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Genetic Profiling of Multidrug -Resistant Uropathogenic *E. coli:* Aminoglycosides, *B*-Lactam, Sulfonamides, and Tetracycline Resistant Genes in Al-Diwaniyah, Iraq

Mohanad Jawad[1*], Rahma Majid Al-Allawi[1], Rashad Imad Al-Zamili[2]
[1]College of Biotechnology, University of Al-Qadisiyah, Iraq
[2]College of Science, University of Al-Qadisiyah, Iraq

Abstract: The present cross-sectional study focuses on unraveling the genetic profile of uropathogenic *E. coli* strains, isolated from UTI patients, regarding aminoglycosides-, *B*-lactam-, tetracyclines, and sulfonamides resistance using polymerase chain reaction and gene specific primer sets. Biochemical profile (API 20E tests) and molecular identification (*16S rRNA* gene sequence analysis) of uropathogenic clinical strains revealed that *E. coli* had 20% prevalence among UTI patients recruited from Al-Diwaniyah Hospital clinic from February 2025 to June 2025. Aminoglycosides resistant genes had frequency of occurrence 70, 25, and 10% for *aac (3)-IIC*, *aaC(6')-Ib*, and *armA* genes, respectively. *B*-lactam resistant genes had a prevalence of 90, 80, and 60% for *blaSHV*, *blaTEM*, and *blaCTX-M*, respectively. Carbapenem resistant genes showed a prevalence of 35, 40, 40, and 45% for *blaNDM*, *blaKPC*, *blaVIM*, and *blaOXA-48*, respectively. The tetracycline resistance gene (*tetA*) exhibited 35% frequency. Sulfonamides resistant genes exhibited 35 and 45% frequency of occurrence for *Sul1* and *Sul2*, respectively. The current findings indicate an alarming increase regarding the circulation of multidrug-resistant uropathogenic *E. coli* within the Al-Diwaniyah region. These findings underscore a crucial necessity to re-assess the empirical antimicrobial therapy strategies for UTIs in the Al-Diwaniyah region, warranting clinical practice is updated to combat the growing circulation of multidrug-resistant uropathogenic E. *coli*.".

Keywords: Uropathogenic *E. Coli*, Aminoglycosides, *B*-Lactam, Tetracyclines, Sulfonamide, Resistance.

Research Paper

***Corresponding Author:**
Mohanad Jawad
College of Biotechnology, University of Al-Qadisiyah, Iraq

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INTRODUCTION

E. coli is the most common etiological agent for human pathogenesis. *E. coli* have strict implications in a variety of infections in human such as intestinal infections, urinary tract infections, etc., (Gaspari *et al*., 2005; Gangoue P 2007). *E. coli* infecting the urinary tract is called uropathogenic *E. coli* (UPEC) (Firoozeh *et al*., 2014). Urinary tract infections (UTIs) have numerous causative bacterial agents, however *E. coli* is responsible for approximately 65% of UTIs (Medina and Castillo-Pino 2019). UTIs are still one of the most communal types of infection both in the community and, mostly, within the healthcare settings (Medina and Castillo-Pino 2019; Grossman 2016). Although conventional antibiotic treatment remains central to managing UTIs, it is associated with incomplete prophylaxis against recurrence and the potential emergence of widespread antimicrobial resistance (Beerepoot *et al*., 2012). Antibiotics resistance phenomenon is a major global health burden worldwide (Djagbare *et al*., 2023). This phenomenon has attracted much attention globally. Aminoglycosides (Thy *et al.,* 2023), *B*-lactam antibiotics (Thakuria and Lahon 2013), carbapenem antibiotics (Nepal *et al*., 2020), tetracyclines (Chopra & Roberts 2001), and sulfonamides antibiotics are the major classes of antibiotics used for therapeutic purposes especially for Gram-negative bacterial infections (UTIs) (Masters *et al*., 2003; Tamma *et al*., 2022).

Developing resistance to each antibiotic class is attributed to certain genetic factors harbored by the bacterial pathogen, particularly UPEC.

The objective of the present work is to unravel the dominant causative agent of uropathogenic clinical strains isolated from UTI patients in Al-Diwaniyah Province, Iraq. The aim is extended to unravel the

genetic profile of some antibiotics resistance genes for five antibiotics groups that would address the antimicrobial resistance phenomenon: aminoglycosides resistance genes (*aac(3)IIC*, *aac(6')-Ib*, and *armA*), *B*-lactam antibiotics resistance genes (*blaTEM*, *blaSHV*, and *blaCTX-M*), carbapenem antibiotics resistance genes (*blaKPC, blaNDM, blaVIM*, and *blaOXA-48*), tetracycline resistance genes (*tetA*), and sulfonamides resistance genes (*Sul1/Sul2*) using conventional PCR and gene specific primer sets.

MATERIALS AND METHODS

Sampling

During the period of February 2025 to June 2025, 100 urine samples were collected from patients diagnosed with UTIs, routinely visiting Al-Diwaniyah Hospital, Iraq. Before the urine sampling, the following criteria were taken into consideration: dysuria, hematuria, proteinuria, and more than 100 colony forming units (CFU) of bacteria/mL urine sample. Sampling of urine was taken from the mid-stream by catheterization after intensive antisepsis of the genital area.

Bacteriological Media

Columbia agar (HiMedia, Canada) supplemented with 5% sheep blood and MacConkey agar (HiMedia, Canada) were used in the course of bacterial culturing from the collected urine samples. Tryptone Bile X-Glucuronide (TBX) agar plates (HiMedia, India) were used in presumptive identification of the clinical strains as *E. coli*.

Gram -Staining

Each clinical strain enrolled in this study was subjected to Gram-staining procedure to unveil its Gram-stain type using the Gram-stain kit (HiMEdia Laboratories, India) according to the manufacturer instructions.

Biochemical Identification

The clinical strains isolated in this study were subjected to biochemical identification by using API20E kit (BioMerieux, France). The steps were conducted according to the manufacturer's instructions. Shortly, a pure young culture from each clinical strain (18-24 hrs) was prepared. A bacterial suspension in saline was prepared, adjusted to a turbidity of 0.5 MacFarland standard tube. For the 20 tubes included in the API20E kit, the tubes were re-hydrated with the prepared bacterial suspension. Then, the strips were incubated with humidity at 37 ºC, for 24 hrs. The results were recorded as a numerical values profile. The API 20E tests are divided into seven groups, triplets each. The numerical values for each group were added to yield a single digit from 0 to 7. By the end, for each bacterial strain, a numerical value of seven digits was obtained. This numerical value was compared to those stored in the database customized for bacterial identification of Enterobacteriaceae members only.

Genomic DNA Isolation

The isolation of genomic DNA from each clinical strain enrolled in this study was conducted using the QIA amp DNA Mini kit (Qiagen, USA) according to the manufacturer's instructions. The quantity of the isolated genomic DNA was assessed using the Nanodrop-Spectrophotometer (ThermoFisher Scientific Co., USA). However, the quality of the isolated genomic DNA was evaluated using UV-Transilluminator (Cleaver, UK) after running on 1% agarose gel electrophoresis and measuring the absorbance ratios Ab260/Ab280 and Ab260/Ab230. The ideal absorbance ratios should be 1.8 to<2.0 and 2.0-2.3 for Ab260/Ab280 and Ab260/Ab230, respectively.

Molecular Identification

The clinical bacterial strains were identified using *16S rRNA* gene sequence analysis. The full length of the gene was amplified using the 16S rRNA universal primer set F27 and R1492 (Table 1). The composition of reaction mixture and the thermocycler conditions were settled as mention previously (Eden *et al*., 1991). The PCR products were subjected to Wizard SV Gel and PCR Clean-Up kit, Promega Co., USA. Then, the purified PCR product was bidirectionally sequenced using the aforementioned primer set. Sequencing was conducted at Eurofin DNA Co., Luxembourg. The obtained nucleotide sequence was cleaned and further analyzed by BioEdit 7.0 and further analyzed using BLASTN similarity sequence analysis search. Finally, the obtained nucleotide sequences of *16S rRNA* gene were deposited in the GenBank database and were assigned accession numbers. A phylogenetic tree was constructed using MEGA 12.0 to portray the genetic relatedness among these 16S rRNA nucleotide sequences and other closely related sequences.

Table 1: Primer sequences and PCR product lengths for amplified genes in this study

Gene	Primer sequence	PCR product (bp)	PCR conditions	References
Ribosomal RNA				
16S rRNA	F8-27:5'- AGAGTTTGATCCTGGCTCAG-3′ R1510-1492: 5′-GGTTACCTTGTTACGACTT-3'	1500	Annealing temp.:55 ºC Extension time.: 1.5 min	Eden *et al*., 1991
Carbapenem resistant genes				
blaNDM	F:5'- GCAGCTTGTCGGCCATGCGGGC-3' R: 5'-GGTCGCGAAGCTGAGCACCGCAT-3'	782	Annealing temp.:58 ºC Extension time.: 45 sec	Erdem *et al*., 2017
blaVIM	F:5'- GTT TGG TCG CAT ATC GCA AC-3' R: 5'- AAT GCG CAG CAC CAG GAT AG-3'	389	Annealing temp.:57 ºC Extension time.: 20 sec	Erdem *et al*., 2017
blaKPC	F: 5'- TGTCACTGTATCGCCGTC-3' R: 5'- CTCAGTGCTCTACAGAAAACC-3'	900	Annealing temp.:59 ºC Extension time.: 50 sec	Erdem *et al*., 2017
blaOXA-48	F: 5'-TTG GTG GCA TCG ATT ATC GG-3' R: 5'- GAG CAC TTC TTT TGT GAT GGC-3'	438	Annealing temp.:60 ºC Extension time.: 25 sec	Sievert *et al*., 2013
***B*-lactam resistant genes**				
blaTEM	F: 5'-ATGAGTATTCAACATTTCCGTGT-3' R: 5'- TTACCAATGCTTAATCAGTGAGG-3'	861	Annealing temp.:55 ºC Extension time.: 45 sec	Kharman and Hawezy 2023
blaSHV	F: 5'-TCGTTATGCGTTATATTCGCC-3' R: 5'-GGTTAGCGTTGCCAGTGCT-3'	868	Annealing temp.:55 ºC Extension time.: 1.5 min	Kharman and Hawezy 2023
blaCTX-M	F: 5'- AACGCACAGACGCTCTACC-3' R: 5'-GGGTAGCCCAGCCTGAAT-3'	517	Annealing temp.:55 ºC Extension time.: 30 sec	Kharman and Hawezy 2023
Aminoglycosides resistant genes				
aac(3)-IIc	F: 5'- ATATCGCGATGCATACGCGG-3' R: 5'- GACGGCCTCTAACCGGAAGG-3'	877	Annealing temp.:55 ºC Extension time.: 45 sec	Arpin *et al*., 2003
aac(6')-Ib	F:5'- TTGCGATGCTCTATGAGTGGCTA-3' R: 5'-CTCGAATGCCTGGCGTGTTT-3'	472	Annealing temp.:55 ºC Extension time.: 25 sec	Park *et al*., 2006
armA	F: 5'-ATT CTG CCT ATC CTA ATT GG -3' R: 5'-ACC TAT ACT TTA TCG TCG TC-3'	315	Annealing temp.:55 ºC Extension time.: 15 sec	Doi *et al*., 2007
Tetracycline- resistant gene				
tetA	F: 5'-GGGTTCTCTATATCGGGCGG-3' R: 5'-AAGCAGGATGTAGCCTGTGC-3'	593	Annealing temp.:60 ºC Extension time.: 35 sec	This study
Sulfonamides resistant genes				
Sul-1	F: 5'-TCTTAGACGCCCTGTCCGAT-3' R: 5'-CCCAAGAAGGATTTCCGCGA-3'	445	Annealing temp.:60 ºC Extension time.: 25 sec	This study
Sul-2	F: 5'- TCCGACACAGAAATCGAGCG-3' R: 5'- AGACAGAAGCACCGGCAAAT-3'	447	Annealing temp.:60 ºC Extension time.: 25 sec	This study

- All extension and annealing steps among different protocols were conducted at 72 ºC and 30 sec, respectively.
- All denaturation steps among different protocols were conducted at 94 ºC for 30 sec.

Amplification of Antibiotics-Resistance Genes

Thirteen antibiotics-resistant genes were detected in the isolated clinical strains using gene specific primer sets. These genes encoded four groups of broad spectrum antibiotics namely aminoglycosides, carbapenem antibiotics, *B*-lactam antibiotics, tetracyclines, and sulfonamides group antibiotics. These gens were designated *aac(3)-IIc, aac(6)-Ib,* and *arm A* for aminoglycosides group, *blaNDM, blaVIM, blaKPC*, and *blaOXa-48* for carbapenems group (a subclass of *B*-lactam group), *blaTEM, blaSHV*, and *blaCTX-M* for *B*-lactam group, and *tetA* for tetracyclines group, and *Sul1*/*Sul2* for sulfonamides group. The PCR mixture for the amplification of each gene contained 30 µL (30 ng genomic DNA, 20 pmole of each forward and reverse primer, 15 µL PCR 2X Master Mix (Promega Co., USA) and the reaction volume was completed to 30 µL with nuclease free water. The PCR conditions were settled in Table 1.

RESULTS

Uropathogenic *E. Coli* Strains

Preliminary Identification

Preliminary isolation of uropathogenic bacteria from 100 urine samples on blood agar revealed β-hemolytic colonies (Figure 1A). Sub-culturing on MacConkey agar showed 20 strains with typical *E. coli* morphology, exhibiting pink colorization due to lactose fermentation (Figure 1B). Further confirmation on TBX

agar showed these 20 strains produced blue to blue-green colorization (Figure 1C) due to the β-Glucuronidase activity on X-Glucuronide. Finally, microscopic examination of Gram-stained cells proved the strains were typical Gram-negative short red rods (Figure 1D).

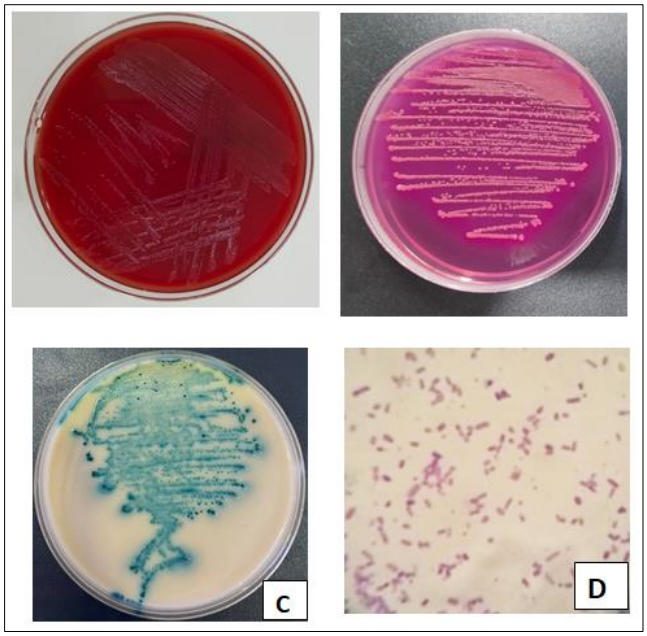

Figure 1: Colonial and cell morphological features of uropathogenic *E. coli* strain, a representative example, isolated from urine samples. A: blood agar plate, B: MacConkey agar plate, C: TBX agar plate, and C: Gram-stained bacterial film visualized under light microscope using oil immersion lens (100X).

Biochemical Profile of E. coli on API20 E

The twenty clinical strains of uropathogenic *E. coli* demonstrated typical profile of *E. coli* on ApI 20E tests (Figure 2 and Table 2).

Figure 2: API20E profile for *E. coli* uropathogenic strain as a representative example

Table 2: Biochemical profile of typical uropathogenic *E. coli* strain on API 20E

Test	Substrate/Reaction	Result
ONPG	β-galactosidase	+
ADH	Arginine Dihydrolase	-
LDC	Lysine Decarboxylase	+
ODC	Ornithine Decarboxylase	+
CIT	Citrate Utilization	-
H_2S	H_2S production	-
URE	Urease	-
TDA	Tryptophan Deaminase	-
IND	Indole Production	+
VP	Voges-Proskauer	-
GEL	Gelatinase	-
GLU	Glucose Fermentation	+
MAN	Mannitol Fermentation	+
INO	Inositol Fermentation	-

Test	Substrate/Reaction	Result
SOR	Sorbitol Fermentation	+
RHA	Rhamnose Fermentation	+
SAC	Sucrose (Saccharose) Fermentation	- or +
MEL	Melibiose Fermentation	-
AMY	Amygdalin Fermentation	+
ARA	Arabinose Fermentation	+

16S rRNA Gene Sequence Analysis

BLASTN similarity sequence analysis revealed that the obtained 16S rRNA nucleotide sequences of the twenty uropathogenic clinical strains were affiliated to *E. coli*. These 16S rRNA nucleotide sequences were submitted in GenBank database under the accession numbers: PX927674- PX927693. The phylogenetic tree, depicted in Figure 3 and constructed based on *16S rRNA* gene partial sequences, revealed that all 20 *Escherichia coli* study isolates from Iraq clustered into two distinct clades alongside reference strains retrieved from the GenBank database. Clade I comprised 11 Iraqi study isolates, namely MQBB13 (PX927686), MQBB20 (PX927693), MQBB15 (PX927688), MQBB11 (PX927684), MQBB12 (PX927685), MQBB1 (PX927674), MQBB5 (PX927678), MQBB9 (PX927682), MQBB2 (PX927675), MQBB10 (PX927683), and MQBB17 (PX927690), supported by bootstrap values ranging from 26 to 93. Clade II encompassed the remaining 9 Iraqi study isolates, including MQBB4 (PX927677), MQBB6 (PX927679), MQBB19 (PX927692), MQBB8 (PX927681), MQBB18 (PX927691), MQBB3 (PX927676), MQBB16 (PX927689), MQBB7 (PX927680), and MQBB14 (PX927687), with bootstrap values ranging from 25 to 79. The remaining reference strains, originating from India, Japan, Russia, China, Bangladesh, and Algeria, were positioned outside the two major clades, forming independent branches at the base of the phylogenetic tree. The overall topology of the tree demonstrated that all study isolates shared a close phylogenetic relationship with globally circulating *E. coli* strains, confirming their identity at the species level.

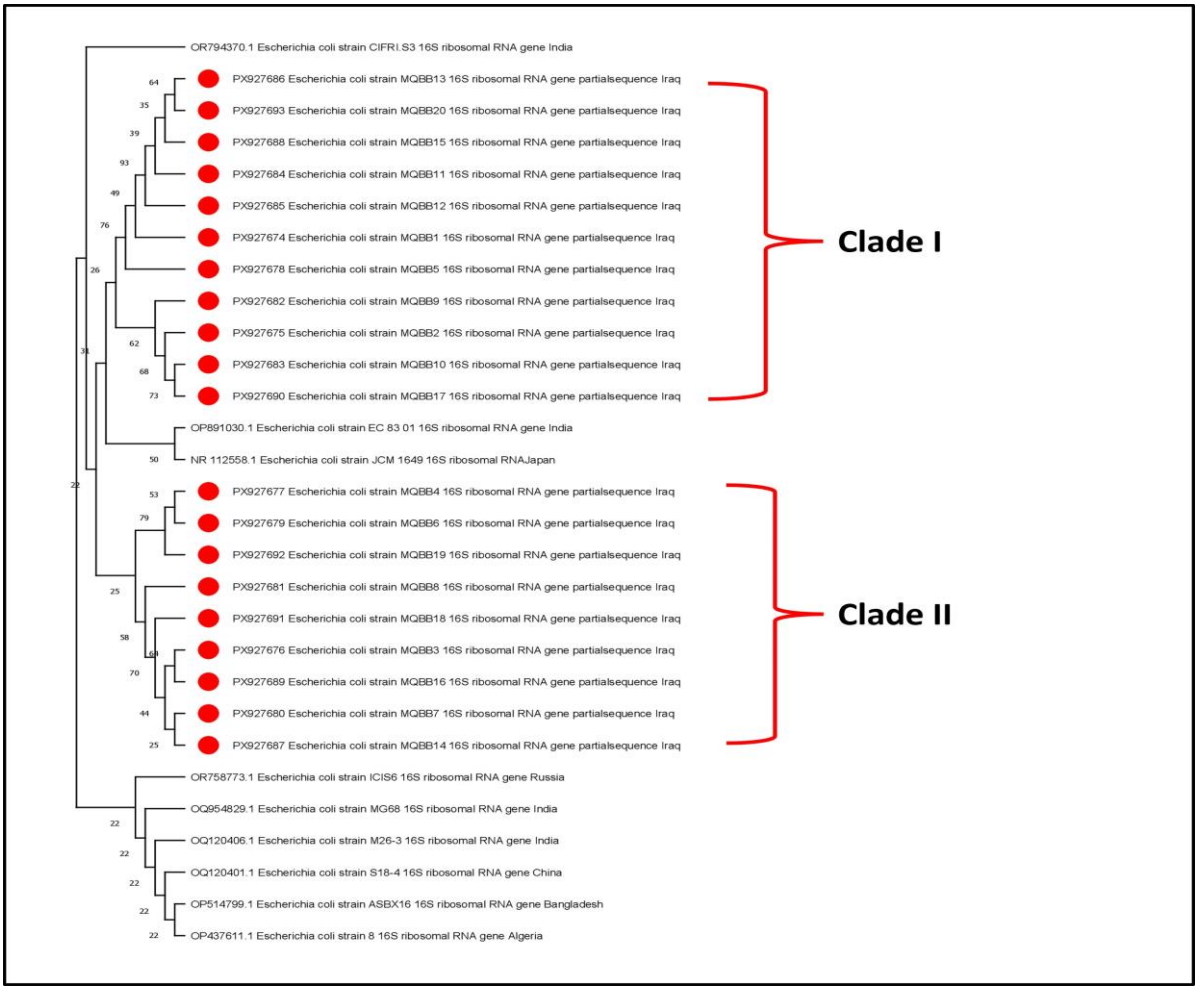

Figure 3: A phylogenetic tree constructed by MEGA 12.0 using the Neighbor-Joining algorithm demonstrates the genetic relatedness among 20 clinical uropathogenic *E. coli* isolated in this study and other closely related *E. coli* strains worldwide. Numbers on branches: bootstrapping values of 1000 re-samplings

Genotyping of Uropathogenic *E. Coli* Strains

i). Aminoglycosides Resistant Genes Prevalence

The prevalence of three aminoglycosides resistant genes were recorded among the twenty uropathogenic *E. coli* strains as shown in Figure 4. A partial fragment of 472 bp of *aac(3)-IIc* gene was successfully amplified from 14 out of 20 (70%) clinical strains (Figure 4A), however, 5 out of 20 (25%) strains harbored the *aac(6')-Ib* gene (Figure 4B), deciphered from a partial fragment of 877 bp. Only 2 out of 20 (10%) clinical strains showed a partial fragment of 315 bp encoding *armA* gene (Figure 4C).

ii). B-Lactam Antibiotics Resistant Genes Prevalence

The prevalence of three *B*-lactam resistant genes were observed among the twenty uropathogenic *E. coli* strains as portrayed in Figure 5. A partial fragment of 861 bp of *blaTEM* gene was successfully amplified from 16 out of 20 (80%) clinical strains (Figure 5A), however, 18 out of 20 (90%) strains carried the *blaSHV* gene (Figure 5B), deciphered from a partial fragment of 868 bp. Only 12 out of 20 (60%) clinical strains displayed a partial fragment of 517 bp encoding *blaCTX-M* gene (Figure 5C).

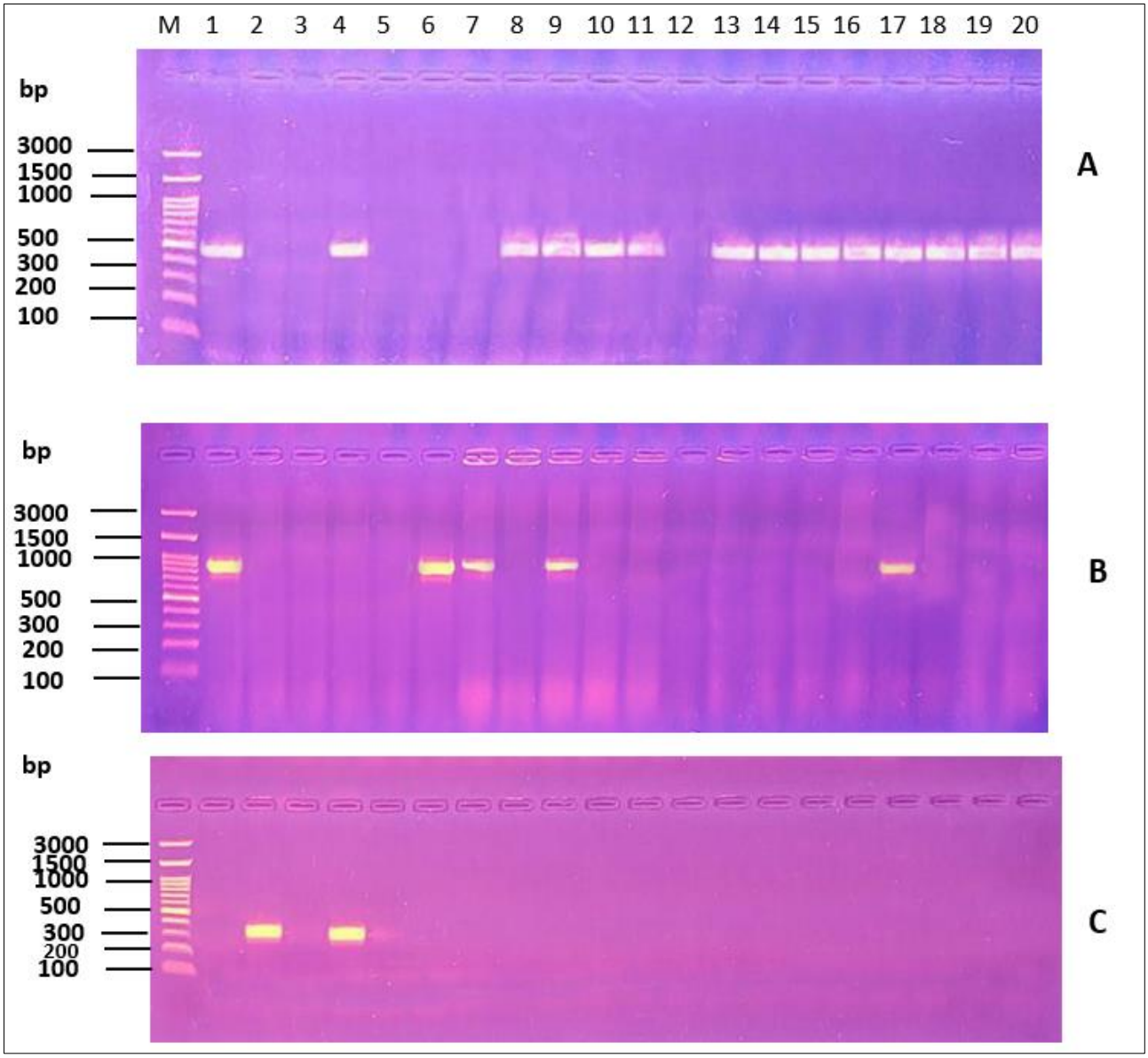

Figure 4: 1% agarose gel electrophoresis showing the amplification of partial fragments of three aminoglycosides resistant genes: *aac(3)-IIc* (A), *aac(6')-Ib* (B), and *armA* (C). Lane M: DNA ladder. Lanes 1-20 in each panel: PCR

products for a partial fragment amplified from the relative aminoglycosides resistant encoding gene among the twenty uropathogenic *E. coli* clinical strains

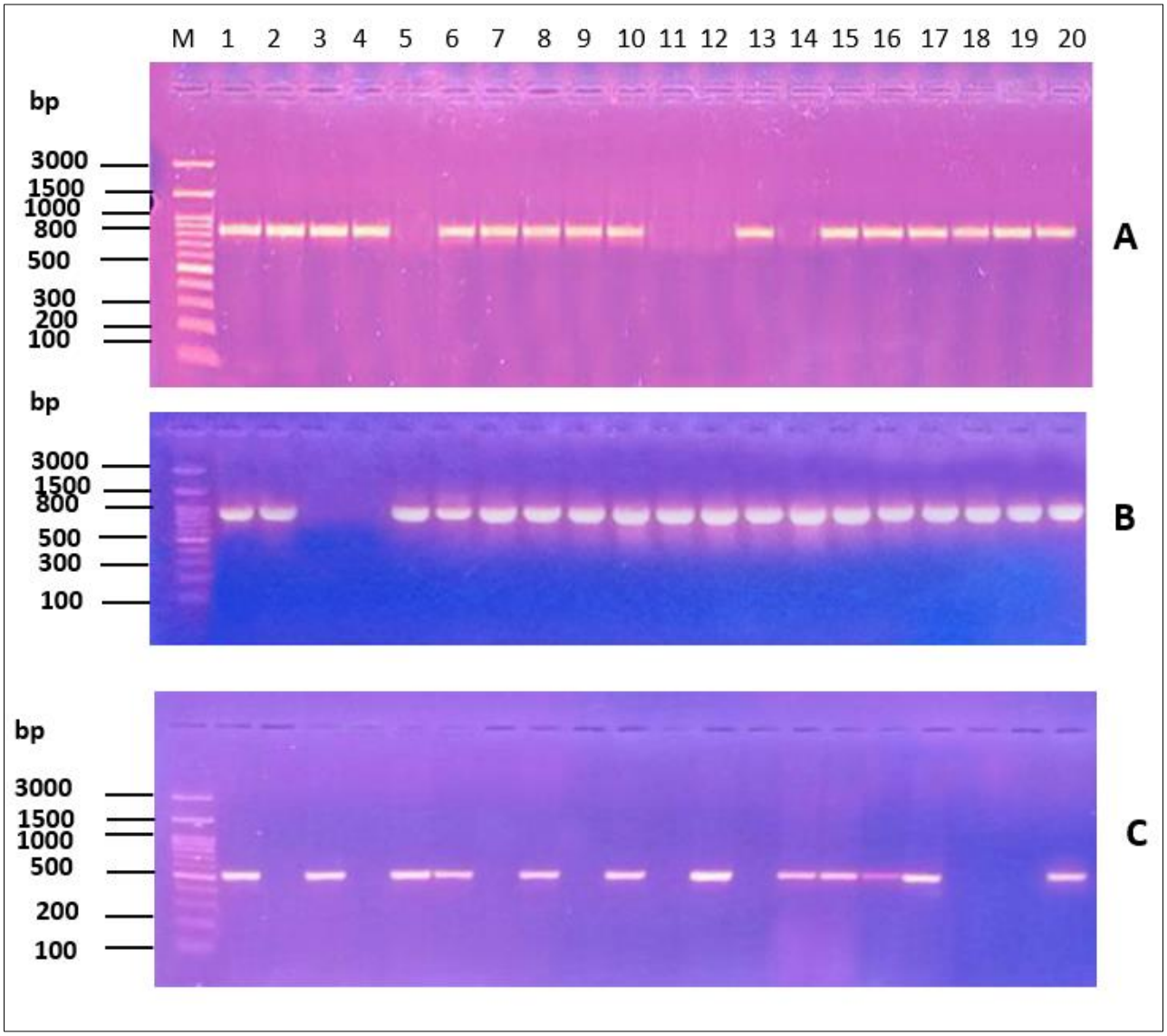

Figure 5: 1% agarose gel electrophoresis showing the amplification of partial fragments of three *B*-lactam antibiotics resistant genes: *blaTEM* (A), *blaSHV* (B), and *blaCTX-M* (C). Lane M: DNA ladder. Lanes 1-20 in each panel: PCR products for a partial fragment amplified from the relative *B*-lactam antibiotic resistant encoding gene among the twenty uropathogenic *E. coli* clinical strains

iii). Carbapenem Antibiotics Resistant Genes Prevalence

The prevalence of three *B*-lactam resistant genes were observed among the twenty uropathogenic *E. coli* strains as portrayed in Figure 6. A partial fragment of 900 bp of *blaKPC* gene was successfully amplified from 8 out of 20 (40%) clinical strains (Figure 6A), however, 8 out of 20 (40%) strains carried the *blaVIM* gene (Figure 6B), deciphered from a partial fragment of 389 bp. Only 9 out of 20 (45%) and 7 out of 20 (35%) clinical strains displayed partial fragments of 438 and 782 bp encoding *blaOXA-48* (Figure 6C) and *blaNDM* (Figure 6D) genes, respectively.

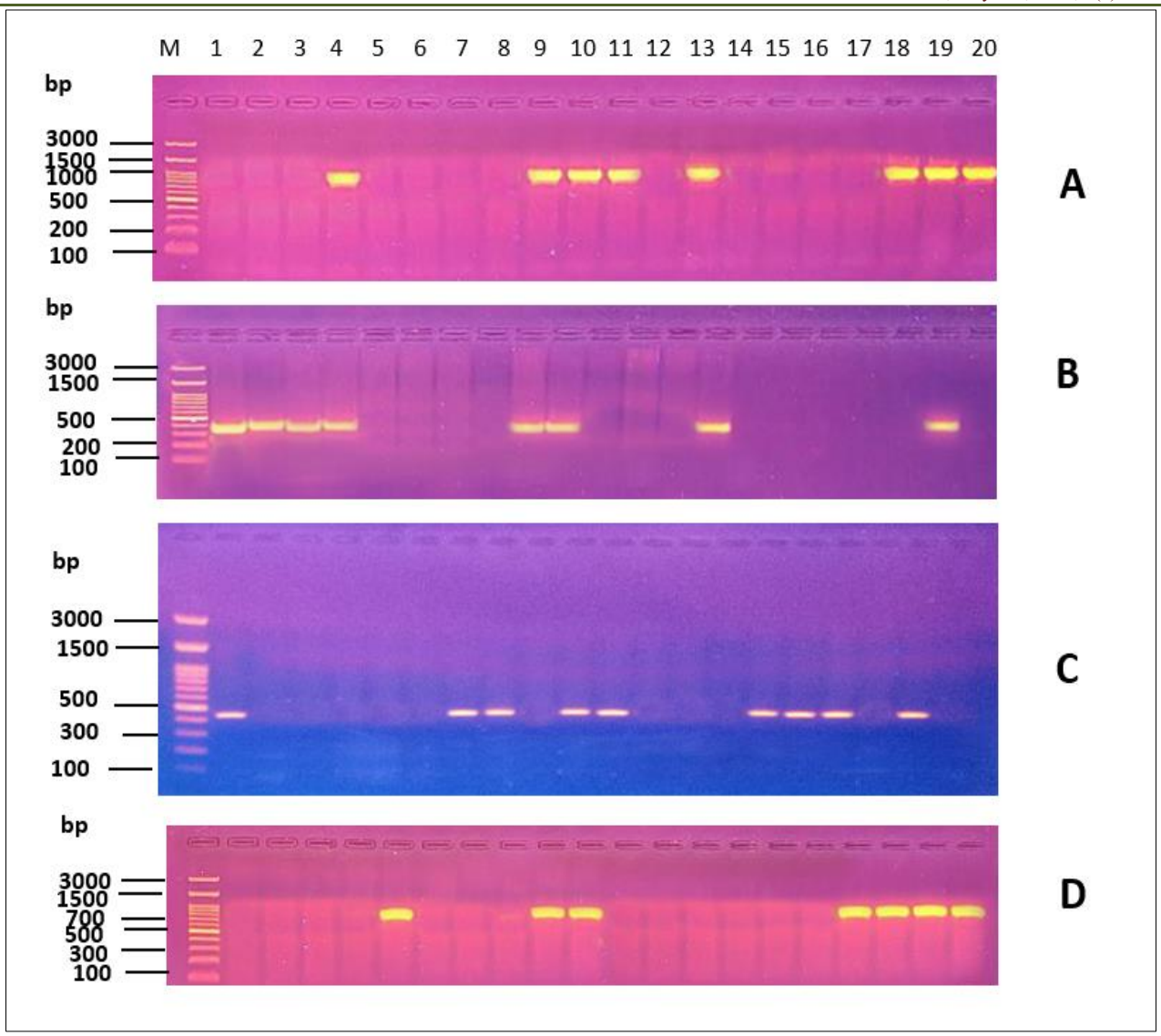

Figure 6: 1% agarose gel electrophoresis showing the amplification of partial fragments of three carbapenem resistant genes: *blaKPC,* 900 bp (A), *blaVIM,* 389 bp (B), *blaOXA-48,* 438 bp (C), and *blaNDM,* 782 bp (D). Lane M: DNA ladder. Lanes 1-20 in each panel: PCR products for a partial fragment amplified from the relative carbapenem resistant encoding gene among the twenty uropathogenic *E. coli* clinical strain

iv) Sulfonamides and Tetracyclines Antibiotics Resistant Genes Prevalence

Finally, the frequency of occurrence of *Sul1/Sul2* and *tetA* sulfonamides and tetracyclines resistance genes, respectively among the twenty clinical strains was recorded (Figure 7). A partial fragment of 445 and 447 bp was successfully amplified from 7 out of 20 (35%) and 9 out of 20 (45%) strains carried sulfonamides *Sul1*(Figure 7A) and *Sul2* (Figure 7B), respectively. Additionally, a partial fragment of 593 bp of *tetA* gene was successfully amplified from 7 out of 20 (35%) clinical strains (Figure 7C).

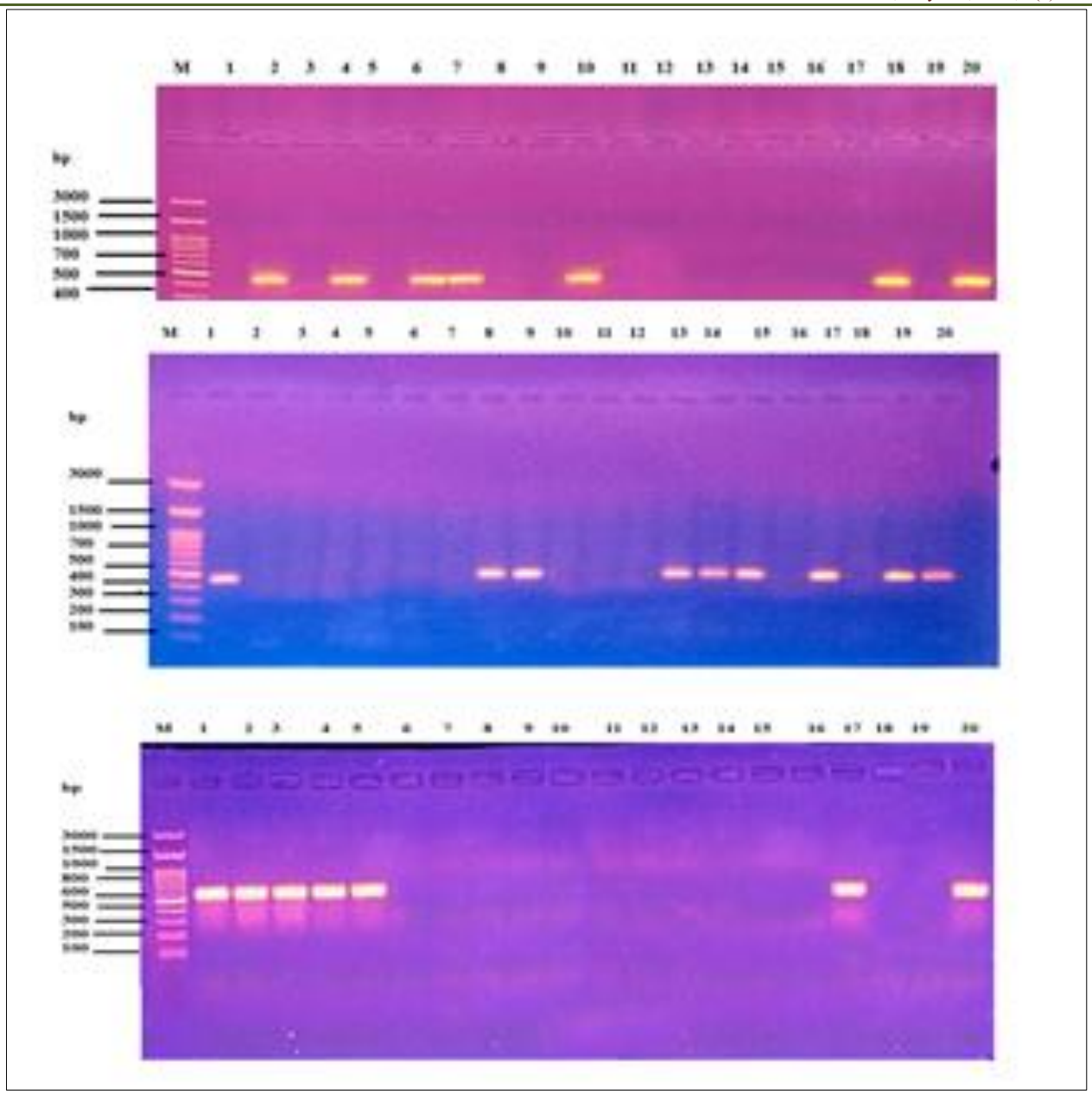

Figure 7: 1% agarose gel electrophoresis showing the amplification of partial fragments of three sulfonamides (*Sul1* and *Sul2*) and tetracyclines (*tetA*) resistant genes: *Sul1*, 445 bp (A), *Sul2*, 447 bp (B), and *tetA*, 593 bp (C). Lane M: DNA ladder. Lanes 1-20 in each panel: PCR products for a partial fragment amplified from the relative sulfonamides and tetracyclines resistant encoding gene among the twenty uropathogenic *E. coli* clinical strain

DISCUSSION

UTIs represent a considerable sector of healthcare associated infections globally each year. Catheterization associated with UTIs leads to a significant increase in mortality and morbidity in intensive care units (ICUs), therefore, UTIs accounts for approximately 30% of infections in ICUs (Genaneh *et al*., 2023). Moreover, the emergence of antibiotics resistance associated with UTIs poses health and economic burdens in ICUs. In 2019, 0.26 million deaths associated with UTIs with antibiotic resistance enforced around $3.5 billion in annual societal expenditures (Li *et al*., 2022). In the context of UTIs and its association with serious public health threats and significant economic burdens due to increases antibiotics resistance for the UTIs etiological agents, the present cross-sectional study was conducted to unveil the most frequent causative agent of UTIs in a sample of 100 patients suffering from UTIs in Al-Diwaniyah Province, Iraq. Moreover, the genetic profile regarding antibiotic resistance genes for the isolated uropathogenic clinical strains were unraveled.

Present findings reveal full agreement between the biochemical profile unraveled by API20E tests and

the molecular identification via *16S rRNA* gene sequence analysis among twenty clinical uropathogenic *E. coli* (Figures 1 & 2 and Table 2). The reason underlying this full agreement may be attributed to the inclusion of the biochemical profile of the present uropathogenic clinical strains, *E. coli*, in API20E database because it is one of the Enterobacteriales group. This criterion leads to the identification of the uropathogenic *E. coli* strains to the species level. However, *16S rRNA* gene sequence analysis approach still encounters a major limitation for discrimination among strains. The findings reinforce the usage of API 20E kits for getting rapid results for 24 hrs for identification of uropathogenic *E. coli* strains in emergency circumstances, especially in poor or medium laboratories lacking DNA sequencing machines.

The clustering of all 20 Iraqi *Escherichia coli* study isolates into two distinct clades (Clade I and Clade II) within the *16S rRNA* gene-based phylogenetic tree is consistent with the well-established clonal diversity characterizing *E. coli* populations in clinical settings, and collectively confirms their unambiguous species-level identification. The topological segregation of the study isolates into two separate clades, rather than a single unified cluster, strongly suggests the potential co-circulation of at least two genetically distinct lineages or phylogroups of *E. coli* within the same geographic locality in Iraq, which is a phenomenon increasingly documented in hospital-associated infections worldwide. Nevertheless, it is of paramount importance to acknowledge that *16S rRNA* gene sequence analysis, despite its well-recognized utility as a universal molecular identification tool, inherently lacks the discriminatory resolution required to differentiate among closely related strains at the intra-species level; accordingly, it failed to resolve the genetic relationships among the 11 study strains clustering within Clade I, as well as the 9 study strains encountered in Clade II. This fundamental limitation is attributed to the highly conserved nature of the 16S rRNA gene across *E. coli* strains, rendering it incapable of detecting the fine-scale genomic variations that distinguish individual strains from one another. The positioning of reference strains from geographically diverse origins, including India, Japan, Russia, China, Bangladesh, and Algeria, at the basal branches of the phylogenetic tree, outside the two major clades, further underscores the genetic distinctiveness of the Iraqi isolates and may reflect unique selective pressures, antimicrobial usage patterns, or transmission dynamics operating within the local epidemiological context. Of particular clinical and epidemiological significance is the possibility that the two circulating lineages represented by Clade I and Clade II may correspond to high-risk epidemic or pandemic clones of *E. coli*, such as the globally disseminated sequence type ST131,a notorious extraintestinal pathogenic *E. coli* (ExPEC) clone associated with multidrug resistance and widespread nosocomial and community-acquired infections, though this cannot be conclusively determined based solely on *16S rRNA* gene sequencing data. Therefore, future prospective studies would inevitably necessitate the adoption of molecular typing approaches with superior discriminatory power at the strain level, such as Multi-Locus Sequence Typing (MLST), Ribosomal Multi-Locus Sequence Typing (rMLST), and Whole Genome Sequencing (WGS), not only to fully resolve the clonal structure and phylogenetic relationships among these isolates, but also to facilitate the identification and surveillance of circulating epidemic, pandemic, or high-risk *E. coli* clones, whether within Iraq or across different countries, thereby contributing to a more comprehensive understanding of the global dissemination dynamics of this clinically significant and genomically versatile pathogen.

The detection of the aminoglycosides resistance genes: *aac(3)-IIc*, *aac(6')-Ib,* and *armA* in the UPEC strains enrolled in this study is of paramount significance (Figure 3). Moreover, these genes indicate key mechanisms portraying the therapeutic failure against these potent antibiotics. The aminoglycosides modifying enzymes are encoded by *aac(3)-IIc* and *aac(3)-IIc. aac(3)-IIc* codes for the AAC(3) enzyme, an acetyltransferase, that performs acetylation at the C3 amino group of the drug. *aac(6')-Ib* codes for the AAC(6') enzyme that generates modifications at the drug C6' amino group (Tolmasky M 2000; Ramirez et al., 2013; Zárate et al., 2018; Nemec et al., 2024). In addition, the presence of the *armA* gene is mostly alarming. *armA* encodes an enzyme namely 16S rRNA methyltransferase that performs by methylating the ribosomal target site itself. This mechanism introduces high-level resistance to almost *all* clinically existing aminoglycosides and is often harbored on large plasmids that can be transmitted easily among bacterial strains and co-harbor other genes of resistance for other antibiotics groups or classes (Galimand *et al.,* 2003; González-Zorn *et al.,* 2005). Aminoglycosides resistant genes had frequency of occurrence 70, 25, and 10% for *aac(3)-IIC*, *aaC(6')-Ib*, and *armA* genes, respectively (Figure 4). Higher level of aminoglycosides resistance genes were reported in China (Xiao and Hu 2017), Norway (Lindemann *et al.,* 2012), and Burkina Faso (Djagbare *et al.,* 2023): *aac(3)-IIC* (79.2, 79.4, and 85.15%) and *aac(6')-Ib* (24.39, 37.9, and 70.3%) on uropathogenic *E. coli* strains. In stark contrast to our findings, a lower prevalence percent was reported in Spain for aminoglycosides resistance genes: *aac(3)-IIC* (16.2%) and *aac(6')-Ib* (14.7%) on uropathogenic *E. coli* strains (Fernández-Martínez *et al.,* 2018). The discrepancy among results may be attributed to the sample size and socioeconomic status of the patients (source of the urine samples). This notable prevalence of aminoglycosides encoding genes underpins intense selective pressure and addresses a troubling circulation of mobile, broad-spectrum resistance determinants, therefore underscoring the reduced usage of aminoglycosides for

empirical UPEC therapeutic purposes in Al-Diwaniyah Province where the study was conducted.

B-lactam antibiotics, drugs with historical backbone of UTIs therapy, resistance constitutes a great challenge in the course of therapy of UPEC infections (Bonfiglio *et al*., 2002). The primary mechanism includes the hydrolysis of the *B*-lactam ring antibiotics, mediated by two enzymes: *B*-lactamases, encoded by *blaTEM, blaSHV*, and *blaCTX-M*, and, carbapenemases, encoded by *blaOXA-48*, *blaNDM*, *blaKPC,* and *blaVIM* (Nasrollahian *et al*., 2022; Mazandarani *et al*., 2024). Therefore, the activity of B-lactamases and carbapenemases are responsible Extended-Spectrum *B*-Lactamase (ESBL) activity (Halat, Moubareck 2020). The spread of ESBL-producing UPEC sends alarms and represent a public health threat burden, directing the clinicians to replace the *B*-lactam antibiotics with non-*B*-lactam antibiotics (Al-Kashef *et al*., 2018; Choi *et al*., 2025). The isolation of *B*-lactamase and carbapenemase encoding genes in this study addresses the indispensable demand for successive molecular surveillances to lead empirical therapy strategies. The prevalence of *B*-lactamases and carbapenemase genes varied among different studies conducted on uropathogenic *E. coli* strains. Our findings revealed that carbapenemase genes exhibited the following frequency among uropathogenic *E. coli* strains: 35, 40, 40, and 45% for *blaNDM*, *blaKPC*, *blaVIM*, and *blaOXA-48*, respectively (Figures 5 &6). A previous study conducted in Wasit Province, Iraq revealed that the prevalence of carbapenemase genes among uropathogenic *E. coli* strains was as follow: *blaOXA-48* (57.8%), *blaKPC* (15.7%), and *blaVIM* (10.5%) (Al-Mayahie *et al.,* 2022). Other study performed in Iraq showed only 1% of the uropathogenic *E. coli* strains were positive for *bla*OXA-48, whereas, *blaKPC*, *blaNDM,* and *blaVIM* were not (Mhawesh *et al.,* 2021). The prevalence of blaOXA-48 was predominant as revealed from other previous studies (Ortega *et al*., 2016; Gauthier *et al*., 2018; El-Shaer *et al*., 2021; Mhawesh *et al.,* 2021). Furtehrmore, the prevalence of carbapenemase genes was noted among different clinical *E. coli* strains (Al-Sa'ady *et al*., 2020; AbouZeid *et al.,* 2017; Mathers *et al*., 2015; O'Hara *et al.,* 2014; Accogli *et al*., 2014; Dimou *et al*., 2012; Mazandarani *et al*., 2024). Similarly, numerous studies revealed the presence of *B*-lactamase genes among various phylotypes of *E. coli* with various prevalence (Sepp *et al*., 2019; Mendes *et al*., 2019; Kubone *et al*., 2020; Ghaderi *et al*., 2020; Jabalameli *et al*., 2021; Perera *et al*., 2022; Afsharikhah *et al*., 2023).

The tetracycline and sulfonamides resistant genes were reported in a previous study conducted on uropathogenic *E. coli* strains isolated in Malaysia where *tetA, Sul1*, and *Sul2* showed prevalence percent of 45, 41.7, and 21.7%, respectively (Chen *et al.,* 2024). Our findings revealed that *tetA* exhibited a prevalence of 35 % of uropathogenic *E. coli* (Figure 7C). Previous findings demonstrated that *tetA* presented slightly higher prevalence in Iraq (Zeadan *et al*., 2022) and Nigeria (Olowe *et al*., 2013). The prevalence of *tetA* gene may be assigned to the superior transferability of *tetA* gene, resulting in an ease in *tetA* gene dissemination among uropathogenic *E. coli* (Olowe *et al*., 2013). Our findings showed a higher prevalence of *Sul2* (45%) compared to *Sul1* (35%) (Figure 7). A similar results were observed in a previous study, where higher prevalence of *sul2* was observed among the co-trimoxazole-resistant clinical strains (Lin *et al*., 2016). A lower prevalence of *tetA*, 20.19%, was observed among uropathogenic *E. coli* in Lebanon (Chreim *et al*., 2025). A higher prevalence of 100% for *tetA* gene was observed among uropathogenic *E. coli* infecting bovine (Yadav *et al*., 2025). A previous study revealed a higher prevalence of *Sul1* (61.91%) and *tetA* (78.57%) genes (Rahman *et al*., 2022) compared to our findings among uropathogenic *E. coli*. Another study demonstrated a higher prevalence of *Sul1* (81%) and *Sul2* (67%) among uropathogenic *E. coli* (Arabi *et al*., 2015).

Based on our findings, we strongly recommend conducting a local, continuous molecular surveillance program for antibiotic resistance genes in UPEC strains to precisely monitor the evolution of resistance and enable evidence-based revisions of the institutional antibiotic stewardship guidelines in Al-Diwaniyah.

CONCLUSION

The present study unveiled an alarming prevalence of multidrug-resistant uropathogenic *E. coli* harboring a diverse arsenal of aminoglycoside, β-lactam, carbapenem, tetracycline, and sulfonamide resistance genes among UTI patients in Al-Diwaniyah Iraq, with the co-existence of carbapenem resistance genes, particularly *bla*NDM, *bla*KPC, *bla*VIM, and *bla*OXA-48, representing an immediate and serious threat to public health, as carbapenems constitute the first line last-resort therapeutic option for multidrug-resistant infections. These findings carry profound clinical significance, urgently mandating the reassessment and revision of empirical antimicrobial therapy protocols for UTIs in the Al-Quadysia region, alongside the implementation of robust antimicrobial stewardship programs and rigorous infection control measures within healthcare facilities. For prospective studies, it is strongly recommended to expand surveillance to encompass a larger, multicenter patient cohort across different Iraqi provinces, coupled with the application of advanced molecular typing tools, including MLST, rMLST, and Whole Genome Sequencing, to unravel the clonal structure, transmission dynamics, and potential epidemic or pandemic lineages of circulating uropathogenic *E. coli* strains, thereby providing the epidemiological intelligence necessary to formulate evidence-based national strategies aimed at curtailing the

escalating tide of antimicrobial resistance in Iraq and the broader Middle Eastern region.

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