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Molecular characterization of *bla*$_{NDM}$ and other carbapenemases genes among carbapenem-resistant *Enterobacterales* from community-and hospital- acquired urinary tract infections

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Abstract

Introduction Extended Spectrum β-lactamase (ESBLs) and/or carbapenemases producing *Enterobacterales* present a significant challenge in treating urinary tract infections (UTIs). Carbapenems, once considered last-resort antibiotics, are now threatened by the emergence of carbapenem-resistant strains.

Methods A total of 181 *Enterobacterales* were collected from (231) urine positive samples of community-acquired (CA) and hospital-acquired (HA) UTI patients from the Urology Department of Jinnah Postgraduate Medical Center (JPMC) Karachi, Pakistan, between January and June 2022. Antimicrobial susceptibility testing (AST) was performed, and carbapenemases production was screened using the Modified Hodge Test (MHT) and Modified Carbapenem Inactivation Method (mCIM). EDTA-Combined Disc Test (CDT) was performed for the phenotypic detection of metallo-β-lactamases. The genes encoding carbapenemases were amplified by PCR, sequenced and submitted to the gene bank.

Results Among 181 isolates 107, (59.1%) were from CA and 74 (40.9%) from HA-UTI cases. *E. coli* (128/181; 70.7%) was the most common uropathogen, followed by *K. pneumoniae* (24; 13.2%). From CA-UTI cases, 68 (63.5%) isolates were found resistant to ampicillin, cefazolin, and cefuroxime. Whereas resistance to cefoxitin (33/107; 30.8%), cefepime (56/107; 52.3%), ceftazidime (58/107; 54.2%), and ceftriaxone (66/107; 61.6%) was lower in CA-UTI. However, in the isolates from HA-UTI the resistance pattern to these antibiotics was significantly higher. Similarly, resistance to fosfomycin was higher (21.6%) in HA than in the isolates from CA-UTI (5.6%). Carbapenem-resistance was more prevalent in HA-UTI isolates (45/74; 60.8%) than CA-UTI isolates (7/107; 6.5%). Chi-square analysis revealed significantly higher antimicrobial resistance rates in HA-UTI isolates compared to CA-UTI cases. The mCIM detected carbapenemases production in 92.3% (48/52) of CRE isolates, while MHT detected only 23.1% (12/52). PCR confirmed

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carbapenemases genes in 98.1% (51/52) of the isolates. The bla_{NDM} gene was found in 86.5% (45/52) of CRE isolates, while bla_{OXA-48} was detected in 11.5% (6/52).

Conclusion CRE prevalence was higher in HA-UTI isolates with bla_{NDM} was the predominant carbapenemase gene detected.

Keywords Carbapenem-resistant *Enterobacterales*, Modified hodge test, Urinary tract infection, Modified carbapenem inactivation method

Introduction

Urinary tract infections (UTIs) are the most prevalent bacterial infections worldwide. Affecting approximately 250 million people each year, accounting for a significant proportion of community and hospital-acquired infections [1]. UTIs are defined by the presence of a significant number of bacteria ($\geq 10^5$) in the urine. UTI can involve any part of the of the urinary tract including, bladder, urethra and kidney, but mostly occurs in lower part of the urinary system [2]. Clinical presentations of UTIs range, from asymptomatic bacteriuria to cystitis or septic shock, which can lead to multi-organ failure and increased mortality [3].

The most frequent uropathogens are the species of *Enterobacteriaceae*, especially *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* [1, 4, 5]. These uropathogens are mostly resistant to commonly prescribed β-lactam antibiotics due to the production of β-lactamases especially extended spectrum β-lactamases (ESBLs). ESBLs hydrolyze the broad spectrum cephalosporins, penicillin and aztreonam [6]. A ten year study from Asia reported that cephalosporins (34.4%) and fluoroquinolones (24.1%) were the frequently prescribed antibiotics for UTI treatment; yet the resistance to these antibiotics was found as high as 54.9% and 42%, respectively [7]. Carbapenems remain a primary treatment for multi-drug resistance (MDR) *Enterobacterales* infections involving ESBLs [5]. However, their overuse especially in countries with inadequate antibiotic stewardship programs and high prevalence of ESBL has driven the emergence of carbapenem resistant *Enterobacterales* (CRE). An ever increasing prevalence of CRE poses a significant challenge to the efficacy of carbapenems, as these resistant pathogens spread rapidly through both clonal and plasmid-mediated mechanisms [8]. A retrospective cohort study conducted in the United States between 2013 and 2018 showed that one in eight hospitalizations for complicated UTIs involved bacteria exhibiting resistance to three different classes of antibiotics, including carbapenems [9]. In some Asian countries such as, Sri Lanka, India, and China, prevalence of CRE in UTIs accounts for 47.4%, 29% and 10%, respectively [10–12]. Resistance to carbapenems mainly occurs through, efflux pumps, modified membrane permeability due to mutations in porin encoding genes, and production of carbapenemases. Generally, CRE are divided into two main subgroups: carbapenemases-producing CRE (CP-CRE) and non-carbapenemases-producing CRE (non-CP-CRE) [13]. CP-CRE are more concerning because of limited treatment options that lead to difficult-to-manage infections associated with significant morbidity and mortality [14].

Within Ambler classification, carbapenemases are classified into class A, class B, and class D [13]. Amongst significant acquired metallo-β-lactamases (MBLs), imipenemase (IMP), New Delhi metallo-β-lactamase (NDM), and Verona integron-encoded metallo-β-lactamase (VIM) types are grouped into subclass B1 (http://www.bldb.eu/BLDB.php?prot=B1#NDM). They hydrolyze all currently available β-lactam antibiotics except mono-bactams aztreonam [15]. The CP-CRE situation varies dramatically in different parts of the world, and also between European countries, from sporadic occurrence to endemic situation [16]. Regions and countries are known to be endemic for a certain carbapenemases, for instance *Klebsiella pneumoniae* carbapenemase (KPC) is routinely found in the USA, Puerto Rico, Colombia, Brazil, Argentina, Greece, and Italy. New Delhi metallo-β-lactamase (NDM) is commonly identified in Indian subcontinent, China, Bangladesh, and Pakistan. Similarly, oxacillinase-48 (OXA-48) is predominantly reported in Morocco, Turkey, and Malta [17]. The pathogens producing carbapenemases especially metallo-β-lactamase (MBL) worsened the situation because of unavailability of clinically relevant inhibitor [18]. Metallo-β-lactamases (MBLs), such as NDM are particularly concerning because they can hydrolyze nearly all β-lactam antibiotics, leaving limited treatment options. The prevalence of bla_{NDM} in CRE is a critical concern, particularly in regions like Pakistan, where AMR is rapidly escalating. Previous studies have highlighted the increasing spread of CPE in Pakistan, with bla_{NDM} being one of the most commonly identified resistance genes often carried on plasmids that facilitate its rapid dissemination within communities and healthcare settings [19]. However, only a few CPE have been found to carry the bla_{NDM} gene on their chromosomes [20].

The present study focuses on the prevalence of carbapenemases genes in CRE isolates from urinary tract infections in both hospitalized and outpatient

populations at a tertiary care hospital in Karachi, Pakistan. By focusing on bla_{NDM}, a key driver of carbapenem resistant, this research provides essential insights into the local resistance patterns. These findings are vital for informing Pakistan's National Action Plan against AMR and for developing targeted strategies to improve infection control and antibiotic stewardship in health care settings. Moreover, this study contributes to global efforts to combat AMR by shedding light on the epidemiology of bla_{NDM} in region with significant antibiotic use and resistance concerns.

Research methodology

Study settings and type

This cross-sectional prospective research work was carried out at the department of Microbiology, University of Karachi, with the collaboration of Basic Medical Sciences Institute (BMSI) and the department of Urology, Jinnah Postgraduate Medical Center (JPMC) Karachi, Pakistan.

Ethical approval

The ethical approval was taken from the Institutional Review Board (IRB) committee of JPMC (No. F.2–81/2021-GENL/57041/JPMC).

Selection criteria

Adult patients with sign and symptoms of UTI were selected in this study. UTI patients were categorized into two groups: community-acquired UTI (CA-UTI) and hospital-acquired UTI (HA-UTI). Patients who attended the outpatient department (OPD) or were admitted with the diagnosed UTI but had no history of previous hospitalization or interventions in the urinary tract were classified as CA-UTI. While those patients who underwent any intervention or who did not have a UTI at the time of hospitalization but developed a UTI after 48 h of admission were included in the HA-UTI group [21].

Sample collection

A total of 181 *Enterobacterales* isolates were isolated from 298 urine samples of patients with UTI who attended the outpatient department (OPD) and/or were admitted to the department of Urology, JPMC during the period from 1st January to 30th June 2022.

Sample processing and identification of the isolates

Urine samples were collected in clean and sterilized containers. These samples were then transported to the department of Microbiology, BMSI, and immediately processed. A loopful of urine was taken with a sterile disposable loop (1µL) and inoculated onto Cysteine-Lactose Electrolyte Deficient (CLED) agar (Oxoid Ltd., Basingstoke, Hampshire, England). A count of $\geq 10^5$ CFU/mL was considered as significant bacteriuria for UTI. Routine laboratory techniques, including cultural, and morphological examination, and a range of biochemical tests, were used to identify *Enterobacterales* isolates. The species identification was confirmed by using the RapID™ one system (Thermo Fisher Diagnostics, Landsmeer, Netherlands). According to the Clinical and Laboratory Standards Institute (CLSI) guidelines and protocol, the disc diffusion method was used to assess the antimicrobial susceptibility (AST). *E. coli* (ATCC 25922) was used as quality control strain for AST.

Screening of carbapenem resistant *Enterobacterales* (CRE)

The *Enterobacterales* resistant to carbapenems including imipenem (10 µg) and/or meropenem (10 µg) were considered CRE and subjected to test the production of carbapenemases.

Modified hodge test (MHT)

A standard McFarland's solution of *E. coli* (ATCC 25922) was prepared. A lawn of this bacterial suspension was prepared onto a Muller-Hinton agar (MHA) plate, using a sterile cotton swab. An imipenem disc (10 µg) was then placed at the center of the MHA plate. Carbapenem-resistant isolates were streaked from the edge of the disc to the periphery of the plate in four different directions. The plate was incubated at 37 °C for 24 h. After the incubation period, the presence of a "clover-leaf-shaped" zone of inhibition by the test isolate was interpreted as a carbapenemase producer [22].

Modified carbapenem inactivation method (mCIM)

A full loop (1 µL) of the CRE isolate was inoculated to a tube containing 2 mL of Tryptic Soy broth (TSB; Oxoid Ltd., Basingstoke, Hampshire, England). A meropenem disc (10 µg) was placed in the tube and then incubated at 35 °C for 4 h ± 15 min. After the incubation period, the disc was removed from the tube and placed on MHA plate, already inoculated with a carbapenem-susceptible *E. coli* (ATCC 25922) strain as indicator. Then the plate was incubated for 18–24 h at 37 °C. After this incubation period, the results were interpreted by measuring the zone of inhibition around the meropenem disc. A reduced or no inhibition zone (≤ 15 mm) indicated carbapenemases production. Conversely, a zone of inhibition (≥ 19 mm) were indicated the absence of carbapenemases [23].

Combined disc test

Carbapenem-resistant isolates were tested for the production of metallo-β-lactamases (ML). An MBL positive *Pseudomonas aeruginosa* ATCC 27,853 was used as a quality control strain. A suspension (correspond to 0.5 McFarland standard) of the test isolate was inoculated onto MHA plate. Two discs of imipenem (10 µg) were

placed on inoculated plates. One disc was added to 10 µL of 0.5 M EDTA solution which was used as an MBL inhibitor. After inoculation, the plates were incubated; at 37 °C for 24 h. The results were interpreted positive, if the zone of inhibition was increased by ≥ 7 mm to imipenem + EDTA compared to the imipenem disc alone [24].

Molecular characterization of carbapenemases encoding genes

Carbapenem-resistant isolates were selected to analyze carbapenemases encoding genes. DNA extraction was performed by the kit method (WizPrepTM DNA Mini Kit, Republic of Korea). A PCR mixture of 20 µL was prepared according to the standard method consisting 0.5 µL of forward and revers primers (Table 1), master mix (10 µL; Dream Taq Green, Thermo Scientific), DNA (2 µL), and PCR grade water (7 µL). Thermo-cycler (Krytac.SC300G-R2) was used with a program consisting of an initial temperature of 94 °C for 5 min, denaturation at 94 °C for 30 s, 35 cycles at the required annealing temperature (30 s), extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The primers, sequence, amplicon size, and annealing temperature conditions are listed in the Table 1.

Gel electrophoresis

Aliquots (10 µL each) of PCR products were separately loaded onto (1%) agarose gel (Bio-World, Suite, Dublin, USA) prepared using Tris-acetate-EDTA (TAE) buffer (RICCA chemical, west Fork Drive Arlington, USA). The gel electrophoresis was conducted at a constant voltage of 85 V for 35 min. Afterward, the gel was visualized using a gel illuminator (Invitrogen, Thermo Fisher, Taiwan) to confirm the presence of DNA fragments. The resulting bands were compared with DNA ladder (GeneRuler 100 bp, Thermo-scientific Baltics, Vilnius, Lithuania) and evaluated alongside the negative and positive controls to assess the results accurately.

Gene sequencing

PCR positive samples of bla_{NDM} and $bla_{\text{OXA-48}}$ were sent to Macrogen (Seoul, Republic of Korea) for Sanger sequencing. Sequences were analyzed and interpreted using DNA baser software (https://www.dnabaser.com). The obtained sequences were validated by comparing them to the reference sequences of bla_{NDM} in the NCBI database (BLAST) and type of variants was confirmed. Confirmed gene sequences were submitted to GenBank, and accession numbers were obtained.

Plasmid extraction

The plasmids were extracted using the GeneJET plasmid miniprep kit (Thermo Scientific™, Waltham, MA, USA). Briefly, a single colony from an overnight culture of CRE was suspended into 5 mL of Luria-Bertani (LB) broth supplemented with (4 µg/mL) meropenem and incubated for overnight at 37 °C. After the incubation period 2 mL of bacterial suspension was transferred into the micro-centrifuge tube and was centrifuged at 8000 rpm to obtain the bacterial cell pallets. The plasmid extraction was carried according to the kit manufacturer's protocol. The PCR mixture and conditions remained the same as described in the previous "Molecular characterization of carbapenemases encoding genes" section for the whole DNA amplification.

Data analysis

The data were entered in Microsoft Excel (version 10) and exported to SPSS (Statistical Package for the Social Sciences, version 22) for analysis. Microsoft Excel was

Table 1 MBL encoding genes and their primer sequences, amplicon size and annealing temperature

Gene	Primer sequence	Amplicon size	Annealing temperature °C	Reference
bla_{NDM}	F: CACCTCATGTTTGAATTCGCC	984	64	[25]
	R: CTCTGTCACATCGAAATCGC			
bla_{VIM}	F: GGTGTTTGGTCGCATATCGC	503	58	
	R: CCATTCAGCCAGATCGGCATC			
bla_{IMP}	F: GGAATAGAGTGGCTTAATTC	327	50	
	R: CAACCAGTTTTGCCTTACC			
bla_{VEB}	F: GTTAGCGGTAATTTAACCAGATAG	1070	56	
	R: CGGTTTGGGCTATGGGCAG			
bla_{GIM}	F: TCGACACACCTTGGTCTGAA	477		[26]
	R: AACTTCCAACTTTGCCATGC			
$bla_{\text{SIM-1}}$	F: TACAAGGGATTCGGCATCG	570	57	
	R: TAATGGCCTGTTCCCATGTG			
bla_{KPC}	F: CTTGTCTCTCATGGCCGCTGG	449	65	[27]
	R: ACGGAACGTGGTATCGCCGAT			
$bla_{\text{OXA-48}}$	F: GCTTGATCGCCCTCGATT	281	57	[28]
	R: GATTTGCTCCGTGGCCGAAA			

Table 2 Distribution of UTI cases by gender and age groups in CA and HA patients ($n=231$)

Categories	CA-UTI *n*(%)	HA-UTI *n*(%)	*p*. Value	Total *n*(%)
Gender				
Female	79 (34.2)	53 (23)	0.97	132 (57.1)
Male	59 (25.5)	40 (17.3)	0.97	99 (42.9)
Age groups (years)				
1st (15–20)	11 (4.8)	7 (3.0)	1.00	18 (7.0)
2nd (21–30)	71 (30.7)	38 (16.5)	0.139	109 (47.2)
3rd (31–40)	32 (13.9)	14 (6.1)	0.135	46 (20.0)
4th (41–50)	18 (7.8)	13 (5.6)	0.846	31 (13.4)
5th (51–60)	4 (1.7)	10 (4.3)	0.022	14 (7.0)
6th (≥61)	2 (0.9)	11 (4.8)	0.001	13 (5.6)
Total *n*(%)	138 (59.7)	93 (40.3)		231 (100)

used for initial data organization and basic calculations, while SPSS was used to compare prevalence rates, chi-square values and comparison of CA and HA-UTI isolates variables.

Results

Burden of UTI in relation to gender and age groups

A total of two hundred ninety-eight (298) urine samples were collected from patients suspected of urinary tract infections (UTIs) in both the outpatient department (OPD) and the hospitalized patients. Of these samples, 193 (64.7%) were from OPD, and 105 (35.3%) were from hospitalized patients. Among these urine samples, 231 (77.5%) were UTI positive, with 138 (out of 193; 71.5%) and 93 (out of 105; 88.6%) samples from CA and HA, respectively. The prevalence of UTI was higher in females (57.1%) compared to males. The percentage of females with UTI was even higher in the CA group (34.2%) than in the HA-UTI (Table 2). The age group with the highest number of UTI cases in the CA-UTI and HA-UTI groups was 21–30 years old, accounting for 109 (47.2%), followed (20%) by the 3rd group age (31–40 year). The significant association between two categorical variables gender and type of UTI CA vs. HA was calculated by chi-square test of independence. The test was used to compare the observed frequencies in contingency to expected frequencies calculated under the assumption that the variables are independent. The results were statistically insignificant ($p \leq 0.05$) in all groups except for the age group 6th (p 0.001).

Prevalence of uropathogens in UTI

The *Enterobacterales* species were the most common uropathogen in both CA- and HA-UTI, (181/231; 78.3%), followed by other Gram-negative bacteria (31/231; 13.4%). The Gram-positive bacteria and *Candida* species appeared less frequently with 4.8% and 3.5% in CA- and HA-UTI, respectively (Fig. 1). The uropathogens other than *Enterobacterales* isolates were excluded from this study.

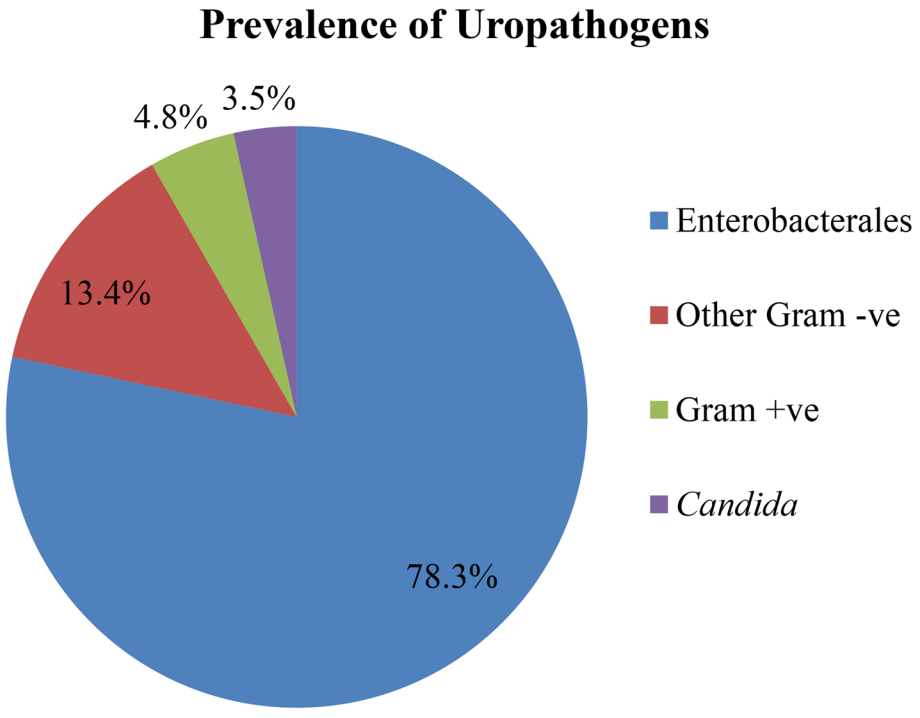

Fig. 1 Prevalence of uropathogen in urine positive samples ($n=231$)

Table 3 Prevalence of *Enterobacterales* species in CA and HA-UTI cases

Enterobacterales	*CA-UTI *n* (%)	*HA-UTI *n* (%)	*P* value	Total *n* (%)
E. coli	79 (73.8)	49 (66.2)	0.347	128 (70.6)
K. pneumoniae	14 (13.4)	10 (13.2)	1.00	24 (13.3)
P. mirabilis	7 (6.6)	2 (2.7)	0.412	9 (4.9)
K. oxytoca	1 (0.9)	4 (5.4)	0.179	5 (2.8)
C. freundii	1 (0.9)	2 (2.7))	0.746	3 (1.7)
E. cloacae	1 (0.9)	2 (2.7)	0.746	3 (1.7)
K. aerogenes	1 (0.9)	2 (2.7)	0.746	3 (1.7)
P. heimbachae	2 (1.9)	1 (1.4)	1.00	3 (1.7)
C. koseri	1 (0.9)	1 (1.4)	1.00	2 (1.1)
P. vulgaris	0 (0)	1 (1.4)	0.852	1 (0.5)
Total	107 (100)	74 (100)		181 (100)

**CA-UTI* community-acquired urinary tract infection, *HA-UTI* hospital-acquired urinary tract infection

Table 4 Antimicrobial resistance pattern of *Enterobacterales* isolates of CA-UTI ($n = 107$)

Antimicrobials	*E. colin*(%)	*K. pneumoniaen*(%)	*P*. mirabilis *n*(%)	*Others *n*(%)	Total *n*(%)
Cefazolin	51 (64.5)	9 (64.2)	4 (57.1)	4 (57.1)	68 (63.5)
Cefuroxime	51 (64.5)	9 (64.2)	4 (57.1)	4 (57.1)	68 (63.5)
Ceftaroline	36 (45.5)	7 (50.0)	1 (14.2)	3 (42.8)	47 (43.9)
Cefepime	44 (55.7)	7 (50.0)	2 (28.5)	3 (42.8)	56 (52.3)
Cefoxitin	27 (34.1)	3 (21.4)	2 (28.5)	1 (14.2)	33 (30.8)
Ceftriaxone	50 (63.2)	8 (57.1)	4 (57.1)	4 (57.1)	66 (61.6)
Ceftazidime	45 (56.9)	8 (57.1)	2 (28.5)	3 (42.8)	58 (54.2)
Aztreonam	44 (55.7)	8 (57.1)	3 (42.8)	3 (42.8)	58 (54.2)
Meropenem	1 (1.2)	1 (7.1)	0	0	2 (1.8)
Imipenem	6 (7.5)	1 (7.1)	0	0	7 (6.5)
Amikacin	9 (11.39)	2 (14.2)	2 (28.5)	0	13 (12.1)
Gentamicin	31 (39.2)	7 (50.0)	2 (28.5)	3(42.8)	43 (40.1)
Nalidixic acid	56 (70.8)	8 (57.1)	3(42.8)	3(42.8)	70 (65.4)
Norfloxacin	50 (63.2)	8 (57.1)	1 (14.2)	4 (57.1)	63 (58.8)
Ciprofloxacin	42 (53.1)	8 (57.1)	4 (57.1)	4 (57.1)	58 (54.2)
Fosfomycin	24 (30.4)	NA	NA	NA	24 (30.4)
Trimethoprim-sulphamethoxazole	48 (60.7)	9 (64.2)	4 (57.1)	4 (57.1)	65 (60.7)
Nitrofurantoin	26 (32.9)	4 (28.5)	7(100)	2 (28.5)	31 (28.9)
Trimethoprim	58 (73.4)	9 (64.2)	6 (85.7)	4 (57.1)	77 (71.9)
Piperacillin/tazobactam	49 (62.0)	8 (57.1)	4 (57.1)	4 (57.1)	65 (60.7)
Amoxicillin/clavulanic acid	52 (65.8)	8 (57.1)	4(57.1)	4 (57.1)	68 (63.5)
Ampicillin	52 (65.8)	14 (100)	4 (57.1)	6 (57.1)	76 (71.0)
Piperacillin	52 (65.8)	8 (57.1)	4(57.1)	4 (57.1)	68 (63.5)

NA not applicable

**C. freundii* (1) *C. koseri* (1) *E. cloacae* (1) *P. vulgaris* (0) *P. heimbachae* (2) *K. oxytoca* (1), *K. aerogenes* (1)

Prevalence of Enterobacterales species in CA and HA-UTI

Out of 181 *Enterobacterales*, *E. coli* was the predominant uropathogen (70.6%) in overall, and in both CA (73.8%) and HA-UTI (66.2%) cases. The prevalence of *K. pneumoniae* in CA- and HA-UTI was 14 (13.4%) and 10 (13.2%), respectively. While fewer species of the genera *Proteus*, *Citrobacter*, and *Enterobacter* were isolated (Table 3). The prevalence of *Enterobacterales* species in CA- and HA-UTI isolates were compared using the chi-square of independence test, which showed no significant association.

Antimicrobial resistant pattern of Enterobacterales of CA-UTI

Among *Enterobacterales* isolated from CA-UTI, *E. coli* (51/79; 64.5%) and *K. pneumoniae* (9/14; 64.2%) exhibited high resistance to cefazolin and cefuroxime. Resistance to carbapenems was relatively lower among CA-UTI as only 2 (1.8%) and 7 (6.5%) isolates were resistant to meropenem and imipenem, respectively. All *P. mirabilis* and other less common species of *Enterobacterales* were sensitive to the imipenem and meropenem (Table 4). In all, 40.1, 30.8 and 12.1% of the *Enterobacterales* were resistant to gentamicin, cefoxitin and amikacin.

Antimicrobial resistance pattern of Enterobacterales isolates of HA-UTI

The antimicrobial resistance patterns in the isolates from HA-UTI cases revealed that *E. coli*, *K. pneumoniae*, *P. mirabilis*, and other species of *Enterobacterales* were highly resistant to β-lactam antibiotics. A total of 70 (94.5%) isolates from HA-UTI were resistant to cefazolin, cefuroxime, ceftriaxone, ceftazidime, amoxicillin/clavulanic acid, ampicillin, and piperacillin. More than half of the isolates were resistant to carbapenems including meropenem (54%) and imipenem (60.8%). *E. coli* and *K. pneumoniae* isolates were resistant to amikacin, with the prevalence in 24 (48.9%) and 7 (70%) isolates, respectively (Table 5). While 48 (64.8%), 61 (82.4%), 47 (95.9%) and 9 (90%) isolates were resistant to ciprofloxacin, norfloxacin, sulfonamide and trimethoprim-sulphamethoxazole, respectively. In contrast, the resistance pattern to nitrofurantoin was relatively low as 22 *E. coli* (55.1%) isolates and 6 (46.15%) isolates of other less common species of *Enterobacterales* were resistant to nitrofurantion.

Comparison of antibiotic resistance pattern in CA and HA-UTI isolates

Statistical analysis using the Chi-square test demonstrated significant differences in the antimicrobial resistance patterns between CA and HA-UTI. Of the 23 antimicrobials evaluated, 21 exhibited statistically significant differences at P value (<0.05), with HA-UTI isolates consistently showing higher resistance rates compared to those from CA-UTI. Notably, the most substantial differences in resistance were observed for meropenem, imipenem, ceftaroline and amikacin indicating a considerable increase in resistance among HA isolates to these antibiotics (Table 6). Whereas the resistance rates for trimethoprim and ciprofloxacin were not significantly different in both setting.

Carbapenem-resistant Enterobacterales

Among the *Enterobacterales*, 52 isolates (28.7%) exhibited resistance to carbapenems both in CA and HA-UTI isolates (Tables 4 and 5). The prevalence of carbapenem-resistance revealed 36 *E. coli* out of 128 isolates (28.1%) and 8 *K. pneumoniae* out of 24 isolates (16.7%) as CRE positive.

Combined disc synergy test (CDT)

The results from CDT indicated the high prevalence (45/52; 86.5%) of MBL among carbapenem-resistant *Enterobacterales* isolates. All the isolates of *P. mirabilis*, *K. oxytoca*, and *K. aerogenes* were found positive for MBL production by CDT. While 33 *E. coli* isolates (87.5%) and 6 *K. pneumoniae* isolates (75%) were found MBL positive.

Table 5 Antibiotics resistance pattern of *Enterobacterales* in HA-UTI ($n=74$)

Antimicrobials	*E. colin*(%)	*K. pneumoniae* *n*(%)	*P.* mirabilis *n*(%)	*Others *n*(%)	Total *n*(%)
Cefazolin	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Cefuroxime	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Ceftaroline	45 (91.8)	10 (100)	1 (50.0)	11 (84.6)	67 (90.5)
Cefepime	46 (93.8)	10 (100)	1 (50.0)	11 (84.6)	68 (91.8)
Cefoxitin	38 (77.5)	7 (70.0)	1 (50.0)	9 (69.2)	55 (74.3)
Ceftriaxone	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Ceftazidime	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Aztreonam	47 (95.9)	9 (90.0)	1 (50.0)	11 (84.6)	68 (91.8)
Meropenem	27 (55.1)	5 (50.0)	1 (50.0)	7 (53.8)	40 (54.0)
Imipenem	30 (61.2)	7 (70.0)	1 (50.0)	7 (53.8)	45 (60.8)
Amikacin	24(48.9)	7 (70.0)	1 (50.0)	7 (53.8)	39 (52.7)
Gentamicin	35 (71.4)	7 (70.0)	1 (50.0)	10 (76.9)	53 (71.6)
Nalidixic acid	40 (81.6)	9 (90.0)	1 (50.0)	10 (76.9)	60 (81.0)
Norfloxacin	40 (81.6)	9 (90.0)	2 (100)	10 (76.9)	61 (82.4)
Ciprofloxacin	28 (57.1)	9 (90.0)	1 (50.0)	10 (76.9)	48 (64.8)
Fosfomycin	30 (61.2)	NA	NA	NA	30 (61.2)
Trimethoprim-sulphamethoxazole	47 (95.9)	9 (90.0)	2 (100)	11 (84.6)	69 (93.2)
Nitrofurantoin	27 (55.1)	8 (80.0)	2 (100)	6 (46.1)	42 (56.7)
Trimethoprim	42 (85.7)	8 (80.0)	2 (100)	10 (76.9)	62 (83.7)
Piperacillin/tazobactam	48 (97.9)	9 (90.0)	1 (50.0)	11 (84.6)	69 (93.2)
Amoxicillin/clavulanic acid	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Ampicillin	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)
Piperacillin	48 (97.9)	10 (100)	1 (50.0)	11 (84.6)	70 (94.5)

NA not applicable

**C. freundii* (2) *C. koseri* (1) *E. cloacae* (2) *P. vulgaris* (1) *P. heimbachae* (1) *K. oxytoca* (4), *K. aerogenes* (2)

Table 6 Comparison of antibiotic resistance pattern in CA and HA-UTI *Enterobacterales* isolates

Antimicrobials	CA-UTI *n* (%)	HA-UTI *n* (%)	*p* value	Total *n* (%)
Cefazolin	68 (63.5)	70 (94.5)	$p < 0.001$	138(76.2)
Cefuroxime	68 (63.5)	70 (94.5)	$p < 0.001$	138(76.2)
Ceftaroline	47 (43.9)	67 (90.5)	$p < 0.001$	114(63.0)
Cefepime	56 (52.3)	68 (91.8)	$p < 0.001$	124(68.5)
Cefoxitin	33 (30.8)	55 (74.3)	$p < 0.001$	88(48.6)
Ceftriaxone	66 (61.6)	70 (94.5)	$p < 0.001$	136(75.1)
Ceftazidime	58 (54.2)	70 (94.5)	$p < 0.001$	128(70.7)
Aztreonam	58 (54.2)	68 (91.8)	$p < 0.001$	126(69.6)
Meropenem	2 (1.8)	40 (54.0)	$p < 0.001$	42(23.2)
Imipenem	7 (6.5)	45 (60.8)	$p < 0.001$	52(28.7)
Amikacin	13 (12.1)	39 (52.7)	$p < 0.001$	52(28.7)
Gentamicin	43 (40.1)	53 (71.6)	$p < 0.001$	96(53.0)
Nalidixic acid	70 (65.4)	60 (81.0)	p 0.0328	130(71.8)
Norfloxacin	63 (58.8)	61 (82.4)	$p < 0.001$	124(68.5)
Ciprofloxacin	58 (54.2)	48 (64.8)	p 0.2014	106(58.6)
Fosfomycin	24 (30.4)	30 (61.2)	p 0.0142	54(29.8)
Trimethoprim-sulphamethoxazole	65 (60.7)	69 (93.2)	$p < 0.001$	134(70.0)
Nitrofurantoin	31 (28.9)	42 (56.7)	$p < 0.0003$	73(40.3)
Trimethoprim	77 (71.9)	62 (83.7)	p 0.0943	139(76.8)
Piperacillin/tazobactam	65 (60.7)	69 (93.2)	$p < 0.001$	134(74.0)
Amoxicillin/clavulanic acid	68 (63.5)	70 (94.5)	$p < 0.001$	138(76.2)
Ampicillin	68 (63.5)	70 (94.5)	$p < 0.001$	138(76.2)
Piperacillin	68 (63.5)	70 (94.5)	$p < 0.001$	138(76.2)

Table 7 Comparison of MHT, mCIM and PCR CRE isolates ($n = 52$)

Isolates	CRE *n*(%)	MHT *n*(%)	mCIM *n*(%)	PCR *n*(%)
E. coli	36 (28.1)	6 (16.7)	34 (94.4)	36 (100)
K. pneumoniae	8 (16.7)	3 (37.5)	7 (87.5)	7 (87.5)
P. mirabilis	1 (11.1)	0 (0)	1 (100)	1 (100)
K. oxytoca	1 (20)	0 (0)	1 (100)	1 (100)
K. aerogenes	2 (66.7)	1 (50)	1 (50)	2 (100)
Other species	4 (30.7)	2 (150)	4 (100)	4 (100)
Total	52 (28.7)	12(23.1)	48 (92.3)	51 (98.1%)

Phenotypic and genotypic detection of carbapenemase

Carbapenemase production was phenotypically detected by modified Hodge test (MHT) and modified carbapenem inactivation method (mCIM). Most of the CRE isolates (48/52; 92.3%) were mCIM positive, which shows higher prevalence of carbapenemase producers. Likewise, 34 out of 36 CR *E. coli* (94.4%) and 7 out of 8 CR-*K. pneumoniae* (87.5%) were carbapenemases producer. The MHT detected fewer cases of carbapenemase producers as only 12 out of 52 (23.1%) CRE isolates were found positive. Similarly 6 out of 36 CR *E. coli* (16.7%) and 3 out of 8 *K. pneumoniae* (37.5%) isolates were found carbapenemase positive by MHT. PCR validated the results obtained by mCIM as 51 isolates out of 52 (98.1%) CRE isolates were positive for carbapenemase production (Table 7).

Specificity and sensitivity

The specificity and sensitivity of three methods for detecting carbapenemase in carbapenem-resistant Enterobacteriaceae were evaluated by using the formula taking the PCR as standard test. A total of 181 *Enterobacterales* isolates were analyzed, of which 52 were CRE, and 129 isolates were non- CRE or sensitive to carbapenems were found true negative. The MHT exhibited a sensitivity of 23.08% and specificity of 100%. While mCIM demonstrated significantly higher sensitivity of 92.32%, and 100% specificity (Table 8).

Molecular characterization of carbapenemase genes

The polymerase chain reaction (PCR) analysis conducted on 52 CRE revealed presence of the bla_{NDM} gene in 45 isolates (86.5%) (Fig. 2) while only 6 (11.5%) carried $bla_{\text{OXA-48}}$. The prevalence of bla_{NDM} in *E. coli* was also higher

Table 8 Sensitivity and specificity of different test for detection of carbapenemases in CRE ($n = 52$)

Methods	*TP	*FN	Sensitivity (%)	*TN	FP	Specificity (%)
MHT	12	40	23.08	129	0	100
mCIM	48	4	92.31	129	0	100
PCR	51	1	98.08	129	0	100

**TP* true positive, *FN* false negative, *TN* true negative, *FP* false positive

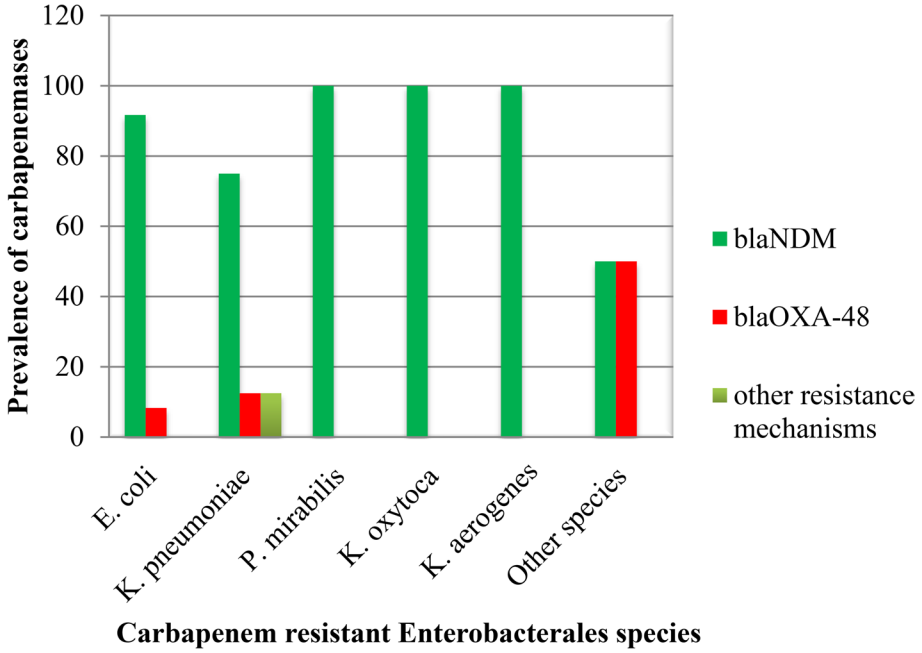

Fig. 2 Prevalence of bla_{NDM} and bla_{OXA-48} gene in CRE isolates * others = includes the other resistance genes

with 91.7% (33/36%). While all the isolates of *P. mirabilis*, *K. oxytoca*, and *K. aerogenes* were bla_{NDM} (100%) producers. Whereas 3 out of 36 (8.3%) *E. coli*, 1 out of 8 *K. pneumoniae* (12.5%) and 2 out of 4 other less common species (50%) were bla_{OXA-48} positive.

Location of bla_{NDM} gene on chromosome or plasmid

Comparative PCR assessments of both whole DNA and plasmid DNA revealed that 44 out of 45 (97%) isolates harbored bla_{NDM} gene on the plasmid. Only one *E. coli* strain was negative for the plasmid PCR.

Prevalence of carbapenemases variants

The sequenced data were analyzed using MEGA 11 and DNA Baser to assess the quality of the sequenced genes. The genes were subjected to BLAST analysis through the NCBI database and compared with available carbapenemases genes. Remarkably, these genes exhibited a 99% match with the previously identified sequences. The results were subsequently submitted to GenBank, and the accession number OR678921.1 was obtained for the complete sequence of bla_{NDM-5}.

Discussion

Urinary tract infections (UTIs) are the most prevalent bacterial infections in both community and healthcare settings, affecting individuals across all age groups and genders. Understanding the demographic distribution of UTI is essential for developing targeted prevention and management strategies, particularly in regions with a high disease burden such as Pakistan. The present study provides valuable insights into the burden of UTI across gender and age groups in both community-acquired UTI and hospital-acquired UTI settings. Out of 298 urine samples, a total of (77.5%) were culture-positive for UTI, with a higher proportion of cases identified among outpatients compared to hospitalized individuals. This high positive rate is consistent with previous study from Pakistan, where UTIs remain a prevalent clinical concern [29]. Our study demonstrated that females accounted for a higher proportion of UTI cases (57.1%), which is consistent with established epidemiological patterns and is largely attributed to anatomical and physiological factors that predispose women to UTI [30]. Age-wise analysis revealed the highest burden of UTIs in the 21–30 years age group (47.2%) followed by the age group 31–40 year group (20%), which aligns with previous studies reporting a peak incidence among young adults, especially women of reproductive age [30]. A particularly significant finding was observed in patients aged ≥ 61 years, where the prevalence of HA-UTI was disproportionately higher than CA-UTI (4.8% vs. 0.9%) with the $p = 0.001$. This underscores the vulnerability of elderly hospitalized patients, likely due to factors such as immunesenscence, chronic illnesses, prolonged hospital stays, and frequent use of invasive devices [31].

UTIS primarily caused by *Enterobacterales*, with *E. coli* identified as the most common etiological agent. In this study, *E. coli* was the predominant (70.7%) followed by *K. pneumoniae* (13.2%). Our results are consistent with prior research conducted in Iran, where *E. coli* (80.8%) was prevalent pathogen in UTI, followed by *K.*

pneumoniae (12.8%). Other less frequently encountered strains included *P. mirabilis* (4.9%), *K. oxytoca* (2.7%), *C. freundii* and *E. cloacae* (1.6%). This distribution also corroborated with a previous study [32].

The rise of antibiotic-resistant bacteria, particularly CRE, poses significant challenges in managing UTIs. Carbapenemases are crucial for treating severe infections due to their broad-spectrum efficacy against resistant pathogens. However, the misuse and overuse of antibiotics have facilitated the emergence of carbapenem-resistant strains through genetic mutations and the acquisition of resistance genes. *E. coli*, *K. pneumoniae* and *P. mirabilis*, along with other less common *Enterobacterales* isolates were found highly resistant to cefazolin, cefuroxime, ampicillin, piperacillin, amoxicillin/clavulanic acid, and piperacillin/tazobactam with a rate of 55.55 to 100%. Similar findings have also been reported in various clinical settings across the world including Bangladesh, China, India and Pakistan [31, 33–35].

In present study, *E. coli* isolates demonstrated significant resistance to ceftaroline (63.2%), ciprofloxacin (65.5%), ceftriaxone (72.6%), and nalidixic acid (64.1%). These findings align with a broader trend in antimicrobial resistance observed in the Asia-Pacific region. A systematic review by Sugianli et al. reported resistance rates ranging from of 33–90% for trimethoprim/sulfamethoxazole, ciprofloxacin, and ceftriaxone among uropathogens, with isolates from Bangladesh, India, Sri Lanka, and Indonesia exhibiting the highest resistance levels. Resistance to nitrofurantoin in this region was comparatively lower (2.7% to 31.4%), while resistance to fosfomycin was reported at lower rates of 1.7–1.8% [36].

In Pakistan, a study highlighted that *E. coli* exhibited the highest resistance to ciprofloxacin (67.4%) and nalidixic acid (70.1%). Our findings are consistent with these observations, particularly for ciprofloxacin and nalidixic acid resistance. However, our study revealed that *E. coli* exhibited alarming high resistance to meropenem (21.87%) and imipenem (28.12%), significantly exceeding those reported in earlier studies from Pakistan [37–39]. Resistant to imipenem than to meropenem, may be due to the differences in their structural stability against β-lactamases and the presence of specific resistance mechanisms that preferentially target imipenem.

A study from Poland reported significant resistance in *K. pneumoniae* against cephalosporins, amoxicillin/clavulanic acid (75.0%), piperacillin/tazobactam (76.2%), cefotaxime (76.2%), cefuroxime (81.0%), ciprofloxacin (81.0%), and trimethoprim/sulphamethoxazole (81.0%). Only 13.6% of the isolates were resistant to meropenem [40]. In our study, *K. pneumoniae* was highly resistant to cefazolin (79.16%), amoxicillin/clavulanic acid (75%), and trimethoprim and ciprofloxacin (70.83%). *K. pneumoniae* isolates were also resistant to other antimicrobials, including meropenem (25%), imipenem (33.3%), amikacin (37.5%), and cefoxitin (41.6%).

In our study HA-UTI isolates exhibited significantly higher resistance rates to all tested antibiotics compared to CA-UTI isolates. Results showed that resistance to cephalosporins including cefazolin, cefuroxime, cefepime, cefoxitin, ceftriaxone, and ceftazidime was markedly elevated in HA-UTI, with resistance rates exceeding 90%, while CA-UTI isolates demonstrated resistance ranging from 30 to 65%. These findings are consistent with other recent studies from Pakistan, which have reported high levels of antimicrobial resistance among uropathogens, particularly in hospital environments [41, 42]. The elevated resistance rates in HA-UTI can be attributed to several factors inherent to the hospital setting. Hospitals frequently use broad-spectrum antibiotics, which creates strong selective pressure favoring the emergence and the persistence of multidrug resistant isolates. Patients admitted to hospitals are often exposed to invasive procedures, have longer durations of stay, and may be immune-compromised, all of which increase the risk of acquiring resistant infections. Additionally, cross-transmission of resistant bacteria is more likely in hospital settings due to close contact between patients and healthcare workers and the presence of environmental reservoirs of resistant pathogens.

The global incidence of (CRE), including, KPC-producing *E. coli* and *K. pneumoniae* is prevalent in the United States, Colombia, Argentina, Greece, and Italy, whereas MBL NDM-1 is the primary carbapenemase-producing strain in India, Pakistan, and Sri Lanka. OXA-48 is widespread in Turkey, Malta, the Middle East, and North Africa [43].

Timely and accurate identification of CRE is essential for optimizing antibiotic therapy and reduce the use of inappropriate medication. Initially, phenotypic methods were employed to diagnose the carbapenemase-producing bacteria, primarily based on reduced carbapenem sensitivity. However, molecular techniques have become the gold standard for confirmation due to the superior accuracy and reliability. Traditional phenotypic approaches, such as the modified Hodge test (MHT), are widely used but have notable limitations, including being time-intensive, challenging to interpret, and exhibiting variable accuracy across bacterial species [44].

In our study, 52 carbapenem-resistant isolates were tested using MHT. Among these, 23.07% were MHT-positive for carbapenemase production, in which, 5.76% of the cases were false positive. Additionally, 65.38% of the isolates were negative for CR. These findings are consistent with those reported by Tsai et al. (2020). The authors concluded that MHT had excellent sensitivity for the detection of $bla_{\text{OXA-48}}$ and bla_{KPC}, and the sensitivity for bla_{NDM} was (46.7%). Another study from India

reported that MHT has a low sensitivity for detecting MBL producers. MHT has excellent sensitivity to detect KPC and OXA-48 carbapenemases but has low sensitivity (50%) for NDM-1 producers [23, 44]. In to our study, MHT exhibited low sensitivity in detecting carbapenemases, particularly bla_{NDM}. This limitation is especially problematic in regions like Pakistan where the prevalence of bla_{NDM} gene is high. Furthermore, the reliability of MHT is further compromised in such settings due to its tendency to produce false-positive results for bla_{NDM} producing-isolates [45].

The modified carbapenem inactivation method was used to detect carbapenemases production. Among the isolates tested, 92.3% were positive for the carbapenemases. Previous studies have demonstrated that mCIM has high sensitivity and specificity for detecting carbapenemase [46]. For the detection of MBLs, the combined disc test (CDT) was used. In the present study, 45 (86.5%) were positive for MBL production using CDT. These findings are consistent with those reported by other researchers [14, 34].

In 2009, bla_{NDM-1} was identified in India, and since then, CRE strains carrying *bla*NDM-1 and its variants have spread to more than fifty-five countries. Most Asian countries, including India, Pakistan, and China, are considered major reservoirs of bla_{NDM} [47].

Our study found that (86.5%) CRE isolates were positive for the presence of bla_{NDM} by PCR. In Pakistan, a study reported the same results on the prevalence of bla_{NDM} [48].

Plasmids have emerged as the primary and perhaps the most prevalent carriers for the bla_{NDM} gene. In this study, plasmid extraction and PCR analysis confirmed that the bla_{NDM} gene predominantly plasmid-mediated. Specifically, 75% of the bla_{NDM} positive isolates carried the gene on plasmids. Our results showed that bla_{NDM} gene (97.56%) is plasmid borne and hence is subjected to rapid transmission through horizontal gene transfer. Our findings are consistent with the previous studies [49] where all tested bla_{NDM} carrying *Enterobacterales* appeared plasmid-mediated. Similarly, Sun et al. characterized bla_{NDM} positive *E. coli* isolates in China and demonstrated that the gene was exclusively carried on plasmids, further supporting the global significance of plasmids as carriers of bla_{NDM} [50].

Conclusion

This research demonstrates a high level of antibiotic resistance among uropathogen. Resistance was particularly pronounced against β-lactam antibiotics, reflecting a significant challenge to effective clinical management of urinary tract infections in the region. Despite these concerning resistance patterns, amikacin, fosfomycin and nitrofurantoin retained comparatively lower resistance rates and may continue to serve as viable options for empirical therapy. Molecular analysis revealed that the most widespread type of carbapenem resistance gene detected is bla_{NDM} among the carbapenem resistant isolates. This is deeply alarming because these resistant genes can easily spread between different species, making the bacteria impervious to common antibiotics used in medical settings.

Limitations and perspectives

This study provides important insights, but it is limited by the absence of molecular typing for strain diversity. Moreover, it is a single-center study and any generalization should be made with this caution. Further research should incorporate advanced molecular epidemiological approaches, such as whole-genome sequencing, to better elucidate resistance mechanisms and transmission dynamics.

Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CRE	Carbapenem Resistant Enterobacterales
CP-CRE	Carbapenemases-producing CRE
CLSI	Clinical and Laboratory Standards Institute
CA	Community-acquired
CLED	Cysteine-Lactose Electrolyte Deficient
CDT	EDTA-Combined Disc Test
ESBLs	Extended Spectrum β-lactamase
HA	Hospital-acquired
IMP	Imipenemase
mCIM	Modified Carbapenem Inactivation Method
MHT	Modified Hodge Test
MHT	Modified Hodge test
MDR	Multidrug resistant
NDM	New Delhi metallo-β-lactamase
OPD	Outpatient department
OXA-48	Oxacillinase-48
TAE	Tris-acetate-EDTA
UTIs	Urinary tract infections
VIM	Verona integron-encoded metallo-β-lactamase

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Authors' contributions

LR, methodology, investigation and writing initial draft; FU, investigation, data curation; resources writing and editing final draft; WAA, data curation; MA, data curation and funding acquisition; MS, conceptualization, resources, writing and editing final draft. All authors approved final draft and submission of the manuscript.

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Data availability

The data and materials can be obtained from the first author upon a reasonable request.

Declarations

Ethical approval and consent to participate

The ethical approval was taken from the Institutional Review Board (IRB) committee of Jinnah Postgraduate Medical Centre (JPMC) with registration No. F.2–81/2021-GENL/57041/JPMC. The written informed consents were obtained from the all the participants. The study adhered the guidelines given in the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Assouma FF, Sina H, Adjobimey T, Noumavo ADP, Socohou A, Boya B, et al. Susceptibility and virulence of Enterobacteriaceae isolated from urinary tract infections in Benin. Microorganisms. 2023. https://doi.org/10.3390/microorganisms11010213.
2. Khatoon I, Khanam S, Azam A, Qadeer S, Naz S, Hassan NU. Incidence pattern, antibiotic susceptibility pattern and associated risk factors of bacterial uropathogens among general population of Pakistan. Infect Drug Resist. 2023;16:4995–5005.
3. Radera S, Srivastava S, Agarwal J. Virulence genotyping and multidrug resistance pattern of Escherichia coli isolated from community-acquired and hospital-acquired urinary tract infections. Cureus. 14(9):e29404. https://doi.org/10.7759/cureus.29404.
4. Toner L, Papa N, Aliyu SH, Dev H, Lawrentschuk N, Al-Hayek S. Extended-spectrum beta-lactamase-producing Enterobacteriaceae in hospital urinary tract infections: incidence and antibiotic susceptibility profile over 9 years. World J Urol. 2016;34:1031–7.
5. Woodford N, Xu-McCrae L, Mushtaq S, Wu HHT, Ellington MJ, Lancaster O, et al. Prevalence of carbapenem resistance and carbapenemase production among Enterobacteriaceae isolated from urine in the UK: results of the UK infection-carbapenem resistance evaluation surveillance trial (iCREST-UK). J Antimicrob Chemother. 2018;73:698–702. https://doi.org/10.1093/jac/dkx471
6. Yengui M, Trabelsi R, Khannous L, Mathlouthi NE, Adnan M, Siddiqui AJ, et al. Rapid detection of Beta-Lactamases genes among enterobacterales in urine samples by using Real-Time PCR. Biomed Res Int. 2022;2022:8612933.
7. Choe H-S, Lee S-J, Cho Y-H, Çek M, Tandoğdu Z, Wagenlehner F, et al. Aspects of urinary tract infections and antimicrobial resistance in hospitalized urology patients in asia: 10-Year results of the global prevalence study of infections in urology (GPIU). J Infect Chemother. 2018;24:278–83.
8. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17:1791.
9. Zilberberg MD, Nathanson BH, Sulham K, Shorr AF. Multiple antimicrobial resistance and outcomes among hospitalized patients with complicated urinary tract infections in the US, 2013–2018: a retrospective cohort study. BMC Infect Dis. 2021;21:1–10.
10. Kumudunie WGM, Wijesooriya LI, Namalie KD, Sunil-Chandra NP, Wijayasinghe YS. Epidemiology of multidrug-resistant Enterobacteriaceae in Sri lanka: first evidence of Bla KPC harboring Klebsiella pneumoniae. J Infect Public Health. 2020;13:1330–5. https://doi.org/10.1016/J.JIPH.2020.04.010.
11. Hoang CQ, Nguyen HD, Vu HQ, Nguyen AT, Pham BT, Tran TL, et al. Emergence of new Delhi Metallo-Beta-Lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC) production by *Escherichia coli* and *Klebsiella pneumoniae* in Southern Vietnam and appropriate methods of detection: a cross-sectional study. Biomed Res Int. 2019. https://doi.org/10.1155/2019/9757625.
12. Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, et al. Global spread of carbapenem-resistant enterobacteriaceae: epidemiological features, resistance mechanisms, detection and therapy. Microbiol Res. 2023;266: 127249.
13. Bologna E, Licari LC, Manfredi C, Ditonno F, Cirillo L, Fusco GM, et al. Carbapenem-resistant Enterobacteriaceae in urinary tract infections: from biological insights to emerging therapeutic alternatives. Medicina (B Aires). 2024;60: 214.
14. Sabour S, Huang JY, Bhatnagar A, Gilbert SE, Karlsson M, Lonsway D, et al. Detection and characterization of targeted carbapenem-resistant health care-associated threats: findings from the antibiotic resistance laboratory network, 2017 to 2019. Antimicrob Agents Chemother. 2021;65:10–1128.
15. Tan X, Kim HS, Baugh K, Huang Y, Kadiyala N, Wences M, et al. Therapeutic options for metallo-β-lactamase-producing enterobacterales. Infect Drug Resist. 2021:125–42.
16. Biotechnol NK-SR. 2020 undefined. CRE (Carbapenem Resistant Enterobacteriaceae) and the Globalization of Antimicrobial Resistance: Problems and Solutions. SuntextreviewsOrg. 2020. https://doi.org/10.51737/2766-5097.2020.003
17. Räisänen K, Lyytikäinen O, Kauranen J, Tarkka E, Forsblom-Helander B, Grönroos JO, et al. Molecular epidemiology of carbapenemase-producing enterobacterales in Finland, 2012–2018. Eur J Clin Microbiol Infect Dis. 2020;39:1651–6.
18. Romero Thomas G, Corso A, Pasterán F, Shal J, Sosa A, Pillonetto M, et al. Increased detection of Carbapenemase-Producing enterobacterales bacteria in Latin America and the Caribbean during the COVID-19 pandemic. Emerg Infect Dis. 2022;28:E1–8. https://doi.org/10.3201/eid2811.220415.
19. Habib A, Phane Lo S, Villageois-Tran K, Petitjean M, Malik SA, Armand-Lefèvre L et al. Dissemination of carbapenemase-producing Enterobacterales in the community of Rawalpindi, Pakistan. JournalsPlosOrgA Habib, S Lo, K Villageois-Tran, M Petitjean, SA Malik, L Arman E RuppePLoS One, 2022•journalsPlosOrg 2022;17. https://doi.org/10.1371/journal.pone.0270707
20. Sakamoto N, Akeda Y, Sugawara Y, Matsumoto Y, Motooka D, Iida T, et al. Role of chromosome-and/or plasmid-located Bla NDM on the carbapenem resistance and the gene stability in *Escherichia coli*. Microbiol Spectr. 2022;10: e00587–22.
21. Aguilar-Duran S, Horcajada JP, Sorlí L, Montero M, Salvadó M, Grau S, et al. Community-onset healthcare-related urinary tract infections: comparison with community and hospital-acquired urinary tract infections. J Infect. 2012;64:478–83.
22. Pasteran F, Gonzalez LJ, Albornoz E, Bahr G, Vila AJ, Corso A. Triton hodge test: improved protocol for modified hodge test for enhanced detection of NDM and other carbapenemase producers. J Clin Microbiol. 2016;54:640–9.
23. Tsai Y-M, Wang S, Chiu H-C, Kao C-Y, Wen L-L. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiol. 2020;20:1–7.
24. Salvia T, Dolma KG, Dhakal OP, Khandelwal B, Singh LS. Phenotypic detection of ESBL, AmpC, MBL, and their co-occurrence among MDR Enterobacteriaceae isolates. J Lab Physicians. 2022;14:329–35.
25. Uddin F, Imam SH, Khan S, Khan TA, Ahmed Z, Sohail M, et al. NDM production as a dominant feature in carbapenem-resistant Enterobacteriaceae isolates from a tertiary care hospital. Antibiotics. 2021;11: 48.
26. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. J Antimicrob Chemother. 2007;59:321–2. https://doi.org/10.1093/JAC/DKL481.
27. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, et al. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the united states: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53:3365–70. https://doi.org/10.1128/AAC.00126-09.
28. Gurung S, Kafle S, Dhungel B, Adhikari N, Shrestha UT, Adhikari B, et al. Detection of OXA-48 gene in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* from urine samples. Infect Drug Resist. 2020. https://doi.org/10.2147/IDR.S259967.
29. Khatoon MA, Karim SMK, Wasim M, Ali R, Zaighum M, Iqbal N. Frequency of urinary tract infection among patients undergoing implant fixation for acute trauma. Cureus. 2023;15:e49817. https://doi.org/10.7759/CUREUS.49817.

30. Czajkowski K, Broś-Konopielko M, Teliga-Czajkowska J. Urinary tract infection in women. Przegląd Menopauzalny = Menopause Rev. 2021;20:40. https://doi.org/10.5114/PM.2021.105382.
31. Huang L, Huang C, Yan Y, Sun L, Li H. Urinary tract infection etiological profiles and antibiotic resistance patterns varied among different age categories: a retrospective study from a tertiary general hospital during a 12-year period. Front Microbiol. 2022;12:813145.
32. Azargun R, Sadeghi MR, Hossein M, Barhaghi S, Kafil HS, Yeganeh F, et al. The prevalence of plasmid-mediated quinolone resistance and ESBL-production in Enterobacteriaceae isolated from urinary tract infections. Infect Drug Resist. 2018;11:1007–14. https://doi.org/10.2147/IDR.S160720.
33. Majumder MMI, Mahadi AR, Ahmed T, Ahmed M, Uddin MN, Alam MZ. Antibiotic resistance pattern of microorganisms causing urinary tract infection: a 10-year comparative analysis in a tertiary care hospital of Bangladesh. Antimicrob Resist Infect Control. 2022;11:1–8.
34. Shirvani M, Keramati A, Esmaeli M. Evaluating the pattern of antibiotic resistance of urinary tract infection (UTI)-causing bacteria in the urine culture samples of patients in the infectious ward of Imam Khomeini hospital, kermanshah, in Iran from 2016–2018. Afr J Urol. 2023;29:1–11. https://doi.org/10.1186/S12301-023-00364-4/TABLES/6.
35. Iqbal Z, Mumtaz MZ, Malik A. Extensive drug-resistance in strains of *Escherichia coli* and *Klebsiella pneumoniae* isolated from paediatric urinary tract infections. J Taibah Univ Med Sci. 2021;16:565–74.
36. Sugianli AK, Ginting F, Parwati I, de Jong MD, van Leth F, Schultsz C. Antimicrobial resistance among uropathogens in the Asia-Pacific region: A systematic review. JAC-Antimicrobial Resist. 2021;3:dlab003.
37. Hussain T, Moqadasi M, Malik S, Zahid AS, Nazary K, Khosa SM et al. Uropathogens antimicrobial sensitivity and resistance pattern from outpatients in balochistan, Pakistan. Cureus. 2021;13. https://doi.org/10.7759/CUREUS.17527
38. Okesola AO. Antibiotic resistance pattern of uropathogenic Escherichia coli in South West Nigeria. Afr J Med Med Sci. 2011;40:235–8.
39. Eezzeldin HM, Badi S, Yousef BA. The antibiotic resistance and multidrug resistance pattern of uropathogenic *Escherichia coli* at Soba university hospital: a descriptive retrospective survey. Sudan J Med Sci. 2022;17:56–69.
40. Kot B, Grużewska A, Szweda P, Wicha J, Parulska U. Antibiotic resistance of uropathogens isolated from patients hospitalized in district hospital in central Poland in 2020. Antibiotics. 2021;10: 447.
41. Khatoon I, Khanam S, Azam A, Qadeer S, Naz S, Hassan NU. Incidence pattern, antibiotic susceptibility pattern and associated risk factors of bacterial uropathogens among general population of Pakistan. Infect Drug Resist. 2023;16:4995. https://doi.org/10.2147/IDR.S418045.
42. Bullens M, de Cerqueira Melo A, Raziq S, Lee J, Khalid GG, Khan SN, et al. Antibiotic resistance in patients with urinary tract infections in Pakistan. Public Heal Action. 2022;12:48–52.
43. Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae infections. Adv clin Immunol med microbiol COVID-19. Big Data. 2021:435–56.
44. Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W, Sabour AA, et al. An overview on phenotypic and genotypic characterisation of carbapenem-resistant enterobacterales. Medicina (B Aires). 2022;58: 1675.
45. Kim H-K, Park JS, Sung H, Kim M-N. Further modification of the modified hodge test for detecting metallo-β-lactamase-producing carbapenem-resistant Enterobacteriaceae. Ann Lab Med. 2015;35:298.
46. Rizvi M, Sami H, Azam M, Ben Khalid D, Jabri AL, Khan Z. Reliability of carbapenem inactivation method (CIM) and modified carbapenem inactivation method (mCIM) for detection of OXA-48-like and NDM-1. Indian J Med Microbiol. 2021;39:451–6. https://doi.org/10.1016/J.IJMMB.2021.07.004.
47. Dong H, Li Y, Cheng J, Xia Z, Liu W, Yan T, et al. Genomic epidemiology insights on NDM-producing pathogens revealed the pivotal role of plasmids on Bla NDM transmission. Microbiol Spectr. 2022;10:e02156–21.
48. Habib A, Lo S, Villageois-Tran K, Petitjean M, Malik SA, Armand-Lefevre L, et al. Dissemination of carbapenemase-producing enterobacterales in the community of Rawalpindi, Pakistan. PLoS One. 2022;17: e0270707.
49. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of Bla NDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother. 2011;55:5403–7.
50. Sun P, Xia W, Liu G, Huang X, Tang C, Liu C, et al. Characterization of Bla NDM-5-positive Escherichia coli prevalent in a university hospital in Eastern China. Infect Drug Resist. 2019;12:3029–38.https://doi.org/10.2147/IDR.S225546.

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