 million AMR-related deaths worldwide in 2050 [41]. Therefore, the findings of the present study would help strengthen surveillance of CRE, which is critical to improving antimicrobial management and patient outcomes. We explicitly acknowledge that the odds ratio analysis of small subgroups of isolates could limit the generalizability of the findings; hence, caution is warranted when interpreting the multivariate regression results. Whole-genome sequencing could be considered in future studies, as it provides detailed data on potential mobile genetic elements, transmission pathways, and relatedness among carbapenem-resistant strains.

Conclusion

Considering the global pooled prevalence of CRE of 14%, a high frequency was observed in the present study, with some also resistant to third-generation cephalosporins. Remarkably, the *bla*_{NDM}- and *bla*_{VIM}-genes were found in non-conventional enterobacterales, indicating their evolving roles as reservoirs of these critical priority AMR genes. A broader scope of surveillance, including environmental samples, is needed to understand the burden of CRE in healthcare settings. It is recommended that actionable plans prioritize enhanced genomic tracking, stringent infection control, rigorous diagnostic stewardship, and stricter antibiotic management to prevent further dissemination of these critical genes.

Notes

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Ethical approval

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

All data generated from this study are presented in the tables and figures; however, further requests could be made through the corresponding author.

AI usage statement

Artificial intelligence tool (PaperPal) was used only to improve the English language of the manuscript. All scientific contents, and references were reviewed, edited, and approved by the authors.

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