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## Article

# Mapping Phenotypic and Genotypic Antimicrobial Susceptibility Profile of *E. coli* and *K. pneumoniae* in Complicated Urinary Tract Infection in the Era of Mounting Drug Resistance

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**Abstract: Background:** Mapping the local etiology and antimicrobial susceptibility profile of pathogens causing complicated urinary tract infection (cUTI) is important for promoting evidence based antimicrobial prescribing. WGS plays an important role in genomic surveillance of AMR and understanding circulating clades. **Methods:** Antimicrobial susceptibility profile of patients presenting with cUTI were analysed. ESBLs, AmpC  $\beta$ -lactamases, and CRE were detected by combined disc test (ESBL), D69C AmpC detection set (AmpC) and KPC/IMP/NDM/VIM/OXA-48 Combo test kit (CRE). Whole genome sequencing (WGS) was carried out in eleven *E. coli* AmpC and twenty-two *K. pneumoniae* CRE isolates. Bioinformatic analysis was performed using online tools. **Results:** The susceptibility patterns of *E. coli* and *K. pneumoniae* were as follows: nitrofurantoin (96%, 38%), fosfomycin (100%, 89%), fluoroquinolones (44%, 47%), aminoglycosides (93%, 82%), piperacillin-tazobactam (95%, 72%) and carbapenems (98%, 83%). The overall prevalence of ESBL among *E. coli* and *K. pneumoniae* was 37.2%, while the estimated prevalence of AmpC was 5.4% and the prevalence of CRE was 6.2%. *E. coli* was the predominant ESBL and AmpC producer, whereas *K. pneumoniae* was the major CRE producer. Nine *E. coli* MLST lineages were identified: ST-10, ST-69, ST-77, ST-131, ST-156, ST-167, ST-361, ST-1125 and ST-2520, while in *K. pneumoniae* 5 MLST lineages were observed: ST-2096, ST-231, ST-147, ST-1770 and ST-111. ST-2096 (12 isolates) and ST-147 (7 isolates) predominated. WGS revealed DHA-1 as the predominant plasmid-mediated AmpC gene, while OXA-232 and NDM-5 as the most common carbapenemase genes. All *E. coli* DHA-1 positive isolates co-harboured the quinolone resistance gene qnrB4 and the sulfonamide resistance gene sul1 while no aminoglycoside resistance genes were detected. The majority of CRE *K. pneumoniae* carried other  $\beta$ -lactamase genes such as blaCTX-M-15, blaSHV, blaTEM, and all co-harboured the quinolone resistance gene OqxAB and 77% carried the aminoglycoside resistance gene armA. **Conclusions:** Fosfomycin is an excellent choice for treating complicated cystitis caused by multi drug resistant pathogens. Nitrofurantoin retains excellent activity against *E. coli* but not *K. pneumoniae*. Aminoglycosides and piperacillin tazobactam are alternatives which spare carbapenems. WGS results revealed DHA-1 was the predominant AmpC while OXA-232 and NDM-5 were the circulating carbapenemases. In AmpC producing *E. coli* no MLST predominated while in CRE *Klebsiella pneumoniae* ST-2096 and ST-147 predominated.

**Keywords:** *E. coli*; *K. pneumoniae*; complicated UTI; whole genome sequencing; AMR

## 1. Introduction

Urinary tract infections (UTIs) rank among the most prevalent bacterial infections worldwide, affecting millions annually and imposing significant healthcare and economic burdens (Kot et al., 2021; López-Montesinos & Horcajada, 2019). Complicated urinary tract infections (cUTIs) are particularly challenging to treat due to their association with risk factors such as anatomical abnormalities, immunosuppression, and the presence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens (Wagenlehner et al., 2020 & Walkty et al., 2022). MDR is defined as acquired non-susceptibility to  $\geq 1$  agent in three or more different classes of antimicrobials while XDR is defined as being non-susceptible to  $\geq 1$  agent in all but remaining susceptible to two or fewer antimicrobial classes (Magiorakos et al., 2012). These infections are predominantly caused by Gram-negative bacilli, including *Escherichia coli* and *Klebsiella pneumoniae*, which frequently exhibit varying levels of resistance through mechanisms such as extended-spectrum beta-lactamase (ESBL) production, AmpC beta-lactamase, and carbapenemase activity. (Zilberberg et al., 2020; Ruppé et al., 2015).

The lack of diagnostic precision in identifying the resistance markers often leads to inappropriate antibiotic prescriptions, further fueling resistance. This necessitates routine identification of the resistance mechanisms so that appropriate antimicrobials can be prescribed to ensure optimum patient care while also applying principles of antimicrobial stewardship and utilising reserve antibiotics judiciously. This study evaluated the susceptibility profile of *E. coli* and *Klebsiella pneumoniae* isolated from cUTI to inform practice, identify cheap, simple and reliable tools to identify ESBL, AmpC and carbapenemase resistant Enterobacterales (CRE) and to understand the circulating MLST and AMR genes in AmpC and carbapenemase producing strains by whole genome sequencing representative isolates.

## 2. Materials and Methods

### 2.1. Study Period and Setting

This study was conducted from September 2022 to August 2023 at the Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman. It was carried out in collaboration with Clinical Microbiology Laboratories at Sultan Qaboos University Hospital (SQUH) and Suhar Hospital, Oman. Ethical approval was obtained from the Medical Research Ethics Committee (MREC), College of Medicine & Health Sciences, Sultan Qaboos University (REF. NO. SQU-EC/377/2021) and the Health Studies and Research Approval Committee, Ministry of Health (MoH/CSR/21/24496).

### 2.2. Study Design

The study evaluated the demographic, and antimicrobial susceptibility profiles of *E. coli* and *K. pneumoniae* isolates from complicated urinary tract infection (cUTI) cases over a one year period.

### 2.3. Bacterial Identification and Susceptibility Testing

Bacterial identification was performed using MALDI-TOF MS (Bruker, Germany) and Phoenix™ (BD Diagnostics, USA) automated systems at SQUH, while VITEK 2 (Biomérieux) was employed at Suhar Hospital as per standard guidelines (Patel et al., 2015; Funke et al., 2005; Singhal et al., 2015). Antimicrobial susceptibility testing was performed and interpreted following CLSI guidelines (2022) (CLSI, M100- ed32., 2022). The automated systems identified the isolates as ESBLs. Carbapenem resistant isolates were subjected to GeneXpert® Carba-R (Cepheid, Sunnyvale, CA) for confirmation of carbapenemase production, while AmpC was estimated on the susceptibility profile.

#### 2.4. Manual Phenotypic Detection of ESBL, AmpC and CRE

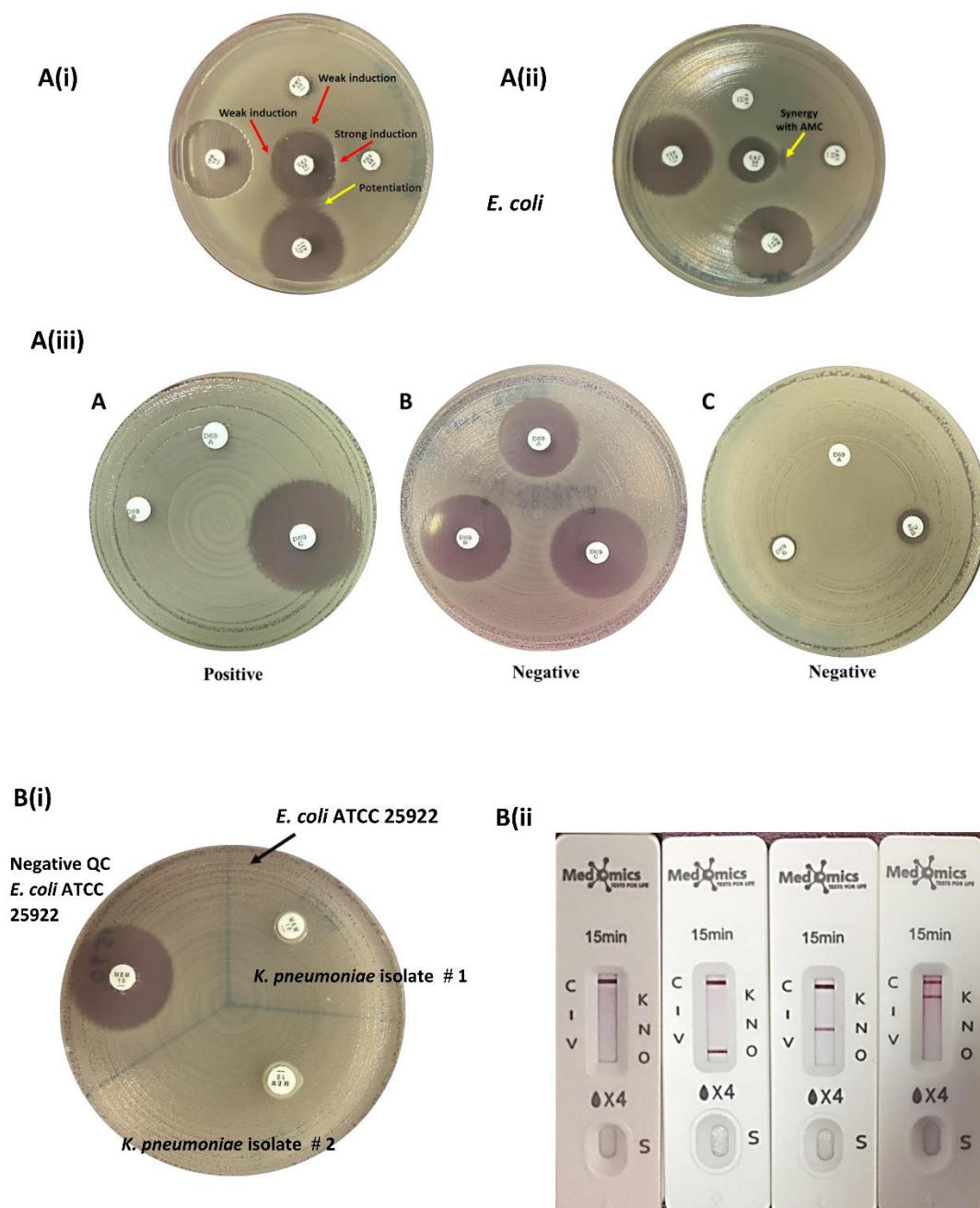
**ESBL Detection:** ESBLs were detected by the double-disk synergy tests. Amoxicillin-clavulanate (AMC, 20/10 µg) (Oxoid, UK) was placed at the center of MHA and the β-lactam antibiotic discs, ceftazidime (CAZ, 30 µg) (Oxoid, UK), cefepime (FEP, 30 µg) (Oxoid, UK), aztreonam (ATM, 30 µg) (Oxoid, UK), cefotaxime (CTX, 30 µg) (Oxoid, UK), cefuroxime (CXM, 30µg) (Oxoid, UK), ceftiofur (FOX, 30 µg) ceftriaxone (CRO, 30 µg) (Oxoid, UK), cefoperazone (CFP, 75 µg) (Liofilchem, Italy) and cefpodoxime (CPD, 30 µg) (Liofilchem, Italy) were placed at a distance of 20mm (edge to edge) from the BL/BLI disk. Plates were incubated at 35 ± 2 °C for 18-24 hours and after incubation, the synergistic activity between BL/BLIs and β-lactam antibiotics were examined. Isolates were phenotypically confirmed as ESBL producers when a clear extension of the edge of the inhibition zone of β-lactam antibiotics was observed towards at least one of the BL/BLI disks or when a zone of inhibition appeared in between β-lactam antibiotic and BL/BLI disk (Waheed et al., 2019; Drieux et al., 2008).

**AmpC Detection:** Disk approximation test: A ceftazidime disk (CAZ, 30 µg) (Oxoid, UK) was placed in the center of the inoculated MHA and disks of the inducing substrates: imipenem (IPM, 10 µg), amoxicillin-clavulanic acid (AMC 20/10 µg) and piperacillin-tazobactam (TZP 110 µg) were placed at a distance of 20 mm apart (edge to edge) from a ceftazidime disk, while the distance between ceftiofur (FOX, 30 µg) and ceftazidime disk was adjusted to 15mm apart (edge to edge) to obtain better induction effect (Gupta et al., 2014; Al Mamari et al., 2023). The plate was incubated at 35°C ± 2 for 18-24 hours. Figure 1

AmpC detection set (D69C): This is a commercial AmpC detection set from Mast Group Ltd, UK was used for the detection of AmpC β-lactamase enzyme production in 16 isolates. The set contained three cartridges A, B and C. The disk content of cartridge A is cefpodoxime (10 µg) with AmpC inducer while in cartridge B the disk contains cefpodoxime (10 µg) with AmpC inducer and ESBL inhibitor, and in cartridge D the disk composed of cefpodoxime (10 µg) with AmpC inducer, ESBL inhibitor and AmpC inhibitors. The inducers and inhibitors in each cartridge were unspecified. The procedure was performed and interpreted as per the manufacturer's instructions (Halstead et al., 2012) Figure 1.

**Carbapenemase Detection:** Modified carbapenem inactivation method (mCIM), (CLSI, M100-ed33., 2023) and a lateral flow immunochromatographic assay KPC/IMP/NDM/VIM/OXA-48 Combo test kit (Medomics, China) for the five major carbapenemase families (KPC, IMP, NDM, VIM, OXA-48) were used for detection of CRE (Figure 1).





**Figure 1.** Disk Approximation test, D69C AmpC detection set for AmpC detection (A), Modified Carbapenem Inactivation Method (mCIM) and Combo test kit for carbapenemase detection (B). Disk Approximation test (A, i), Strong and weak induction (Red arrows) towards the ceftazidime (CAZ) as substrate and imipenem (IPM), ceftazidime (FOX), amoxicillin/clavulanic acid (AMC) and piperacillin/tazobactam (TZP) as inducers. The potentiation between drugs is indicated by (Yellow arrows), (A, ii) Isolate exhibits resistance to ceftazidime rendering AmpC production undetectable by the disk approximation method. Synergistic activity between the substrate (ceftazidime) and amoxicillin-clavulanate is indicated by (Yellow arrows). (A, iii) D69C AmpC detection set. (A) AmpC positive. (B and C) AmpC negative (exhibiting other type(s) of resistance). Disk A (cefpodoxime 10 µg+AmpC inducer), disk B (cefpodoxime 10µg+AmpC inducer+ESBL inhibitor), disk C (cefpodoxime 10 µg+AmpC inducer+ESBL inhibitor+AmpC inhibitors). Modified Carbapenem Inactivation Method (mCIM) (B, i), Identification of carbapenemase-producing strains via mCIM test. The control strain *E. coli* ATCC 25922 was carbapenemase negative, having a zone size of > 19 mm. Two target isolates carried carbapenemase, which broke down the 10 µg meropenem after incubation for 4 h ± 15 min at 35 °C. Combo test kit for carbapenemase detection (B, ii), From left to right – carbapenemase not detected (C= control), OXA 48 detected (O= OAX-48), NDM detected (N= NDM), KPC detected (K=KPC), (V=VIM), (I=IMP).

### 2.5. Whole Genome Sequencing (WGS)

Representative AmpC and CRE *E. coli* and *K. pneumoniae* strains were subjected to WGS. It was carried out in MicrobesNG by Illumina next-generation sequencing (<https://microbesng.co.uk>, Birmingham, United Kingdom). The DNA samples were prepared and then sequenced following the manufacturer's protocol. After sequencing, the samples were assembled using the De Novo assembly by the SPAdes program (Bankevich et al., 2012). The assembled contigs were then annotated by Prokka (Seemann, 2014). The assembled and annotated sequences were uploaded to the website to carry out further analysis.

Detailed resistance gene profiling, with bioinformatics analysis was conducted using Center for Genomic Epidemiology (CGE) website (<https://www.genomicepidemiology.org/>) for identifying multi-locus sequence typing (MLST) using MLST tool (Larsen et al., 2012), plasmids using PlasmidFinder tool (Carattoli et al., 2014) and antibiotic resistance genes using ResFinder tool (Bortolaia et al., 2020).

### 2.6. Statistical Analysis

Data were analyzed using SPSS (version 23). Continuous data were expressed as mean  $\pm$  SD, while categorical variables were presented as percentages. Sensitivity and specificity analyses of diagnostic tests were performed, and results were visualized using Microsoft Excel.

## 3. Results

### 3.1. Demographics and Sample Characteristics

During the study period (September 2022–August 2023), 1,194 non-duplicate urine cultures from patients with suspected complicated urinary tract infections (cUTIs) were analyzed. Of these, 60% were from females (n=712) and 40% from males (n=482). Adults comprised the majority (89%, n=1,066), while 11% (n=128) were pediatric patients. The highest prevalence of cUTIs was observed in patients aged >60 years (47.2%), followed by those aged 30–60 years (32.7%) and  $\leq$ 13 years (10.7%). Key complicating factors included urinary instrumentation, hypertension, diabetes, chronic kidney disease, dyslipidemia, sickle cell anaemia, malignancy, renal stones, urinary obstruction, benign prostatic hyperplasia and thalassemia.

### 3.2. Microbial Etiology

A total of 1,233 bacterial isolates were identified amongst which Gram-negative bacilli predominated with *E. coli* accounting for 406 (33%) and *K. pneumoniae* 163 (13.5%). In midstream urine (MSU) samples, *E. coli* was the most common pathogen in both adults (35.5%) and children (49.4%) while in catheter specimen urine (CSU) samples, *Candida* spp. predominated in adults (41.9%) and *E. coli* in the pediatric age group (31.1%).

### 3.3. Antimicrobial Susceptibility Profiles

*E. coli* exhibited high susceptibility to fosfomycin (100%), nitrofurantoin (96%), piperacillin-tazobactam (95%), gentamicin (87%), amikacin (98%) and meropenem (99%). *K. pneumoniae* had lower susceptibility rates than *E. coli*, with 38% to nitrofurantoin, fosfomycin 89%, gentamicin (80%), amikacin (84%) and 84% for meropenem susceptibilities, as shown in Table 1.

**Table 1.** Susceptibility profile of *E. coli* and *Klebsiella pneumoniae* isolated from patients with cUTI.

	Nitrofurantoin	Cotrimoxazole	Fosfomycin	Ampicillin	Cefazolin	Cefuroxime	Ceftriaxone	Ceftazidime	Cefepime	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin-tazobactam	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Ertapenem	Imipenem	Meropenem
<i>E. coli</i> n=406	96%	63%	100%	29%	27%	51%	54%	57%	56%	66%	65%	95%	87%	98%	44%	46%	98%	98%	99%
<i>K. pneumoniae</i> n=163	38% (157)	65% (162)	89% (119)		33% (92)	55% (161)	61% (161)	60% (162)	63% (163)	65% (77)	52% (103)	72% (163)	80% (163)	84% (163)	47% (133)	48% (136)	81% (146)	83% (163)	84% (163)

Lower susceptibility was observed for fluoroquinolones (ciprofloxacin (44%), levofloxacin (46%) The susceptibility rates to cephalosporins was slightly lower in *E. coli* (cefazolin (27%), cefuroxime (51%), ceftriaxone (54%), ceftazidime (57%), and cefepime (56%)) than in *K. pneumoniae* (cefazolin (33%), cefuroxime (55%), ceftriaxone (61%), ceftazidime (60%), and cefepime (63%)). Similar susceptibility to amoxicillin-clavulanate in *E. coli* and *K. pneumoniae* was observed: 66% and 65% respectively.

### 3.4. Prevalence of ESBL, AmpC Beta-Lactamases, and Carbapenemases

**ESBL:** The overall prevalence of ESBL among *E. coli* and *K. pneumoniae* (n=569) was 37.2%, with *E. coli* accounting for 40.4% and *K. pneumoniae* 20.2%. (Table 2)

**Table 2.** Prevalence of ESBL, AmpC and CRE among *E. coli* and *K. pneumoniae* in patients with cUTI.

Phenotype	<i>E. coli</i> , n (%)	<i>K. pneumoniae</i> , n (%)	Total prevalence among <i>E. coli</i> (n=406) and <i>K. pneumoniae</i> (n=163)
<b>ESBL</b>	164 (40.4%)	33 (20.2%)	197 (34.6%)
<b>AmpC</b>	10 (2.5%)	6 (3.7%)	16 (2.8%)
<b>ESBL+AmpC</b>	14 (3.4%)	1 (0.6%)	15 (2.6%)
<b>CRE</b>	7 (1.7%)	28 (17.2%)	35 (6.2%)
<b>Total</b>	195	68	263

The ESBL prevalence increased to 43.8% in *E. coli* and 20.8% when isolates co-harboured ESBL and AmpC were included.

**AmpC:** AmpC alone was identified in 16 (2.8%) isolates while AmpC and ESBL co-carriage was found in 15(2.6%) cases.

**Carbapenemases:** The overall prevalence of carbapenemase producers was 35 (6.2%). *K. pneumoniae* predominated as a carbapenemase producer, accounting for 17.2% of the total *K. pneumoniae* isolated, compared to *E. coli* 1.7% as seen in Table 2.

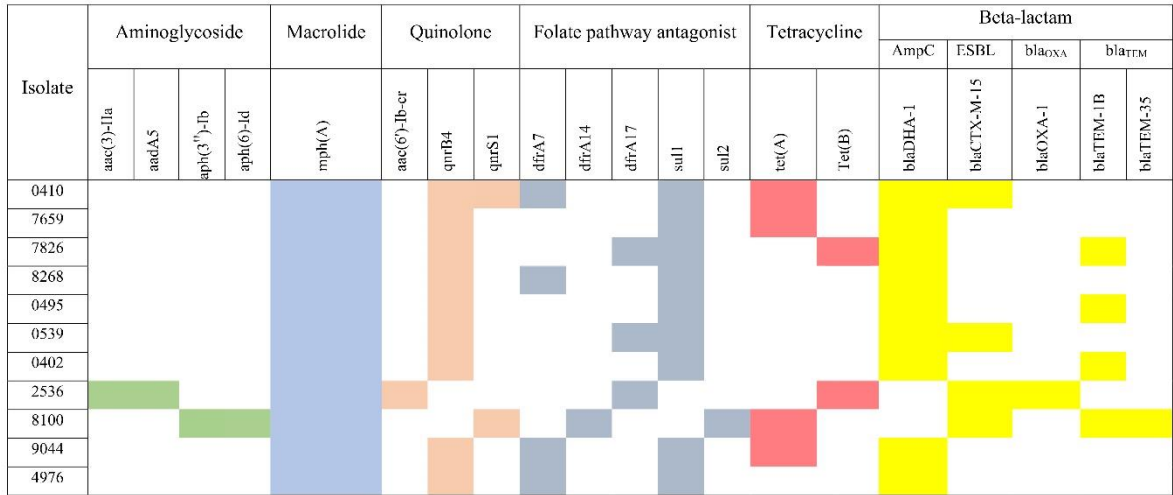
### 3.5. Detection of Resistance Genes in AmpC Beta Lactamase Producing *E. coli* and Carbapenemase Producing *K. pneumoniae*

Diverse antimicrobial resistance genes were detected in the 11 AmpC producing *E. coli* and 22 carbapenemase producing *Klebsiella pneumoniae* isolates.

The MLST analysis of the AmpC strains identified the presence of 9 *E. coli* lineages: ST-10, ST-69, ST-77, ST-131, ST-156, ST-167, ST-361, ST-1125 and ST-2520, with ST-10 and ST-69 being observed in two isolates each.

The beta-lactam resistance genes were detected in all isolates as seen in the heat map (Figure 2). Five different beta-lactam resistance genes were found blaDHA1, blaCTX-M-15, blaOXA-1, blaTEM-1B and blaTEM-35. The AmpC gene blaDHA-1 was detected in 9 isolates. The two isolates, that tested

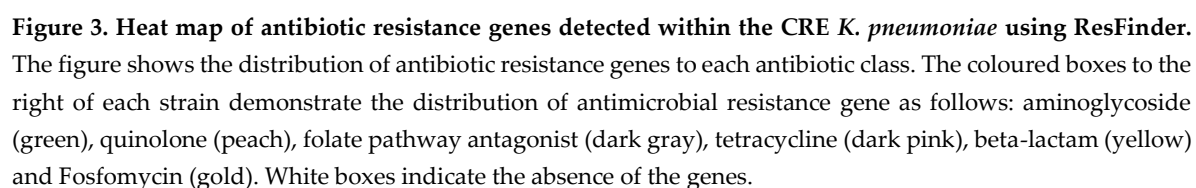
negative for AmpC genes co-harboured blaCTX-M-15 and blaOXA-1, and blaCTX-M-15, blaTEM-1B and blaTEM-35 respectively (Figure 2). Three were resistant to piperacillin-tazobactam *E. coli* 0410, 2536 and 8100 and all co-harboured more than one  $\beta$ -lactamases. However only one carried bla OXA-1. Three different quinolone resistance genes were identified, aac(6')-Ib-cr, qnrB4 and qnrS1. The majority of isolates harboured only one of these quinolone resistance genes and qnrB4 was the predominant gene found in 9 isolates. Regarding ciprofloxacin susceptibility, only five isolates (5/11) remained susceptible and all carried the qnrB4 gene. Among the 11 analysed isolates, only 2 were resistant to cotrimoxazole. The tetracycline resistance genes (tet(A) and tet(B)) were detected in 7 isolates and tet(A) was the most frequent. Only two carried aminoglycoside-resistant genes, *E. coli* 2536 (aac(3)-IIa and aadA5) and *E. coli* 8100 (aph(3'')-Ib and aph(6)-Id). The marA gene which is responsible for antibiotic efflux as well as reduced cellular permeability to antibiotics was detected in the majority of isolates (10/11). The most frequently detected efflux pump genes belonged to resistance-nodulation-cell division (RND) and major facilitator superfamily (MFS) efflux gene families (Supplementary Table S1). Overall, H-NS was the predominant efflux gene detected in all isolates which confers resistance to macrolide, fluoroquinolone, cephalosporin, cephamycin, penam, and tetracycline, followed by evgA (10 isolates), TolC (9 isolates) and emrB (9 isolates). The efflux gene qacEdelta1 was only found in one isolate. Four antibiotic target alteration genes were detected (bacA, PmrF, ugd and eptA) of which PmrF was the predominant gene found in 7 isolates.



**Figure 2. Heat map of antibiotic resistance genes detected within the FOX-resistant *E. coli* using ResFinder.** The figure shows the distribution of antibiotic resistance genes to each antibiotic class. The coloured boxes to the right of each strain demonstrate the distribution of antimicrobial resistance genes as follows: aminoglycoside (green), macrolide (light blue), quinolone (peach), folate pathway antagonist (dark gray), tetracycline (dark pink), beta-lactam (yellow) and fosfomycin (gold). White boxes indicate absence of these genes.

In the 22 carbapenemase producing *K. pneumoniae*, MLST revealed the presence of 5 lineages: ST-2096, ST-231, ST-147, ST-1770 and ST-111 with ST-2096 (12 isolates) and ST-147 (7 isolates) being the most common. The ST-1770 was identified as *Klebsiella quasipneumoniae*. The resistance genes' heatmap using resfinder is shown in Figure 3. The  $\beta$ -lactam resistance genes were the most predominant among all identified antibiotic-resistant genes. In total, 18 different  $\beta$ -lactam resistance genes were found (blaSHV-11, blaSHV-28, blaSHV-40, blaSHV-56, blaSHV-67, blaSHV-89, blaSHV-106, blaTEM-1, blaCTX-M-8, blaCTX-M-15, blaDHA-1, blaOXA-1, blaOXA-9, blaOXA-48, blaOXA-232, blaNDM-5, blaKPC-2 and blaOKP-B-3). Among SHV genes, blaSHV-28 and blaSHV-106 were the most common as only detected in isolates of ST-2096. All isolates of ST-147 carried the blaSHV-11 and blaSHV-67. The blaTEM-1 detected in 72.7% (16/22) of the isolates. All isolates of ST-2096, ST-147 and ST231 carried the ESBL gene blaCTX-M-15. All isolates of ST-2096 carried the blaOXA-1 while the majority (6/7) of isolates of ST-147 harboured blaOXA-9. The blaDHA-1 and blaCTX-M-8,





Patients with cUTIs are at a higher risk of treatment failure, since these infections are frequently caused by MDR Gram-negative bacteria (Li et al., 2017). Mapping susceptibility of uropathogens in cUTI is imperative to institute optimum empiric treatment. *E. coli* and *K. pneumoniae*, the most frequently detected pathogens in UTIs are commonly associated with  $\beta$ -lactamases such as ESBLs, AmpC  $\beta$ -lactamases and carbapenemases (Wang & Palasik, 2022). Optimizing their detection is critical for appropriate management of cUTIs and promoting antimicrobial stewardship.

Our study revealed similar as well as key critical differences in their susceptibility rates. *E. coli* exhibited a higher susceptibility rate to nitrofurantoin (96%) than *K. pneumoniae* (38%). However both displayed excellent rates to fosfomycin (100% vs 89%). Babiker et al.,(2019) too advocated fosfomycin

as a good option for management of UTI. Fosfomycin has a low incidence of collateral damage and a broad antimicrobial spectrum. Both exhibited susceptibility greater than 60% to cotrimoxazole and less than 50% to fluoroquinolones which significantly limits their utility in empiric management. Similar rates were reported by Li et al. (2017). Susceptibility to cephalosporins were slightly lower in *E. coli* [cefazolin (27%), cefuroxime (51%), ceftriaxone (54%), ceftazidime (57%), and cefepime (56%)] than in *K. pneumoniae* [cefazolin (33%), cefuroxime (55%), ceftriaxone (61%), ceftazidime (60%), and cefepime (63%)] which indicates higher prevalence of ESBL in *E.coli* than *K. pneumoniae* which was corroborated by further testing. Mohamed et al. (2020) reported a similar trend.

Conversely the lower piperacillin-tazobactam, aminoglycoside and carbapenem susceptibility rates in *K. pneumoniae* [piperacillin-tazobactam 72%, amikacin (84%), gentamicin (80%), ertapenem (81%), imipenem (83%), and meropenem (84%)] compared to *E. coli* [piperacillin-tazobactam (95%), amikacin (98%) and gentamicin (87%), ertapenem (98%), imipenem (98%), and meropenem (99%)] suggests *K. pneumoniae* is more likely to carry blaOXA-1, aminoglycoside and carbapenem resistance genes. Fernández-Martínez et al., 2018; Abo-State et al., 2018; Arumugam et al., 2024 corroborated these findings.

An interesting epidemiology emerged. *E. coli* ESBL prevalence (43.8%) was double that in *K. pneumoniae* (20.8%). An Egyptian study reported a higher prevalence of ESBL, (62%-69% in *E. coli*, which too was double that of *K. pneumoniae* (30%-37%) (Shash et.al., 2019). AmpC prevalence too was higher in *E. coli* (5.9%) than in *K. pneumoniae* (4.3%). CRE however predominated among *K. pneumoniae* (17.2% vs 1.7% in *E. coli*). AL Mamari et al., 2022 reported similar prevalence in this region. As in other parts of the world, XDR *K. pneumoniae* is a grave public health concern. (Kaza et al., 2024; Chapelle et al., 2021).

A surprisingly pronounced genetic heterogeneity was observed in the 11 AmpC carrying strains, with 9 MLST types observed (ST-10, ST-69, ST-77, ST-131, ST-156, ST-167, ST-361, ST-1125 and ST-2520), indicating the great flux in the *E. coli* clades circulating in this region. Surprisingly all AmpC producing *E. coli* carried blaDHA-1 with only two isolates co-harboring blaCTX-M-15 and three blaTEM-1B. Only ST-167, ST-361, ST-131, and ST-156 have been reported previously from this region (Al-Farsi et al., 2020). Our study highlights the high prevalence of the plasmid-mediated AmpC gene, blaDHA-1, among FOX-resistant *E. coli* isolates in cUTI. Similar high prevalence of blaDHA-1 in uropathogenic *E. coli* have been reported from Egypt, Iran and India although Saudi Arabia reported predominance of CMY-2. Mohamed et al. (2020), (Jomehzadeh et al., 2021), (Mohamudha et al., 2012; Bala et al., 2020) (Abdalhamid et al. 2017).

Surprisingly they did not carry aminoglycoside modifying/resistance genes, supporting the empirical use of gentamicin and amikacin in managing *E. coli* AmpC mediated cUTI infections. On the other hand quinolones should be prescribed with caution as all blaDHA-1 carried the qnrB4 gene. The predominant gene qnrB4 was detected only in AmpC-producing isolates harbouring the blaDHA-1 gene. Similar findings were reported by Kamruzzaman et al. (2013), who found *E. coli* isolates carried both blaDHA-1 and qnrB. These findings were in agreement with Mata et al. (2011) who found a close association between blaDHA-1 and qnrB genes. Our results support these findings as all blaDHA-1 isolates carried the qnrB gene.) The aac(6')-Ib-cr is a multifunctional gene that can induce resistance to both ciprofloxacin and aminoglycosides (Eftekhari et al., 2015).

Much lower MLST heterogeneity was observed in the CRE *K. pneumoniae*, with 5 lineages being identified: ST-2096, ST-231, ST-147, ST-1770 and ST-111 with ST-2096 predominating. These findings are starkly different from a previous study in Oman where the majority of XDR/PDR *K. pneumoniae* belonged to ST-231 (Al-Quraini et al., 2022). Al Fadhli et al., 2023 reported that the ST-14 and ST-231 were the predominant STs in the Arabian Peninsula with ST-231 predominating in Oman and ST-2096 in Saudi Arabia. A study from India found that ST-231 was the most common ST (34.8%) among CRE *K. pneumoniae*, followed by ST-147 (23.5%) and ST-14 (Nagaraj et al., 2021). Maybe uropathogenic *K. pneumoniae* are characterised by different MLSTs compared to other invasive *K. pneumoniae* infections. Further studies are needed in cUTI to delineate any ST predominance.

Among the carbapenemases, *bla*<sub>OXA-232</sub> predominated, followed by *bla*<sub>NDM-5</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>KPC-2</sub>. All isolates of ST-2096 and ST-147 carried the ESBL gene *bla*<sub>CTX-M-15</sub> which reflects the strong association and co-localization of *bla*<sub>OXA-232</sub> and *bla*<sub>NDM-5</sub> with *bla*<sub>CTX-M-15</sub> in these clades, an association also observed in other studies. (Shukla et al., 2023; Shankar et al., 2022; Sundaresan et al., 2022). ST-231 co-harboured ESBL gene *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-232</sub> which was also reported by Al-Quraini et al. (2022) and Mancini et al. (2018). All isolates of ST-2096 carried the *bla*<sub>OXA-1</sub> while the majority (6/7) of isolates of ST-147 harboured *bla*<sub>OXA-9</sub>. Shankar et al. (2022) found that *bla*<sub>OXA-1</sub> integrated in the chromosome of isolates of ST-2096 and Di Pilato et al. (2022) reported *bla*<sub>OXA-1</sub> and *bla*<sub>OXA-9</sub> in ST-147. *K. pneumoniae* ST-2096 predominantly carried OXA-232 carbapenemase, a frequent variant of OXA-48-like carbapenemase in Oman while ST-147 predominantly carried NDM-5. Studies from Qatar and India corroborate our findings. (Abid et al., 2021) (Shukla et al., 2023). Surprisingly ST111 co-harboured *bla*-DHA-1 with *bla*<sub>CTX-M-8</sub> but did not possess carbapenemases. It was interesting to note that all the other carbapenemase carrying ST types did not carry AmpC beta lactamases. *Klebsiella quasipneumoniae* ST-1770 carried KPC-2 carbapenemase. A study from China too reported *Klebsiella quasipneumoniae* ST-1770 from ICU patients (Wang et al., 2023). *Klebsiella quasipneumoniae* is considered an emerging pathogen in healthcare settings which can acquire carbapenemase plasmids from other CRE isolates (Mathers et al., 2019). Venkitapathi et al., 2022, have reported this pathogen in recurrent UTI in women.

Uniform aminoglycoside resistance was mediated by *armA*, *aac*(6')-Ib, *aadA1*, *aadA2*, *aph*(3')-Ia, *ph*(3')-VI, *aph*(6)-Id, and *rmtF*. Alarming, majority (17/22 isolates) carried the *armA* gene. Among 16S rRNA methyltransferase genes, *armA* and *rmtB* are the most prevalent in Asia (Shen et al., 2020; Saadatian et al., 2018; Al Sheikh et al., 2014). In contrast, Al-Quraini et al. (2022), detected *armA* and *rmtB* genes only in one isolate which co-harboured *bla*<sub>OXA-232</sub> and *bla*<sub>NDM-5</sub>. These findings indicate the increasing prevalence of plasmids carrying the *armA* gene among the CRE *K. pneumoniae* in Oman which may be attributed to the excessive antimicrobial use during the COVID-19 pandemic. The findings in the current study indicated the emergence of CRE *K. pneumoniae* strains harbouring *armA* and *rmtF* genes that retain resistance to all currently available aminoglycosides which is a cause of great concern.

Fluoroquinolone resistance was mediated by *OqxA* and *OqxB* genes while ST-2096 co-harboured the *aac*(6')-Ib-cr and ST-147 predominantly co-harboured *qnrS*. Urooj et al., 2022 reported similar findings. The *aac*(6')-Ib-cr gene was found integrated in the chromosome of *K. pneumoniae* of ST-2096 which was transferred through MGEs (Shankar et al., 2022). A study from India reported *qnrB*, *aac*(6')-Ib-cr, *oqxA*, *oqxB* in XDR *K. pneumoniae* belonging to ST147 (Dey et al., 2020).

The *fosA6* was the predominant gene in ST-2096 and ST-231 clones which agrees with previous studies from California and India (Ceron et al., 2023; Boonyasiri et al., 2021). On the other hand, the *fosA5* was only found in the ST-147 clone, similar to Al-Quraini et al's report. (2022). Although all isolates harboured the *fosA* gene, only four isolates phenotypically expressed resistance to fosfomycin.

Identifying the molecular types of AmpC  $\beta$ -lactamase and CRE at the national level is needed for understanding the burden and epidemiology of resistance and in optimising the antimicrobial therapy as well as in promoting antimicrobial stewardship and greater vigilance in infection control.

## 5. Conclusions

Fosfomycin emerges as an excellent choice for the management of UTI as it has a broad spectrum of action. Nitrofurantoin remains an excellent oral treatment option for cystitis caused by *E. coli* but not for *K. pneumoniae*. Empirical fluoroquinolone therapy is best avoided, only to be prescribed after susceptibility confirmation. ESBL and AmpC prevalence was higher in *E. coli* while CRE in *K. pneumoniae*. DHA-1 was the only incriminating AmpC while OXA-232 and NDM-5 were the predominant circulating carbapenemases. In AmpC producing *E. coli* significant MLST heterogeneity was observed while in CRE *Klebsiella pneumoniae* ST-2096 and ST-147 predominated. Routine monitoring of the circulating clades is important for tracking the spread of drug-resistant variants in

different regions. Concerning emergence of CRE *K. pneumoniae* strains harbouring armA and rmtF genes that are resistant to all currently available aminoglycosides was observed. Tracking the extent of dissemination of these stains at the national level is essential along with the implementation of effective infection prevention control measures.

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