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Original Article

Phenotypic detection of ESBL, AmpC, and MBL in Enterobacterales uropathogens: a two-center experience in Karachi, Pakistan

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Abstract

Introduction: The emergence of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases; and their co-existence among Enterobacterales uropathogens present new diagnostic and therapeutic challenges. This study aimed to elucidate the phenotypic detection and co-occurrence of ESBL-, AmpC-, and carbapenemase-producing uropathogens.

Methodology: A cross-sectional study was conducted from 3 January to 26 July 2024, at the Department of Microbiology, Basic Medical Sciences Institute, in collaboration with the Department of Urology at Jinnah Postgraduate Medical Center and National Medical Centre, Karachi, Pakistan. A total of 260 non-repetitive urine samples were collected from hospitalized and community patients. Antimicrobial susceptibility was determined by disc diffusion; ESBL, AmpC, and carbapenemase producers were identified using the double disc synergy test (DDST), modified three-dimensional method, and lateral flow immunochromatographic (LFI) assay, respectively.

Results: Among the 260 cases, 207 (80%) showed positive growth, yielding 240 isolates. Out of 189 Enterobacterales, *E. coli* (131; 69.3%) was the most prevalent, followed by *K. pneumoniae* (46; 24.3%), *P. mirabilis* (3; 1.6%), *E. cloacae* (3; 1.6%), *K. oxytoca* (2; 1.1%), *C. freundii* (2; 1.1%), *C. werkmanii* (1; 0.5%), and *P. rettgeri* (1; 0.5%). There were 89 (47.1%) ESBL producers, 10 (5.2%) AmpC producers, and 59 (31.2%) carbapenem-resistant isolates. New Delhi metallo-beta-lactamase (NDM) was the dominant carbapenemase (33; 56%). Co-production of ESBL and NDM was the most common, and detected in 30 (19%) isolates.

Conclusions: The prevalence of ESBLs and carbapenemase producers was high, with frequent co-production of ESBL and NDM. Rapid, cost-effective phenotypic methods are crucial for timely detection and appropriate antimicrobial treatment.

Key words: AmpC; *E. coli*; Enterobacterales; ESBLs; MDR.

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Introduction

The emergence and dissemination of multidrug-resistant (MDR) pathogens is a grave global health threat. Antibiotic therapy is the keystone for any bacterial infection, including urinary tract infections (UTIs). The rampant use of antibiotics, both devoted to human consumption, and veterinary or agricultural-industry, has directed to a momentous public health threat of the rise in antibiotic resistance among bacterial pathogens [1,2]. The World Health Organization (WHO) mentions antibiotic resistance as one of the foremost threats to global security, after global warming [3]. The European Center for Disease Prevention and Control (ECDC) estimates more than half a million cases of UTI per annum by antibiotic resistant bacterial pathogens [4]. The prevalence of antimicrobial resistance of Gram-negative uropathogens is 76.9% in Pakistan [5].

Extended spectrum β-lactamases (ESBLs), AmpC, and carbapenemase-producing Enterobacterales have increased dramatically globally over the past decade, with reports from both community and hospital settings. WHO has divided microorganisms into priority categories on the basis of antimicrobial resistance. Carbapenem-resistant and extended-spectrum cephalosporin-resistant Enterobacterales are listed in critical priority [6]. ESBLs are β-lactamases exhibiting an extended spectrum of resistance towards the extended spectrum β-lactam antibiotics; excluding carbapenems, cephamycins, and β-lactamase inhibitor combinations of penicillin, aztreonam, and cephalosporins [7]. AmpC β-lactamases inactivate cephamycin, cephalosporins, aminopenicillins, and monobactams; but are less inhibited by clavulanic acids [8]. Carbapenems play a vital role in the treatment of ESBL and AmpC producing Enterobacterales.

Nowadays carbapenem resistance alone, as well as, co-associated with ESBL or AmpC β-lactamase is alarming for global health security [9].

Culture and susceptibility testing of bacterial pathogens is a prerequisite for appropriate therapeutic strategies to treat bacterial infections and to control antimicrobial resistance. However, the simultaneous presence of various β-lactamase types in a single organism could result in inadequate therapy particularly in critically ill patients. Therefore, the co-occurrence of resistance markers must be determined to prescribe an effective treatment regimen. Antibiotic susceptibility testing is however ineffective in detecting these resistant species. Therefore, additional testing to detect the presence of multiple drug resistance markers using a variety of phenotypic assays is needed. Considering the challenges faced by the healthcare sector due to the co-occurrence of β-lactamases in bacterial pathogens, this study aimed to elucidate the phenotypic detection of co-harbored resistance markers in pathogens associated with UTIs in two tertiary care hospitals in Karachi.

Methodology

Study design and period

A cross-sectional study was conducted at the Department of Microbiology, Basic Medical Science Institute (BMSI), in collaboration with the Department of Urology, Jinnah Postgraduate Medical Center (JPMC), and National Medical Centre (NMC), Karachi, Pakistan from 3 January to 26 July 2024. The non-probability convenient sampling technique was applied. The estimated sample size [10] was calculated as 260 with 95% confidence interval and 5% margin of error by using the WHO calculator [11].

Inclusion criteria

The study enrolled hospitalized and community-dwelling patients of both genders who were suspected to have UTI. The UTI cases were divided into two groups: hospital-associated (HA) and community-acquired (CA) UTIs. HA-UTIs are infections that arise when a patient is undergoing medical treatment and must not have existed before the patient was admitted to the hospital. Consequently, HAIs typically manifest more than 48 hours following hospitalization [12]. CA-UTIs are UTIs that develop in the community or within 48 hours after hospital admission [13]. Urine samples were included only if the culture results were clinically significant and accompanied by pyuria, indicating true infection.

Exclusion criteria

Patients with UTIs caused by uropathogens other than Enterobacterales were excluded from this study. Patients who were already on antibiotic therapy were also excluded from this study. Repeat samples were not included in this study. Colonizers and contaminants were excluded from the study to ensure the accuracy of microbiological and clinical correlation.

Ethical approval

Ethical approval was obtained from the Institutional Review Board (IRB) of JPMC (NO F. 2-81/2023-GENL/211/JPMC).

Collection and inoculation of urine samples

Midstream urine was collected in a wide mouth sterile container following aseptic technique. The samples were transported to the lab and were processed for urine detail report (D/R) and culture in accordance with the standard operating procedures [14]. The urine samples were cultured on cystine-lactose-electrolyte-deficient (CLED) agar plates (Oxoid, Hampshire, United Kingdom) at 37 ± 2 °C for 24–48 hours. Growth was observed after the incubation period, and preliminary identification was based on the cultural characteristics, motility, and traditional biochemical tests (triple sugar iron (TSI), citrate, indole, and urea). The desired isolates were further identified by the RapID ONE System (Thermo Fischer Diagnostic, Lenexa, USA) and VITEK 2 Compact (Biomerieux, Marcy-I'Etoile, France) at species level. The VITEK 2 GN test cards (BioMerieux, Marcy-I'Etoile, France) were performed at the National Medical Center (NMC) Karachi. The cards were filled with cell suspension according to the manufacturer's instructions, and the results were interpreted accordingly.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing (Kerby-Bauer disc diffusion) was performed on Muller Hinton agar (MHA) plates (Oxoid, Hampshire, United Kingdom) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [15]. The antibiotics used in this study included ampicillin (AMP; 10 µg), amoxicillin-clavulanic acid (AMC; 20/10 µg), ceftazidime (CAZ; 30 µg), cefepime (FEP; 30 µg), imipenem (IPM; 10 µg), meropenem (MEM; 10 µg), amikacin (AK; 30 µg), gentamicin (CN; 10 µg), nalidixic acid (NA; 30 µg), ciprofloxacin (CIP; 5 µg), nitrofurantoin (F; 300 µg), Fosfomycin (FOT; 200 µg), aztreonam (ATM; 30 µg), ceftriaxone (CRO; 30 µg), cefoxitin (FOX; 30 µg), ceftaroline (CPT; 30 µg),

trimethoprim-sulphamethoxazole (SXT; 1.25/23.75 µg), cefotaxime (CTX; 30 µg), cefazolin (KZ; 30 µg), levofloxacin (LEV; 5 µg), ceftazidime-avibactam (CZA; 30/20 µg), meropenem-veborbactam (MEV; 20/10 µg), temocillin (TEM; 30 µg), cefiderocol (FDC; 30 µg), and ceftolozane-tazobactam (C/T; 30/10 µg). *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as quality control (QC) strains [15].

Screening for ESBLs

Isolates exhibiting zone of growth inhibition ≤ 22 mm for ceftazidime, ≤ 25 mm for ceftriaxone and aztreonam, and ≤ 27 mm for cefotaxime by disc diffusion technique were deemed ESBL producers [15].

Phenotypic detection of ESBL production by double disc synergy test (DDST)

The ESBL producers were phenotypically confirmed using the double-disc synergy test as described previously [16]. Briefly MHA plates were lawned with the standard (0.5 McFarland) bacterial suspension of the isolates. Five antibiotic discs were used, including ceftriaxone (30 µg), ceftazidime (30 µg), aztreonam (30 µg), cefepime (30 µg), and amoxicillin-clavulanic acid (20/10 µg) which served as a source of clavulanic acid. The first 4 antibiotic discs were positioned 15 mm apart from the amoxicillin-clavulanic acid disc, which was placed in the center of the MHA plate. After incubation at 35 °C for 24 hours, ESBL producers were identified by considering an expansion of the zone of inhibition of any 3rd or 4th generation cephalosporin or aztreonam towards amoxicillin/clavulanate. The reference strains of ESBL-positive *Klebisella pneumoniae* (ATCC 700603) and ESBL-negative *E. coli* (ATCC 25922) were used as QC strain [15].

Screening of Amp-C β-lactamase producers

An inhibition zone diameter of less than 18 mm for the cefoxitin (30 µg) disc suggested the potential presence of AmpC β-lactamase production in the tested organism [15].

Phenotypic detection of AmpC β-lactamases by modified three-dimensional test (MTDT)

A 0.5 McFarland standard suspension of *E. coli* ATCC 25922 was prepared and uniformly inoculated onto MHA plates using a sterile cotton swab. A cefoxitin disc (30 µg) was positioned in the center, and 3 cm linear slits were cut 3 mm apart from the disc. Eight to 10 identical colonies of test bacteria were inoculated into the slit using a sterile wire loop, and incubated for 24 hours at 37 °C. The reference strains AmpC-positive *E. coli* MIR-1 and AmpC-negative ATCC *E. coli* 11775 were used as QC strains [17].

Screening of carbapenem-resistant isolates

The isolates were screened for resistance to meropenem and imipenem using the disc diffusion method. A zone of growth inhibition of ≤ 23 mm around meropenem and imipenem indicated carbapenem-resistant isolate [15].

Phenotypic detection of carbapenemase

Carbapenemase production in carbapenem-resistant pathogens was detected by phenotypic assays:

Modified carbapenem inactivation method (mCIM)

mCIM was used for the determination of carbapenemases in carbapenem-resistant isolates in accordance with CLSI guidelines [15]. A loopful (1 µL) of tested organism from an overnight MacConkey agar plate was emulsified in 2 mL trypticase soy broth (TSB, Oxoid, Hampshire, England). The bacterial suspension was vortexed for 10–15 seconds. Then meropenem disc (10 µg) was added aseptically into TSB bacterial suspension and incubated at 35 ± 2 °C for 4 hours (± 15 minutes). Before the completion of the 4 hours carbapenem inactivation step, a suspension (0.5 McFarland standards) of mCIM indicator organism *E. coli* (ATCC 25922) was prepared. The bacterial suspension was spread on the MHA plate to make a lawn. The meropenem disc was removed from TSB bacterial suspension with the help of the inoculating loop and placed on the MHA plate, which was incubated at 35 ± 2 °C for 24 hours. A zone of inhibition of less than 15 mm around the meropenem disc was interpreted as carbapenemase positive isolate [15]. *K. pneumoniae* BAA-2146 and *K. pneumoniae* BAA-1706 were used as positive and negative quality control strains [15].

Simplified carbapenem inactivation method (sCIM)

sCIM originated from the mCIM with the improvement of laboratory procedures. The isolate to be tested was smeared directly onto the imipenem and meropenem discs, rather than incubating it in the TSB culture media for 4 hours, as in the case of mCIM. To execute the sCIM, a bacterial suspension of *E. coli* (ATCC 25922) strain of 0.5 McFarland standard suspension was prepared and applied on an MHA plate. The plate was placed to dry for 3–10 minutes. Next, 1–3 overnight colonies of the test organism grown on

MacConkey agar was smeared onto meropenem and imipenem discs, and the side of the disc coated with the test bacteria was placed on the MHA plate. Meropenem and imipenem discs without smearing of test isolate were used as positive control [18]. This plate was incubated at 35 °C for 24 hours. Bacterial strains which produced carbapenemase can hydrolyze meropenem and imipenem and a < 20 mm zone of inhibition around the meropenem and imipenem discs was considered as positive [19].

Phenotypic detection of metallo-β-lactamase by combined disc test (CDST)

The MHA plate was inoculated with the test isolate (0.5 McFarland opacity standards). Two imipenem (10 µg) and 2 meropenem (10 µg) discs were positioned 25 mm apart from each other on the inoculated plate. Ten microliters of 0.5 M EDTA solution were applied to 1 imipenem and 1 meropenem disc. The plates were incubated at 37 °C for 24 hours. When the zone of inhibition of imipenem + EDTA and meropenem + EDTA discs were greater than 7 mm in comparison to imipenem and meropenem alone, the isolate was considered to be a metallo-β-lactamase producer [20].

Lateral flow immunochromatographic (LFI) assay

The carbapenem-resistant K.N.I.V.O detection or K-set (lateral flow immunochromatographic assay, Genobio Pharmaceutical Co, Tianjin, China) was used for qualitative detection of KPC-type, New Delhi metallo-beta-lactamase (NDM)-type, IMP-type, VIM-type, and OXA-48-type-carbapenemases in carbapenem-resistant isolates. The test was performed in accordance with manufacturer's instructions. Briefly, 5 drops of sample treatment solution were added into a microcentrifuge tube. The bacterial colony was taken and dipped into the tube and mixed well. Cassettes A and B were placed horizontally and 50 µL of the mixture was added into each cassette. The results were noted after 10–15 minutes. The presence of one or more red lines in the test area, regardless of the intensity of test line, indicated a positive result of its corresponding carbapenemase [21].

Statistical analysis

The data was analyzed by using IBM SPSS statistics version 27 (IBM Corp, Armonk, NY, USA). Descriptive statistics, including frequencies and percentage, were determined. The association between variables was analyzed using Chi-square and t-tests. $p < 0.05$ was considered statistically significant in the case of Chi-square tests. Additionally, the 95% confidence interval and Youden's index were calculated. Youden's Index was used to evaluate the effectiveness of diagnostic tests.

Results

Demographic distribution

A total of 260 urine samples were collected from both hospitals during the study period, out of which 190 (73.1%) were obtained from HA-UTI and 70 (26.9%) were from CA-UTI cases. A total of 139 urine samples were from JPMC, out of which 111 (79.9%) were HA-UTI and 28 (20.1%) were CA-UTI patients. The total number of samples from the NMC were 121, out of which 79 (65.3%) were from HA-UTI and 42 (34.7%) were from CA-UTI cases. An overall female predominance (175; 67.4%) was observed. Of these 175 female patients; 125 (48.1%) and 50 (19.2%) were HA- and CA-UTI cases, respectively; while males accounted for 65 (25%) in HA and 20 (7.7%) in CA-UTI cases. Most of the patients (55; 21.1%) were in the 21–30 years age group; followed the 31–40 years, and 41–50 years age groups, with 21% and 20%, respectively. There were more females than males across all age categories. The mean age of the patients was 43.06 ± 17.45 years (Table 1).

Table 1. Descriptive statistics of study population (n = 260).

Study population	Female (%)	Male (%)	Total (%)
Age (years)			
Group 1 (12–20)	14 (5.4)	7 (2.7)	21 (8)
Group 2 (21–30)	45 (17.3)	10 (3.8)	55 (21.1)
Group 3 (31–40)	34 (13.1)	20 (7.7)	54 (21)
Group 4 (41–50)	32 (12.3)	20 (7.7)	52 (20)
Group 5 (51–60)	32 (12.3)	5 (2)	37 (14.2)
Group 6 (61–70)	21 (8.1)	6 (2.3)	27 (10.4)
Group 7 (71–80)	9 (3.5)	1 (0.3)	10 (3.8)
Group 8 (> 80)	3 (1.2)	1(0.3)	4 (1.5)
Mean age ± standard deviation			43.06 ± 17.45
Gender	175 (67.4)	85 (32.6)	260 (100)
Source			
Hospital associated (HA)	125 (48.1)	65 (25)	190 (73.1)
Community acquired (CA)	50 (19.2)	20 (7.7)	70 (26.9)

Table 2. Distribution of mono and poly-uropathogens growth positive isolates (n = 240).

Isolates	Positive cases		Isolates (%)
	HA-UTI[a] (%)	CA-UTI [a] (%)	
Mono-culture growth			
E. coli	100 (62)	21 (47)	121 (50.4)
K. pneumoniae	25 (15)	15 (33.3)	40 (17)
P. mirabilis	1 (0.6)	2 (4.4)	3 (1.3)
E. cloacae complex	3 (1.9)	ND	3 (1.3)
K. oxytoca	2 (1.2)	ND	2 (0.8)
C. freundii	1 (0.6)	1(2.2)	2 (0.8)
C. werkmanii	1 (0.6)	ND	1(0.4)
P. rettgeri	1 0.6	ND	1(0.4)
P. aeruginosa	1 (0.6)	ND	1(0.4)
Poly-microbial growth			
E. coli + *Candida*	6 (4)	ND	12 (5)
E. coli + *P. aeruginosa*	2 (1.2)	ND	4 (1.6)
E. coli + *Staphylococcus* sp.	2 (1.2)	ND	4 (1.6)
K. pneumoniae + *Enterococci*	ND	2 (4.4)	4(1.6)
K. pneumoniae +*Staphylococcus* sp.	ND	2 (4.4)	4 (1.6)
K. pneumoniae + *P. aeruginosa*	ND	2 (4.4)	4 (1.6)
Candida sp. + *Staphylococcus* sp.	2 (1.2)	ND[b]	4 (1.6)
Staphylococcus sp.+ *Candida sp.*	2 (1.2)	ND	4 (1.6)
P. aeruginosa, Staphylococcus sp.	8 (5)	ND	16 (7)
Staphylococcus sp.+ *A. baumannii*	2 (1.2)	ND	4 (1.6)
Staphylococcus aureus + *Enterococci*	3 (1.9)	ND	6 (2.5)
Total	162 (100)	45 (100)	240 (100)

[a]Hospital associated urinary tract infection; [b]Not detected. HA-UTI: hospital acquired urinary tract infection; CA-UTI: community acquired urinary tract infection.

Frequency of Enterobacterales species

Out of the 260 samples analyzed, 207 (80%) were positive for growth. A total of 240 uropathogens were isolated from these growth positive samples. Among the 240 uropathogens, 189 (79%) belonged to Enterobacterales, while 15 (6%) were non-Enterobacterales Gram negative rods. *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Burkholderia cepacia* complex were the notable non-Enterobacterales. Moreover, 26 (11%) were Gram-positive and 10 (4%) were *Candida* species (Table 2).

Among the 189 Enterobacterales isolates, the most common uropathogen was *E. coli* (131; 69.3%), followed by *Klebsiella pneumoniae* (46; 24.3%), *Proteus mirabilis* (3; 1.6%), *Enterobacter cloacae* (3; 1.6%), *Klebsiella oxytoca* (2; 1.1%), *Citrobacter freundii* (2; 1.1%), *Citrobacter werkmanii* (1; 0.5%), and *Providencia rettgeri* (1; 0.5 %). 70 *E. coli* (53%) isolates were obtained from HA-UTI cases from JPMC, while 40 *E. coli* isolates (31%) were obtained from HA-UTI cases from NMC. *K. oxytoca*, *E. cloacae* complex, *C. freundii*, *C. werkmanii*, and *P. rettgeri* were not found in CA-UTI cases (Table 3).

Species identification

Species identification was carried out using two distinct phenotypic methods: the Remel RapID ONE system and the VITEK 2 GN cards system. These methods were compared to evaluate their accuracy and reliability in correctly identifying bacterial species. In the case of *E. coli*, both systems showed a perfect identification rate, with 100% correct identification achieved by both systems. However, a notable discrepancy was observed when identifying members of the *E. cloacae* complex, which include a group of closely related species. While the Remel RapID ONE system successfully identified only 20% of these strains; the VITEK 2 GN system demonstrated a significantly higher accuracy, correctly identifying 100% of the *E. cloacae* complex strains. The VITEK 2 GN system also provided higher reliability, with a probability of correct identification reported at 93%,

Table 3. Frequency of Enterobacterales species (n = 189).

Enterobacterales	JPMC HA (%)	NMC HA (%)	JMPC CA (%)	NMC CA (%)	Total 189 (%)
E. coli	70 (53)	40 (31)	15 (11)	6 (5)	131(69.3)
K. pneumoniae	20 (43)	5 (11)	5 (11)	16 (35)	46 (24.3)
K. oxytoca	2 (100)	0	0	0	2 (1.1)
C. freundii	2 (100)	0	0	0	2 (1.1)
C. werkmanii	0	1 (100)	0	0	1 (0.5)
E. cloacae complex	3 (100)	0	0	0	3 (1.6)
P. mirabilis	1(33.3)	0	2(66.7)	0	3 (1.6)
P. rettgeri	1 (100)	0	0	0	1 (0.5)
Total	99(52)	46(24)	22(12)	22(12)	189(100)

CA: community acquired; JPMC: Jinnah Postgraduate Medical Center; HA: hospital acquired; NMC: National Medical Center.

95%, and 99% in various instances, further underscoring its superior performance in this particular case. This study followed the results of VITEK 2GN. A discrepancy was observed in the identification of species within the genus *Enterobacter*. The Remel RapID ONE system identified *Cronobacter sakazakii*, *K. pneumoniae*, and *P. mirabilis;* while the VITEK 2 GN system classified all these species under the *E. cloacae* complex. Additionally, VITEK 2 identified two *K. pneumoniae* strains as *K. oxytoca* and one as *E. coli*. The Remel RapID ONE system identified *P. mirabilis*, but VITEK 2 classified it as *Providencia rettgeri*. Furthermore, VITEK 2 identified *C. werkmanii* in place of *C. freundii* (Table 4).

Antimicrobial resistance pattern of Enterobacterales

The majority of isolates showed resistance to several antibiotics, with the highest resistance rates observed in HA-UTI. The Chi-square test indicated a significant difference in antibiotic resistance between the CA and HA-UTI isolates ($p < 0.05$) in the case of *E. coli*. The Chi-square test revealed insignificant difference in the case of *K. pneumoniae* for some antibiotics including, cefepime, gentamicin, ciprofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole (Table 5A).

K. oxytoca, *E. cloacae* complex, *C. freundii*, *C. werkmanii*, and *P. rettgeri* were only detected in HA-UTI. These isolates exhibited almost 100% susceptibility to cefiderocol and β-lactam/β-lactamase inhibitors (Table 5B).

Phenotypic detection of ESBL producers

Phenotypic detection revealed that 47.1% (89/189) of Enterobacterales isolates were ESBL producers (Figure 1 and Table 6). Of these ESBL producers, 60 (67.4%) and 29 (32.6%) isolates were HA-UTI and CA-UTIs, respectively. ESBL producing Enterobacterales uropathogens were prevalent (60; 67%) in HA-UTI isolates. Out of these 60 ESBL producers, 45 (75%) were *E. coli.*

Phenotypic detection of AmpC-β-lactamases

AmpC producing uropathogens were detected at a

Table 4. Comparison of species identification by two phenotypic methods.

Sample No.	remel RapID ONE	Probability of identification	VITEK 2 GN	Probability of identification
004-SB	*E. cloacae* complex	> 99.9%	*E. cloacae* complex	99%
11-SB	*K. pneumoniae*	> 99.9%	*K. a pneumoniae*	99%
30-SB	*K. pneumoniae*	97.70%	*K. pneumoniae*	98%
38-SB	*E. coli*	> 99%	*E. coli*	99%
51-SB	*K. pneumoniae*	> 99%	*K. pneumoniae*	99%
55-SB	*K. pneumoniae*	95%	*E. coli*	96%
63-SB	*K. pneumoniae*	95%	*K. pneumoniae*	99%
78-SB	*P. mirabilis*	95%	*E. cloaece* complex	95%
94-SB	*Corona sakazakii*	> 99.9%	*E. cloacae* complex	99%
99-SB	*E. coli*	92%	*E. coli*	93%
101-SB	*K. pneumoniae*	98%	*E. cloaece* complex	95%
108-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
117-SB	*K. pneumoniae*	99%	*K. pneumoniae*	98%
135-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
150-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
163-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
164-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
189-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
192-SB	*P. mirabilis*	92%	*Providencia rettgeri*	99%
196-SB	*K. pneumoniae*	98.30%	*K. pneumoniae*	99%
203-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	98%
210-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
213-SB	*K. pneumoniae*	> 99.9%	*K. pneumoniae*	99%
223-SB	*E. coli*	99%	*E. coli*	99%
261-SB	*C. freundii*	> 99%	*C. freundii*	99%
288-SB	*C. freundii*	> 99%	*C. freundii*	99%
296-SB	*K. pneumoniae*	99%	*K. pneumoniae*	99%
309-SB	*E. coli*	99%	*E. coli*	97%
310-SB	*K. pneumoniae*	99%	*K. pneumoniae*	93%
386-SB	*K. pneumoniae*	99%	*K. oxytoca*	98%
387-SB	*K. pneumoniae*	98%	*K. pneumoniae*	99%
389-SB	*K. pneumoniae*	99%	*K. pneumoniae*	995
390-SB	*E. coli*	99%	*E. coli*	97%
397-SB	*C. fraundii*	99%	*C. werkmannii*	99%
17-SB	*P. mirabilis*	99%	*P. mirabilis*	99%
17-SB	*P. mirabilis*	99%	*P. mirabilis*	99%
17-SB	*P. mirabilis*	99%	*P. mirabilis*	99%
401-SB	*K. pneumoniae*	99%	*K. oxytoca*	98%

Table 5A. Antimicrobial resistance patterns of Enterobacterales uropathogens (n = 189).

Antibiotics	*E. coli*		*p*	*K. pneumoniae*		*p*	*P. mirabilis*	
	HA n (%)	CA n (%)		HA n (%)	CA n (%)		HA n (%)	CA n (%)
Ampicillin	99 (99)	12 (39)	0.00	29 (97)	5 (31)	0.00	1 (100)	2 (100)
Amoxicillin / clavulanic acid	85 (85)	14 (45)	0.00	9 (30)	2 (6.2)	0.02	1 (100)	2 (100)
Cefazolin	80 (80)	25 (81)	0.00	24 (80)	2 (6.2)	0.00	1 (100)	1 (50)
Cefuroxime	90 (90)	11 (35)	0.00	26 (86.6)	2 (6.2)	0.00	1 (100)	2 (100)
Ceftaroline	32 (32)	2 (6.4)	0.00	30 (100)	14 (88)	0.01	1 (100)	2 (100)
Ceftriaxone	92 (92)	0 (0)	0.00	12 (40)	0 (0)	0.00	1 (100)	1 (50)
Ceftazidime	50 (50)	16 (52)	0.00	30 (100)	5 (31.2)	0.00	1 (100)	2 (100)
Cefepime	32 (32)	2 (6.4)	0.00	30 (100)	10 (62.5)	0.11	1 (100)	2 (100)
Cefoxitin	34 (34)	2 (6.4)	0.00	30 (100)	3 (18.7)	0.03	0	0
Ciprofloxacin	39 (39)	10 (32.2)	0.02	30 (100)	15 (94)	0.22	0	0
Levofloxacin	39 (39)	10 (32.2)	0.02	24 (80)	9 (56)	0.05	0	0
Nalidixic acid	55 (55)	20 (65)	0.00	23 (76.6)	3 (18.7)	0.02	0	0
Amikacin	48 (48)	11 (35.4)	0.00	13 (43.3)	2 (12.5)	0.02	0	0
Gentamicin	48 (48)	11 (35.4)	0.00	13 (43.3)	3 (18.7)	0.07	0	0
Aztreonam	56 (56)	10 (32.2)	0.00	30 (100)	9 (56)	0.03	0	2 (100)
Fosfomycin	48 (48)	2 (6.4)	0.00	NA	NA	NA	NA	NA
Nitrofurantoin	28 (28)	2 (6.4)	0.00	10 (33)	6 (38)	0.64	NA	NA
Meropenem	24 (24)	6 (10.1)	0.00	19 (63.3)	7 (43.7)	0.00	0 (0)	0
Imipenem	24 (24)	6 (10.1)	0.00	19 (63.3)	7 (43.7)	0.00	0 (0)	0
Temocillin	39 (39)	2 (6.4)	0.00	13 (43.3)	2 (12.5)	0.02	1 (100)	0
Trimethoprim-sulfamethoxazole	89 (89)	27 (87)	0.00	30 (100)	16 (100)	0.07	1 (100)	2 (100)
Piperacillin/Tazobactam	30 (30)	7 (23)	0.00	18 (60)	2 (12.5)	0.01	0	0
Cefiderocol	39 (39)	2 (6.4)	0.00	15 (50)	0 (0)	0.00	1 (100)	0
Meropenem-veborbactam	21 (21)	2 (6.4)	0.00	15 (50)	0 (0)	0.00	0	0
Ceftazidime-avibactam	21 (21)	2 (6.4)	0.00	15 (50)	0 (0)	0.00	0	0
Ceftolozane-tazobactam	21 (21)	2 (6.4)	0.00	15 (50)	0 (0)	0.00	0	0

NA: not applicable because of no recommendations by CLSI; HA: hospital acquired; CA: community acquired.

Table 5B. Antimicrobial resistance patterns of isolates of hospital associated (HA)-UTI samples.

Antibiotics	*K. oxytoca*	*E. cloacae*	*C. freundii*	*C. werkmanii*	*P. rettgeri*
	n (%)	n (%)	n (%)	n (%)	n (%)
Ampicillin	2 (100)	3 (100)	1 (50)	1 (100)	1 (100)
Amoxicillin / clavulanic acid	2 (100)	3 (100)	1 (50)	1 (100)	1 (100)
Cefazolin	2 (100)	2 (67)	1 (50)	1 (100)	1 (100)
Cefuroxime	2 (100)	2 (67)	1 (50)	1 (100)	1 (100)
Ceftaroline	2 (100)	3 (100)	1 (50)	1 (100)	1 (100)
Ceftriaxone	1 (50)	2 (67)	1 (50)	1 (100)	1 (100)
Ceftazidime	2 (100)	2 (67)	1 (50)	1 (100)	1 (100)
Cefepime	2 (100)	3 (100)	2 (100)	1 (100)	1 (100)
Cefotaxime	2 (100)	3 (100)	2 (100)	1 (100)	1 (100)
Cefoxitin	1 (50)	0	0	0	0
Aztreonam	1(50)	3 (100)	1 (50)	1 (100)	1 (100)
Nitrofurantoin	1 (50)	2 (67)	1 (50)	0	NA
Meropenem	0	2 (67)	0	1 (100)	0
Imipenem	0	2 (67)	0	1 (100)	0
Temocillin	0	2 (67)	0	0	0
Trimethoprim-sulfamethoxazole	2 (100)	3 (100)	2 (100)	1 (100)	1 (100)
Piperacillin/Tazobactam	0	2 (67)	0	0	0
Cefiderocol	0	2 (67)	0	0	0
Meropenem-veborbactam	0	1 (33)	0	0	0
Ceftazidime-avibactam	0	1 (33)	0	0	0
Ceftolozane-tazobactam	0	1 (33)	0	0	0

UTI: urinary tract infection.

very low rate (10; 5.3%). Out of these AmpC producing isolates, 6 (4.6%) were *E. coli* and 4 (8.7%) were *K. pneumoniae*.

Phenotypic detection of carbapenem-resistant Enterobacterales (CRE)

Phenotypic detection revealed that 31.2% (59/189) of Enterobacterales were CRE (Table 6). Of these CRE, 19 (76%) and 7 (33%) *K. pneumoniae* were obtained from HA-UTI and CA-UTIs cases, respectively. There were 24 (22%) and 6 (29%) carbapenem resistant *E. coli* among HA-UTIs and CA-UTIs respectively. The *E. cloacae* complex (2; 66.6%) and *C. werkmanii* (1; 100%) were only found in HA-UTI cases and exhibited resistance to carbapenem antibiotics.

Table 6. Comparison of ESBL, AmpC producing, and CRE isolates from HA-UTI and CA-UTI.

Organisms	HA-UTI (%)		Total (%)	CA-UTI (%)		Total (%)	*p* value*
	Positive	Negative		Positive	Negative		
ESBL n = 89							
E. coli	45 (41)	65 (59)	110 (100)	6 (29)	15 (71)	21 (100)	0.002
K. pneumoniae	13 (52)	12 (48)	25 (100)	21 (100)	0 (0)	21 (100)	0.077
P. mirabilis	1 (100)	0 (0)	1 (100)	2 (100)	0 (0)	2 (100)	1.0
P. rettgeri	1 (100)	0 (0)	1 (100)	ND	ND	ND	NA
CRE n = 59							
E. coli	24 (22)	86 (78)	110 (100)	6 (29)	15 (71)	21 (100)	0.52
K. pneumoniae	19 (76)	6 (24)	25 (100)	7 (33)	14 (67)	21 (100)	0.003
E. cloacae complex	2 (67)	1 (33)	3 (100)	ND	ND	ND	NA
C. werkmanii	1 (100)	0 (0)	1 (100)	ND	ND	ND	NA
Total							

HA: hospital acquired; CA: community acquired; UTI: urinary tract infection; ESBL: extended spectrum β-lactamase; CRE: carbapenem-resistant Enterobacterales; NA: not applicable. *p value significant at ≤ 0.05.

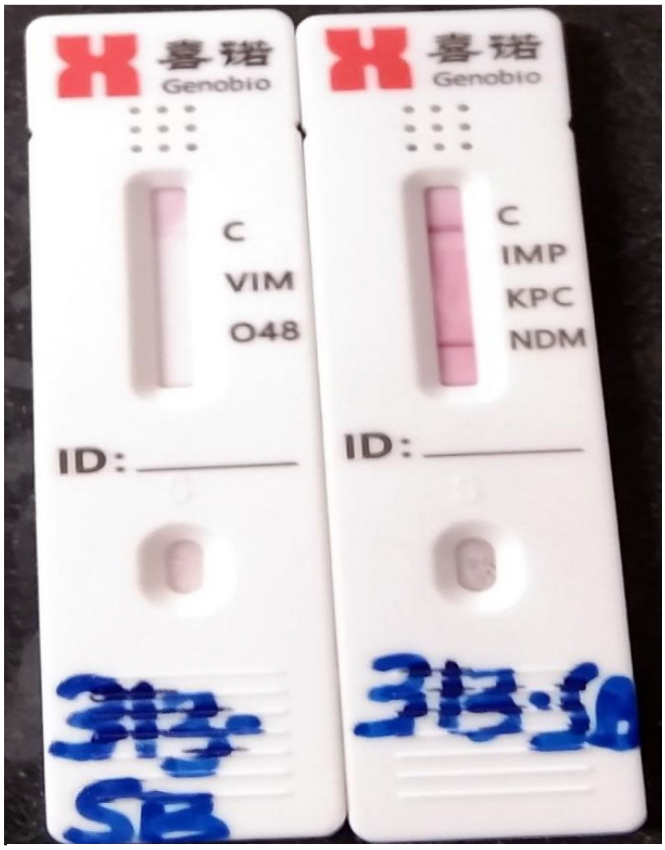
Comparison of ESBL producing and CRE isolates from CA- and HA-UTI

The distribution and comparison of ESBL-producing pathogens in HA-UTI and CA-UTI cases was analyzed by the Chi-square test of independence which assessed whether the distribution of pathogens was independent of the type of UTI (HA-UTI vs. CA-UTI). The *p* values for each pathogen were computed and this determined that there was a statistically

Figure 1. Phenotypic detection of ESBL producing uropathogens by DDST.

ESBL: Extended spectrum β-lactamase; DDST: double disc synergy test.

Figure 2. Lateral Flow Immunochromatographic assay for the detection of carbapenemases



C: control line; KPC: *Klebsiella pneumoniae* carbapenemase; VIM: Verona integron-mediated metallo- β-lactamase; NDM: New Delhi metallo-b-lactamase; OXA: oxacillinase-48-like carbapenemase (OXA-48).

Table 7. Prevalence of metallo-β-lactamase (MBL) producing Enterobacterales (n = 59).

Enterobacterales	MBL		Total n (%)
	Positive n (%)	Negative n (%)	
E. coli	20 (67)	10 (33)	30 (100)
K. pneumoniae	6 (23)	20 (77)	26 (100)
E. cloacae complex	1 (50)	1 (50)	2 (100)
C. werkmanii		1 (100)	1 (100)
Total	27 (45.8)	32 (54.2)	59 (100)

Table 8. Evaluation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of phenotypic tests.

Tests	Enterobacterales (CPE)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
mCIM	30	75.86	100	100	12.5	0.7586
sCIM	41	81.58	100	100	30	0.8158
LFI	41	94.87	100	100	50	0.9487

mCIM: modified carbapenem inactivation method; sCIM: simplified carbapenem inactivation method; LFI: lateral flow immunochromatography assays.

significant relationship between the infection type and the pathogens. ESBL producers were more common in HA-UTI (60; 67.4 %) than in CA-UTI (29; 33%). ESBL producing *K. pneumoniae* was prevalent (13; 52%) in HA-UTI. Similarly, there was a statistically significant association between HA-*K. pneumoniae* and CA-*K. pneumoniae* harboring carbapenem resistance (p = 0.003). The *E. cloacae* complex and *C. werkmannii* were not found in CA-UTI (Table 6).

Prevalence of metallo-β-lactamase producing Enterobacterales

Of the 59 carbapenem-resistant isolates, 20 (67%), 6 (23%), 1 (50%), and 1 (100%) were metallo-β-lactamase producing *E. coli*, *K. pneumoniae*, *E. cloacae* complex, and *C. werkmanii* respectively; as determined by combine disc test (CDT) with EDTA (Table 7).

Prevalence of carbapenemase-producing Enterobacterales

Of 59 carbapenem-resistant isolates, 39 (66%) were from JPMC and 20 (34%) were from NMC. Among these, 41 (69.5%) were carbapenemase producers (CPs) while 18 (30.5%) appeared negative for all the carbapenemases in the test spectrum, including NDM, OXA-48, VIM, IMP, and VIM variants. The most prevalent carbapenemase among the CPs (41) were NDM (33; 80.5%), followed by the OXA-48 (8; 19.5%). Among the 30 carbapenem-resistant *E. coli*, majority (23; 77%) were NDM producers (Figure 2), followed by OXA-48 producers (3; 10%); while 4 (13%) were negative for NDM. Among the 26 carbapenem-resistant *K. pneumoniae*, 9 (35%) were NDM producers, 5 (19%) were OXA-48 producers, and 12 (46%) appeared negative. NDM was detected in only 1 (50%) isolate of the *Enterobacter cloacae* complex. The remaining three enzymes VIM, IMP, and VIM were not present in any isolate (Figure 3).

Performance of carbapenemase detection assays

Of the 59 carbapenem-resistant isolates, carbapenemase production was detected in 30 (50.8%) by mCIM and 41 (69.5%) by sCIM; whereas, 18 (30.5%) were negative for carbapenemase production. The Youden's index of each test was calculated as 0.7586 for mCIM and 0.8158 for sCIM. sCIM detected 41 carbapenemase producing isolates, with 81.58% sensitivity and 100% specificity. LFI also identified 41 carbapenemase-producing organisms with 94.87% sensitivity and 100% specificity and the Youden's Index of LFI was 0.9487 (Table 8).

Co-occurrence of β-lactamases in different Enterobacterales isolates

Out of 158 β-lactamases producing uropathogens, ESBL, AmpC, and CR producing Enterobacterales were 89, 10, and 59 respectively. Co-occurrence of ESBL + AmpC, ESBL + MBL, AmpC + MBL, and ESBL + AmpC + MBL was detected in 4/158 (2.5%), 30/158 (19%), 2/158 (1.2%), and 1/158 (0.6%) isolates, respectively (Table 9).

Figure 3. Prevalence of carbapenemase producing Enterobacterales

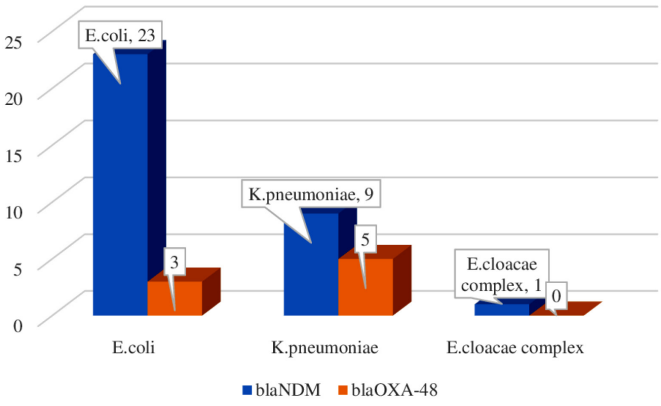


Table 9. Co-existence of β-lactamases in different Enterobacterales species.

Enterobacterales species	ESBL + AmpC (%)	ESBL + MBL (%)	AmpC + MBL (%)	ESBL + AmpC + MBL (%)
E. coli	2 (1.3)	19 (12.1)	1 (0.6)	1 (0.6)
K. pneumoniae	1 (0.6)	10 (6.3)	1 (0.6)	–
E. cloacae complex	1 (0.6)	1 (0.6)	–	–
Total (%)	4 (2.5)	30 (19)	2 (1.2)	1 (0.6)

ESBL: extended spectrum β-lactamase; AmpC: ampicillin C beta-lactamase; MBL: metallo-β-lactamase.

Discussion

UTIs have become a major concern due to the rise of MDR pathogens [22]. The related factors that may have contributed to the global antimicrobial resistance crisis include the rising incidence of infections, inadequate hospitals or healthcare facilities, inadequate infection control procedures, a lack of suitable diagnostic techniques, and clinicians' inclination toward empirical treatment methods [23].

Most of the patients in this study were younger (55; 21.1%), followed by middle, and old age groups. The mean age of the patients was 43.06 ± 17.45 years and this corroborated findings from Pakistan [24]. The proportion of UTIs in female (73.1%) was significantly higher. The potential reasons for this disparity were anatomical differences, hormonal fluctuations, and behavioral aspects [25]. In the current study, *E. coli* was the most prevalent Enterobacterales species in both CA- and HA-UTIs, followed by *K. pneumoniae*; these findings are in agreement with previous studies [26–28]. The prevalence of ESBL-producing and CR isolates were higher in hospitalized patients than CA-UTI cases. Similar findings were reported from Sri Lanka [29]. Around 50% isolates were ESBL producers by phenotypic assay in the present study, which has also been reported in other studies [14,29,30]. ESBL producers were more prevalent (97%) in 5 sites in the US [31]. This was often driven by factors such as overuse of antibiotics, especially in healthcare settings; recent hospitalizations; prior antibiotic exposure; and the ability of these bacteria to rapidly share resistance genes through plasmids, facilitating their swift spread both in the community and within hospitals. Resource constraints in healthcare settings hinder the implementation of effective infection control and antimicrobial stewardship programs. Barriers to implement antibiotic surveillance include lack of trained personnel, inadequate funding, and insufficient diagnostic facilities, leading to overuse and misuse of antibiotics [32]. Clinicians often resort to empirical treatment due to delays in obtaining microbiological results. This practice can contribute to the overuse and misuse of antibiotics, further promoting the development of resistance [32]. AmpC producers were at lower prevalence (5.3%) in this study; similar trends were reported earlier with a prevalence of 7.8–9% [33,34]. The present study revealed 31.2% carbapenem resistance which was close to the 28.4% resistance to imipenem and 37.2% to meropenem reported in Nigeria [35]. The emergence of ESBL and CRE has been reported to be higher in Karachi [23], as was affirmed in the present study and corroborated another study from Central Texas [36]. The current study identified 69.4% carbapenemase producing organisms; and a similar finding was reported from Eastern Romania [37]. This study revealed that sCIM demonstrated higher accuracy, along with excellent sensitivity, specificity, and Youden index. These findings were consistent with those of a study conducted in China by [38], which also reported that sCIM was superior to mCIM. In the present study, rapid immune-chromatographic assay had good sensitivity and specificity as many other studies had showed 100% sensitivity and specificity [20,39,40]. The findings in this study revealed 56% NDM and 14% OXA-48 in this geographical area, which was in agreement with a study conducted at Mayo hospital, Lahore [41]. This study also revealed co-occurrence of ESBL + MBL, ESBL + AmpC, AmpC + MBL, and ESBL + AmpC + MBL; and this was supported by another recent study [7]. The in vitro antibiotic susceptibility of newly launched antibiotics, including temocillin, cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam were assessed in this study. These antibiotics exhibited low resistance rates and have been approved as effective alternatives for treating ESBL, AmpC, and carbapenemase producing Enterobacterales uropathogens, as highlighted by [42].

Conclusions

This study revealed that there is a high prevalence of ESBL-producing uropathogens, followed by carbapenemase producers, in UTIs; AmpC β-lactamase strains were comparatively infrequent. Among CRE, NDM emerged as the predominant resistance determinant, followed by OXA-48-like enzymes. Co-expression of ESBL and NDM enzymes within Enterobacterales isolates represented the most commonly encountered resistance phenotype. Additionally, the study substantiated the diagnostic utility of the lateral flow immunoassay (LFI), demonstrating its complete concordance with the World Health Organization's Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users (ASSURED) criteria [43] encompassing affordability, sensitivity, specificity, user-friendliness, rapidity, equipment-free operation, and deliverability to end users. The LFI platform also offers the advantage of long-term stability under ambient storage conditions, further supporting its applicability in diverse healthcare settings, including those with limited laboratory infrastructure.

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Conflict of interest

No conflict of interest is declared.

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