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ORIGINAL ARTICLE

The evaluation of antimicrobial resistance rates in infections caused by uropathogenic *Escherichia coli* strains collected from the south of Lebanon

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ABSTRACT

Background and Objectives: Uropathogenic *Escherichia coli* (UPEC) is a leading cause of urinary tract infections, which are a significant public health concern worldwide. Antibiotic resistance among UPEC isolates is an increasing challenge, necessitating a better understanding of the resistance patterns and underlying genetic mechanisms. This study examined the prevalence of antibiotic resistance phenotypes and the detection of specific resistance genes among patients with UPEC infections in Sheikh Ragheb Harb University Hospital in south Lebanon.

Materials and Methods: Antimicrobial resistance phenotype of 104 urine samples was tested to determine the resistance percentages for various antibiotics including ampicillin, gentamicin, ciprofloxacin, tetracycline, bactrim, meropenem, and imipenem using disk diffusion test. Additionally, molecular analysis like polymerase chain reaction (PCR) was performed to detect the presence of bla_{SHV}, *qnrA, tetA, dfrA1, aac3,* bla_{OXA} and bla_{IMP} resistance genes.

Results: The antimicrobial resistance testing revealed the following resistance percentages for various antibiotics: ampicillin (100%), gentamicin (15.38%), ciprofloxacin (34.61%), tetracycline (48.07%), bactrim (17.3%), meropenem (0.96%) and imipenem (0.96%). The analysis of resistance genes showed the presence of bla_{SHV} (7.96%), *qnrA* (0.96%), *tetA* (20.19%), and *dfrA1* (0.96%) genes, while the *aac3,* bla_{OXA}, and bla_{IMP} genes were not detected.

Conclusion: The high rates of antibiotic resistance observed, particularly to ampicillin and tetracycline, highlight the need for more judicious antibiotic use and the development of alternative treatment strategies to combat UPEC infections. These results can inform antimicrobial stewardship programs and guide the selection of appropriate empiric therapy for urinary tract infections.

Keywords: Urinary tract infection; Uropathogenic *Escherichia coli*; Antibiotic resistance; Resistance genes; Polymerase chain reaction

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INTRODUCTION

Urinary tract infections (UTIs) are a widespread health concern globally, affecting an estimated 150 million people each year (1). They are particularly prevalent among women, with up to 50% experiencing a UTI at least once in their lifetime (1). UTIs can occur anywhere in the urinary system, with symptoms including frequent urination, pain or burning during urination, cloudy or bloody urine, and pelvic discomfort (2).

The primary bacteria responsible for UTIs is the Gram-negative bacteria, *Escherichia coli (E. coli)* especially the uropathogenic *E. coli* strain, accounting for approximately 80-90% of community-acquired cases (1). Other bacteria such as *Klebsiella, Proteus, Enterococcus,* and *Staphylococcus saprophiticus* can also cause UTIs, albeit less frequently (1).

Uropathogenic *E. coli* strains possess virulence factors enabling them to adhere to and colonize the urinary tract, evading immune responses and causing tissue damage. Uropathogenic *Escherichia coli*'s dominance in UTIs underscores the significance of their virulence mechanisms, which include adhesins, toxins and iron acquisition systems (1). This understanding is pivotal as research endeavors aim to develop therapies and vaccines targeting uropathogenic *E. coli*, addressing the urgent need for effective UTI management strategies.

There are many antibiotics used to treat uropathogenic *E. coli*, including: trimethoprim/sulfamethoxazole (TMP/SMX), this combination antibiotic is frequently used as a first-line treatment for uncomplicated UTIs (1). Often prescribed for uncomplicated UTIs, nitrofurantoin is effective against many uropathogenic *E. coli* strains and is especially suitable for lower urinary tract infections (7). The third-generation cephalosporin antibiotic like ceftriaxone may be used for complicated UTIs patients (1). Sometimes amoxicillin/clavulanate is prescribed for UTIs, especially if the infection is suspected to involve more resistant bacteria or if the patient has allergies to other antibiotics (1). However, the widespread and often indiscriminate use of these antimicrobials has contributed to the alarming rise of antibiotic-resistant uropathogenic *E. coli* strains. Many uropathogenic *E. coli* isolates have now developed resistance to multiple classes of antibiotics, rendering common treatment regimens ineffective (1). This emergence of multidrug-resistant uropathogenic *E. coli* is a major public health concern, as it significantly limits the available therapeutic options and increases the risk of treatment failure, prolonged illness and serious complications (1).

So, the prevalence of antibiotic-resistant uropathogenic *E. coli* is a growing public health concern, particularly in south Lebanon, where data on local resistance patterns remains limited. Our study aims to address this critical knowledge gap by providing a comprehensive assessment of the prevalence of antibiotic resistance phenotypes and underlying resistance genes among uropathogenic *E. coli* isolates in this region. The findings from this research will have direct clinical relevance, as they can guide the selection of empiric antibiotic therapies and inform the development of evidence-based antibiotic stewardship programs. Given the urgent need for up-to-date, locally relevant data to drive effective infection control and antimicrobial resistance mitigation strategies, the results of this study will be a timely and important addition to the scientific literature. We will examine the prevalence of antibiotic resistance phenotypes and the detection of specific resistance genes among patients with uropathogenic *E. coli* infections in Sheikh Ragheb Harb University Hospital in south Lebanon.

MATERIALS AND METHODS

Sample collection. 104 urine samples are collected from patients suffering from UTI at Sheikh Ragheb Harb University Hospital in south Lebanon.

Bacterial isolation and identification. A urine sample was collected and streaked onto MacConkey agar (TM MEDIA, TMG 337), then incubated at 37°C for 18 to 24 hours. The IMViC tests (TM MEDIA) were performed to differentiate UPEC from other *E. coli* strains. For the indole test, the culture was inoculated into tryptophan broth (TM 468) and incubated for 24 hours, followed by the addition of Kovac's reagent (TR 008). The isolate was then inoculated into MR-VP broth (TM 2421) for the methyl red and Voges-Proskauer tests, also incubated for 24 hours. Finally, the isolate was inoculated into Simmons' citrate agar (TM 348) and incubated for 24-48 hours.

Antimicrobial susceptibility testing. The disk diffusion test, or Kirby-Bauer method, determines

the antimicrobial susceptibility of bacterial isolates, including UPEC. Mueller-Hinton agar (TM MEDIA, TM 339) is prepared in sterile Petri dishes, and UPEC isolates from urine cultures are swabbed onto the agar. Sterile paper disks (Bioanalyse) impregnated with various antibiotics—ampicillin (10 µg, ASD00200), ciprofloxacin (5 µg, ASD04800), tetracycline (30 µg, ASD08900), bactrim (25 µg, ASD09320), meropenem (10 µg, ASD05400), imipenem (10 µg, ASD03650), and gentamicin (10 µg, ASD05000)—are placed on the agar surface. The plates are incubated at 37°C for 16 to 18 hours. After incubation, the zones of inhibition are measured and compared to the Clinical and Laboratory Standards Institute (CLSI 2023) criteria.

Incubation of UPEC in Luria-Bertani broth. Luria-Bertani (HI MEDIA, M1245-500G) broth is prepared according to the manufacturer's instructions. 10 ml of the UPEC isolate suspension is added to 3ml Luria-Bertani broth. The inoculated Luria-Bertani broth tubes are placed in an incubator set at 37°C for 16 to 24 hours.

DNA extraction. QIAamp DNA Mini Kit, Cat. No. 51304 was used to extract the bacterial genomic DNA.

PCR protocol. The primers and the PCR master mix that contain dNTPs, DNA polymerase, buffer, $MgCl_2$ and loading dye are ordered from Pishgam Institute in Iran (pishgambc@gmail.com). 2 ml of the extracted UPEC DNA, 7.2 ml of H_2O, 0.4 ml of forward, 0.4 ml of reverse primers and 10 ml of the master mix (Ampliqon master mix, ID 5200350) are mixed. The PCR reaction mix is placed in a thermal cycler; where the denaturation (95°C/ 5 mins), cycling (35 cycles-95°C/ 1 min), annealing (temperature specific to each primer mentioned in Table 1 /45 secs), extension (72°C/ 1 min) and final extension (72°C/ 10 mins) steps are performed.

Gel electrophoresis. 0.75 g of agarose (1.5%) (Sigma-Aldrich, A2576-5G) is dissolved in 50 ml diluted TAE buffer (242 g Tris-HCl, 57.1 ml acetic acid, and 100 ml of 500 mM EDTA pH 8) according to the manufacturer's instructions. The heated gel is stained with 2 ml of Nancy DNA-specific fluorescent dye (Sigma-Aldrich, 01494-500UL) and allowed to solidify for 30 minutes in the gel tray. The tray is then placed in an electrophoresis chamber filled with TAE buffer. In the first well, 3 µl of ladder (Solis BioDyne, 07-11-0000S) is loaded, followed by 10 µl of DNA samples. A constant voltage of 80-120 volts is applied to initiate electrophoresis.

Data analysis. In this study, bar charts were created using Microsoft Excel to visually represent the comparative data across different categories. And data analysis was conducted using IBM SPSS Statis-

Table 1. The sequence and the annealing temperature of primers used for PCR protocol.

Antimicrobial Class Agent	Antimicrobial resistance gene	Primer Sequence (5'-3')	Annealing Temperature (°C)	References
beta lactam (Ampicillin)	*bla*$_{SHV}$	F-TCGCCTGTGTATTATCTCCC R-CGCAGATAAATCACCACAATG	58	AF148850 (1)
aminoglycosides (gentamicin)	*aac(3)*	F- TGCTGGTCCACAGCTCCTTC R- CGGATGCAGGAAGATCAA	59	ALS39198 (2)
fluoroquinolones (ciprofloxacin)	*qnrA*	F-ATTTCTCACGCCAGGATTTG R-GATCGGCAAAGGTTAGGTCA	55	CAL30210 (3)
tetracycline	*tet(A)*	F-GGTTCACTCGAACGACGTCA R-CTGTCCGACAAGTTGCATGA	56	P02982 (4)
sulfonamides (bactrim)	*dfrA1*	F-TGGTAGCTATATCGAAGAATGGAGT R- TATGTTAGAGGCGAAGTCTTGGGTA	60	AQS26669 (5)
carbapenem (meropenem)	*bla*$_{OXA}$	F- GCTTGATCGCCCTCGATT R- GATTTGCTCCGTGGCCGAAA	60	QGJ97581 (6)
carbapenem (imipenem)	*bla*$_{IMP}$	F-GGAATAGAGTGGCTTAATTCTC R-GGTTTAAYAAAACAACCACC	52	AGZ83333 (7)

tics 23. The results were presented as mean ± SD for quantitative variables and were summarized by frequency (percentage) for categorical variables.

Ethics. Ethical approval is not applicable for this article. There are no human subjects in this article and informed consent is not applicable.

RESULTS

Identification of UPEC. UPEC colonies typically appear as pink to dark-red colonies on MacConkey agar due to their ability to ferment lactose.

The results of the IMViC tests for UPEC typically show positive indole and methyl red reactions, and negative Voges-Proskauer and citrate results (IMViC: + + - -).

Antimicrobial susceptibility profile. The antimicrobial susceptibility profile among the 104 patients analyzed is illustrated in Fig. 1. All 104 patients (100%) demonstrated resistance to ampicillin, indicating a widespread issue with this antibiotic in the treatment of infections caused by uropathogenic *E. coli*. Among these, 16 patients (15.38%) exhibited resistance to gentamicin. Resistance to ciprofloxacin was observed in 3 patients (34.61%). Tetracycline resistance was noted in 50 patients (48.07%), indicating that nearly half of the isolates were resistant to this antibiotic. Additionally, 18 patients (17.3%) showed resistance to bactrim, a combination of trimethoprim and sulfamethoxazole that is frequently used for UTIs. Interestingly, resistance to meropenem and imipenem was observed in one patient each (0.96%).

Prevalence of antibiotic resistance genes. The prevalence of antibiotic resistance genes among the 104 patients analyzed is depicted in Fig. 2. Of the patients exhibiting ampicillin resistance, 4 patients (3.84%) were found to harbor the bla_{SHV} resistance gene. In contrast, none of the 16 patients with gentamicin resistance displayed the *aac3* resistance gene. Among the 36 patients exhibiting ciprofloxacin resistance, only 1 patient (2.77%) carried the *qnrA* resistance gene. For the group of 50 patients showing tetracycline resistance, a significant 15 patients (30%) possessed the *tetA* resistance gene. Additionally, 1 out of the 18 patients with bactrim resistance (5.55%) exhibited the *dfrA1* resistance gene, which is associated with trimethoprim resistance. Notably, the single patients resistant to meropenem and imipenem did not demonstrate the presence of the bla_{OXA} or bla_{IMP} resistance genes.

The DNA bands labeled 1-4 in Fig. 3 indicate the presence of the *tetA* gene, which confers tetracycline

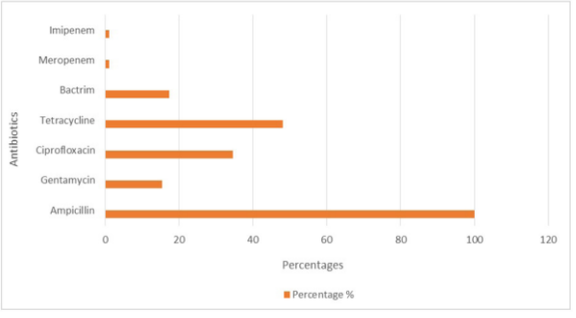

Fig. 1. Percentages of antibiotic resistance phenotype among patients.

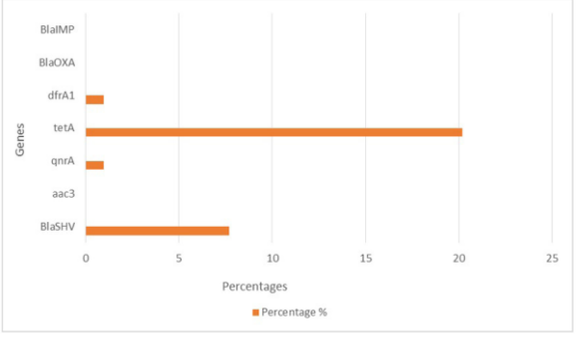

Fig. 2. Percentages of antibiotic resistance genes among patients.

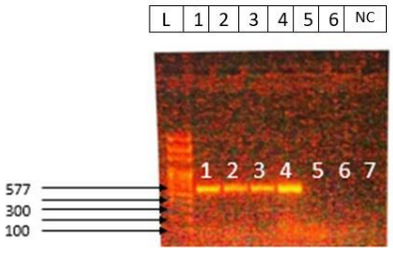

Fig. 3. DNA bands observation of patients suffering from tetracycline resistance phenotype. An agarose gel (1.5%) was run at 80-120 volts for 30 minutes. Molecular weight ladder (Solis BioDyne) is indicated in lane L, while samples 1,2,3 and 4 show amplification products at approximately 577 bp. The gel was stained with Ethidium Bromide for visualization. L: ladder DNA (100bp to 1000bp), 1-6: tested patients, NC: negative control.

resistance. The alignment of these bands at 577 bp suggests the presence of the *tetA* gene in these samples.

DISCUSSION

UPEC poses a serious public health concern due to the growing problem of antibiotic resistance. UPEC has demonstrated an alarming ability to develop resistance to many commonly prescribed antibiotics for urinary tract infections (UTIs), including Ampicillin, fluoroquinolones, and trimethoprim sulfamethoxazole (11). The rise of multidrug-resistant UPEC isolates greatly limits the effective treatment options available, increasing the risk of treatment failure (1).

In a 2009-2011 study conducted in China, resistance rates to ceftazidime, gentamicin, ciprofloxacin and sulfonamides were reported at 31%, 20%, 33% and 47%, respectively (1). Similarly, the resistance rate to fluoroquinolones in India exceeded 60% in 2019 (1). Turning to the United States, the prevalence of fluoroquinolone-resistant uropathogenic *E. coli* strains was documented at approximately 31%. In 2013-2014, 18.8% of isolated strains were resistant to ciprofloxacin in Brazil, while in the same period in the United States, 12.1% of *E. coli* isolates from patients with acute pyelonephritis exhibited ciprofloxacin resistance (1). Resistance to fluoroquinolones was observed in around 30% of uropathogenic *E. coli* isolates in Poland (11). Higher rates of ciprofloxacin resistance have been reported in specific patient populations, reaching 42.8% among elderly hospitalized patients in Argentina and 47.3% in community and hospital-acquired uropathogenic *E. coli* infections in Mexico (1). In Switzerland, ciprofloxacin resistance increased significantly from 1.8% in 1997-2007 to 15.9% and 17.4% in subsequent studies during 2012-2015 (1). Alarmingly, resistance to ciprofloxacin appears to be substantially higher in developing countries, with rates exceeding 50% in Ethiopia, Nepal, Pakistan, Mongolia and Jordan, compared to 5.1-24.8% in the United States, Germany, Switzerland and France (1). In the United States, from 2009 to 2013, the rates of resistance to amoxicillin or Ampicillin /beta-lactamase inhibitors were approximately 40%. Between 2015 and 2017 in Romania and Bosnia in 2016, 29.0% and 19.6% of uropathogenic *E. coli* isolates collected from outpatients were resistant to amoxicillin-clavulanic acid. In Poland in women with uncomplicated UTIs, 3.3% of uropathogenic *E. coli* were resistant to amoxicillin-clavulanic acid in 2003-2006, while the percentage was 13.9% in 2007-2008 for hospital-acquired UTIs (1).

In 2022, in the GCC region, CTX-M (53.8%) appeared to be the most common antimicrobial resistance gene followed by TEM (40.6%), NDM-1 (28.4%), OXA (24.3%), VIM (8.5%) and SHV (7.8%), respectively (1). In 2022 in Saudi Arabia, antimicrobial susceptibility testing revealed that 82% (41/50) of all UPEC isolates were resistant to fluoroquinolones, and 60% (30/50) of the isolates were resistant to ampicillin. Moreover, 44% (22/50) of all UPEC isolates showed resistance to trimethoprim/sulfamethoxazole, and 38% (19/50) of the isolates exhibited resistance to cephalosporin. In contrast, very low resistance to gentamicin (12%), amoxicillin/clavulanic acid (8%), and piperacillin/tazobactam (4%) was observed. None of the 50 UPEC isolates examined in the same study exhibited resistance to carbapenems (imipenem, meropenem, and ertapenem) or amikacin (24).

In our study, ampicillin resistance was observed in 100% of the 104 UPEC isolates, which is the highest antibiotic resistance level, also, the resistance rates reported in similar studies from North Lebanon and Iran, were the highest at 67.6% and 80%, respectively (23). Conversely, imipenem appears to be the most effective antibiotic against UPEC, with a resistance level of only 0.96% in our study and 3% in a separate Iranian study (24).

Analysis of antibiotic resistance genes revealed that *tetA* had the highest distribution at 20.19%, while *qnrA* and *dfrA1* were much lower at 0.96% each. Notably, the resistance genes *aac3*, bla_{OXA}, and bla_{IMP} were not detected. These findings contrast with an Iranian study that reported a much higher prevalence of the *qnrA* gene (46.34%) and a lower frequency of *dfrA1* (21.95%), compared to our results (25).

Overall, these results highlight the complexity of antibiotic resistance mechanisms in uropathogenic *E. coli*. The varying prevalence of resistance genes across different antibiotics underscores the need for continued genetic surveillance to better understand the resistance landscape and inform treatment strategies.

There are a few possible explanations for the discrepancy between the observed resistance phenotypes and the detection of specific resistance genes through gel electrophoresis analysis. Firstly, bacteria can develop resistance to antibiotics through non-ge-

netic resistance mechanisms. These mechanisms do not necessarily involve the presence of specific resistance genes but rather changes in the bacteria's physiology that confer reduced susceptibility to the antibiotic. For example, alterations in membrane permeability, the upregulation of efflux pumps, or the enzymatic inactivation of the antibiotic can all contribute to a resistant phenotype without the involvement of identifiable resistance genes (1). In such cases, the resistance phenotype would be observed, but the specific resistance genes may not be detectable through the gel electrophoresis technique. Secondly, genetic mutations can also play a role in antimicrobial resistance, independent of the presence of known resistance genes. Bacteria can acquire point mutations or other genetic changes that modify the target site of the antibiotic or alter its mode of action, thereby conferring resistance (1). These genetic alterations may not necessarily involve the acquisition of specific resistance genes that are typically targeted in gel electrophoresis assays. It is important to recognize that the relationship between genotype (the presence of resistance genes) and phenotype (the observed resistance) is complex and can vary among different bacterial strains and antibiotics. In some cases, the presence of a resistance gene may not necessarily translate into a fully expressed resistant phenotype, while in other cases, resistance can arise through mechanisms that do not involve the presence of the targeted resistance genes (1).

UPEC infections can lead to severe consequences, particularly in vulnerable groups such as the elderly (1). This highlights the urgent need to understand the factors contributing to antibiotic resistance in UPEC and to develop effective strategies to address this growing public health concern. The high prevalence of resistance underscores the necessity for ongoing surveillance and the importance of antibiotic stewardship to reduce the spread of resistant strains. Additionally, there is a critical need for alternative treatment options and the development of new antibiotics to effectively tackle infections caused by resistant UPEC.

CONCLUSION

In summary, the discrepancies observed between resistance phenotypes and the detection of specific resistance genes through gel electrophoresis analysis can be attributed to the multifaceted nature of antimicrobial resistance in bacteria. Considering non-genetic resistance mechanisms and the role of genetic mutations, in addition to the presence of resistance genes, is crucial for a comprehensive understanding of the complex interplay between genotype and phenotype in the context of antimicrobial resistance.

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