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A Molecular and Genetic Study of Antibiotic Resistance Genes in Escherichia coli Isolated from Selected Iraqi Patients

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ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections and, as such, Escherichia coli (E. coli) is the most frequently found uropathogen. The aim of this study was to evaluate the resistance gene prevalence of bacterial isolates in 40 urine samples from patients with UTIs, as well as antibiotic resistance rates. E. coli comprised 50%; Klebsiella pneumoniae, 20%; Proteus spp., 15%; Staphylococcus spp., 10%; and Enterobacter spp., 5%. We confirmed the presence of resistance genes by molecular identification using PCR; in 90% of the isolates, we found Sul1 and in 5% dfrA. Sulfamethoxazole resistance (85%) and trimethoprim resistance (12.5%) were found by antibiotic susceptibility testing. No significant differences in bacterial prevalence or resistance patterns by gender or age were found in these results by statistical analysis. To combat the expanding threat of antibiotic resistance in UTIs, these findings highlight the need for antimicrobial stewardship programs and molecular diagnostic algorithms. Results from this study help to define the genetic mechanisms of resistance, underscore the continued need for sustained surveillance and appropriately targeted interventions to reduce the spread of resistant pathogens and improve treatment outcomes.

1. INTRODUCTION

Urinary tract infections (UTIs), in spite of being among the most frequently reported bacterial infections, continue to heavily infringe on the quality of life of millions of individuals and have a major repercussion on public health and its apparatus. Escherichia coli (E. coli), a Gram-negative bacterium which is well adapted to colonize the urinary tract, is an important cause of UTIs. It is promoted by the presence of a range of virulence factors including adhesins, toxins, and biofilm formation, which allow the pathogen to overcome the host immune response and establish persistent infections (1).

However, in recent years, the spread and emergence of antimicrobial resistance (AMR) in E. coli have escalated global burden of UTI. Recent resistance to common antibiotics, such as tetracyclines and fluoroquinolones, has diminished the effectiveness of standard treatment protocols to the point that infections last a much longer time and the risk for complications increases. The resistance is genetically determined, being mainly due to genetic factors conferring survival adavantages under antibiotic pressure (2).

The acquisition and expression of resistance genes represent a key aspect of the resistance mechanisms in E. coli. Mobile genetic elements carry genes encoding various efflux pumps, enzymatic antibiotics deactivation and alterations of target sites, which are often horizontally transferred among bacterial populations (3). These genetic adaptations have been linked to the worldwide spread of AMR, particularly in regions with high antibiotic misuse (3,4). For this study, we characterized molecularly the three genes in E. coli isolates causing UTIs in Iraq. The 16S rRNA gene is one of these and is being used as a universal marker for bacterial identification and the confirmation of E. coli. The other two genes are known to have important roles in sulphonamide and trimethoprim resistance, respectively, and may be involved in MDR. This research applies advanced molecular techniques, including polymerase chain reaction (PCR), to establish the prevalence of these genes, their relationship with antibiotic resistance pattern and their role on therapeutic outcome (5,6,7).

It is of great importance to understand the genetic basis of resistance to aid the designing of appropriate antimicrobial stewardship strategies in addition to further helping clinical management of UTIs. This study, in addition, provides a foundation for additional work in the molecular epidemiology of E. coli and its role in the global AMR crisis (8,9).

2. MATERIAL AND METHODS

Sample Collection

Over a four months period from January to April 2024, a total of 125 urine samples from patients who had been diagnosed with urinary tract infection (UTI) in Ghazi Al-Hariri Hospital, Medical City in Baghdad, were collected. Of these, 100 samples had bacterial isolates, which were analyzed for identification. Diagnostic methods at the outset were culturing on various selection media including MacConkey agar, blood agar, mannitol salt agar, and EMB agar. Preliminary bacterial identification (10,11) was done by biochemical tests i.e. urease test, KIA agar test, Simon Streat test, etc.

The bacterial isolates were identified as follows: The majority of the isolates, 50, were identified as Escherichia coli followed by 20 isolates of Klebsiella pneumoniae (20%), 15 of Proteus spp. (15%), 10 of Staphylococcus spp. (10%) and 5 of Enterobacter spp. (5%) (12,13).

The molecular identification was furthered in the VITEK2 system, to confirm that 40 out of the 50 nucleic acid source that were first identified as E. coli, in fact, was E. coli (14, 15).

Bacterial Isolation and Identification

Urine samples were cultured on MacConkey agar, blood agar, mannitol salt agar and EMB agar to isolate bacterial pathogens. Standard biochemical tests such as the urease test, KIA agar test, Simon Streat test were used to identify the Bacterial isolates. The presence of E. coli was confirmed using the VITEK 2 system for the automated identification of bacterial species in 40 of the 50 E. coli preliminary isolates revealed from biochemical testing. Next, 40 of these 40 confirmed E. coli isolates were molecularly characterized to assess antibiotic susceptibility and resistance genes. (16,17).

PCR and Gene Sequencing

Polymerase Chain Reaction (PCR) was employed to detect antibiotic resistance genes associated with E. coli. The targeted genes included 16S rRNA, dfrA (Trimethoprim resistance), and Sul1 (Sulfamethoxazole resistance) (Table 3). The primers used for amplifying these genes are listed in Table 2

PCR Procedure: Primer specific for each gene were used for the PCR reactions. The amplification conditions were optmized for each primer set, and the PCR products were analyzed by 1.5% agarose gel electrophoresis staining with Ethdium Bromide (Eth.Br) and photograph under UV light.

Table 1: The result of conventional pcr for 40 samples

Gene Name	Numbers of total samples	No. of positive samples By PCR deduction	No. of negative sample By PCR deduction
16s	40	40	0
dfrA	40	2	38
Sul1	40	36	4

Table 2: The study designed primers

Primer	Sequence (5′→3′direction)	Primer size bp	Annealing Temp. (°C)	Accession number
sul1-F	AGGACTCCTTCTTCGATGAG	508	56	NG_048081
sul1-R	CTCAAGAAAAATCCCATCCCC	508	56	NG_048081
dfrA-F	AACCTACAGTATGCGGTCTTA	203	56.2	KY366689
dfrA-R	CGGCTTGGATGTCTATTGTAG	203	56.2	KY366689
16S-F	GGTGAGTAATGTCTGGGAAAC	354	55	NR_024570
16S-R	TAACTTTACTCCCTTCCTCCC	354	55	-

Antibiotic Susceptibility Testing

Antimicrobial susceptibility was tested by the Kirby Bauer method (disk diffusion). Antibiotic discs were placed on Mueller–Hinton agar plates pre inoculated with the bacterial isolates and then tested against Sulfamethoxazole and Trimethoprim.

Statistical Analysis

SPSS (version 2019) and statistical analyses were conducted. Groups of data were made, based on gender, age and bacterial gene distribution. These groups were compared by chi square test (χ^2) with any statistically significant differences (18).

Gender Analysis: Bacterial isolation was compared between male and female patients.

Age Group Analysis: The samples were further broken down in three age groups of less than 30 years, 30–50, and more than 50. The effect of age on bacterial isolation (19,20) was tested using the Chi square test.

Bacterial Gene Analysis: The Chi-square test was used to assess the presence of antibiotic resistance genes (16S rRNA, dfrA, and Sul1) among the samples (20,21).

Antibiotic Resistance Analysis: The relative contribution of specific genes for resistance to Sulfamethoxazole and Trimethoprim relative the resistance profile for each mutant was analyzed (22,23).

Determinations of statistical significance were made at $P \leq 0.05$ and a more stringent comparison was performed at $P \leq 0.01$. Statistically significant was defined as a P value of less than 0.05.

3. RESULTS

Prevalence of Bacterial Isolates

Urine samples from patients with urinary tract infections (UTIs) were analyzed in a total of 40 samples. Of these, Escherichia coli (E. coli) was the most prevalent pathogen, present as 50% of all isolates. Isolates of Klebsiella pneumoniae were found in 20%, Proteus spp. in 15%, Staphylococcus spp. and Enterobacter spp. in 10% and 5% respectively. Therefore, the high prevalence of E. coli as a leading uropathogen in this population emphasizes its critical role Table (3,4).

Table 3: Gender an there numbers

Total numbers of all genders	Genders	Numbers of genders
40	Male	21
	Females	19

Table 4: classification of age groups

Total number of all age	Age grops	Numbers
40	Less than 30 y	10
	30-50 y	10
	More than 50 y	20

Molecular Identification of E. coli

The identity of E. coli and presence of specific antibiotic resistance genes were confirmed on all 40 isolates utilizing conventional PCR. To identify and confirm E. coli utilizing a universal approach, 16S rRNA gene was targeted whilst two resistance genes, Sul1 and dfrA were studied for their role in sulfonamide and trimethoprim resistance respectively. Agarose gel electrophoresis of amplification results was visualized for all three genes..

16S rRNA Gene

In all 40 of the E. coli isolates, the 16S rRNA gene was successfully amplified producing a band size of 354 bp, confirming our identity of Escherichia coli.

Figure 1 shows the result of the amplification in lanes 1–10, these lanes correspond to the amplified PCR product of the 16S rRNA gene.

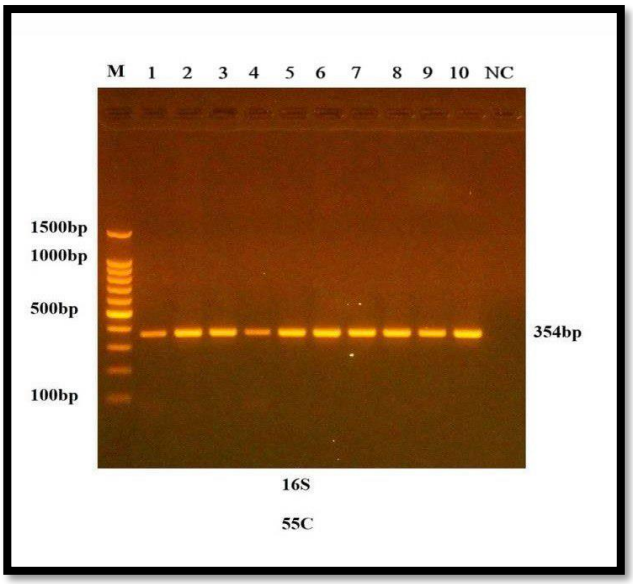

Figure 1: A 1.5% agarose gel electrophoresis of the Escherichia coli DNA amplification results of 16S rRNA genes were stained with Eth.Br, M: 100bp ladder marker. Lanes 1-10 resemble 354bp PCR

Sul1 Gene

The Sul1 gene, associated with resistance to sulfonamides, was detected in 36 out of 40 E. coli isolates. The PCR amplification produced bands of 508 bp, indicating the presence of this resistance gene in the majority of isolates. The results are shown in Figure 2, where lanes 1–17 illustrate the amplified PCR products for the Sul1 gene.

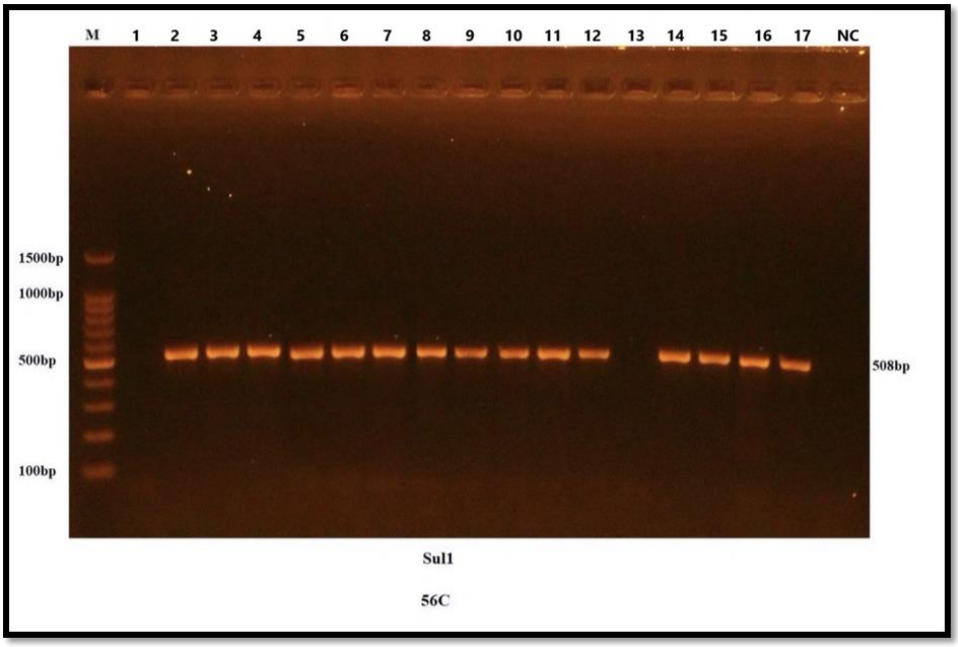

Figure 2: Results of the amplification of Sul1 genes of Escherichia coli were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-17 resemble 508bp PCR products.

dfrA Gene

The dfrA gene, related to trimethoprim resistance, was amplified in 2 out of 40 E. coli isolates. The PCR amplification produced bands of 203 bp, indicating a lower prevalence of this resistance gene. (Figure 3) displays the results, with lanes 1–9 showing the amplified PCR products of the dfrA gene. The overall PCR results for the three targeted genes are summarized in Table 5, and their statistical significance is highlighted in Table 6.

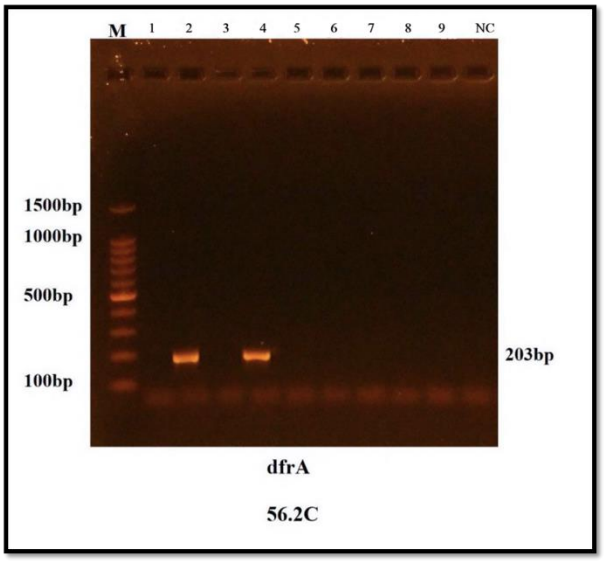

Figure 3: Results of the amplification of dfrA genes of Escherichia coli were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-9 resemble 203bp PCR products

Table 5: The result of conventional pcr for 40 samples

Gene Name	Numbers of total samples	No. of positive samples By PCR deduction	No. of negative sample By PCR deduction
16s	40	40	0
dfrA	40	2	38
Sul1	40	36	4

Table 6: Result of conventional PCR for 40 samples with difference genes

Gene Name	No. of total samples	Positive By PCR deduction No. (%)	Negative By PCR deduction No. (%)	P-value
16s	40	40 (100%)	0 (0.00%)	0.0001 **
dfrA	40	2 (5.00%)	38 (95.00%)	0.0001 **
Sul1	40	36 (90.00%)	4 (10.00%)	0.0001 **
P-value	---	0.0001 **	0.0001 **	---
** (P≤0.01).				

Antibiotic Susceptibility Testing

The antibiotic resistance profiles of the isolates were evaluated using the disk diffusion method on Mueller-Hinton agar.

Sulfamethoxazole: Resistance was observed in 85% of the isolates, while 15% showed intermediate resistance. No isolates were sensitive to this antibiotic.

Trimethoprim: A lower resistance rate was observed, with 12.5% of isolates being resistant, 25% intermediate, and 62.5% sensitive.

The AST test results for both antibiotics are summarized in Table 7. These findings suggest a high prevalence of resistance to Sulfamethoxazole and a moderate resistance to Trimethoprim.

Table7: AST test on Molar Hinton Agar with difference Antibiotics

Antibiotics name	Total No.	Resistance-R No. (%)	Intermediate-I No. (%)	Sensitive- S No. (%)	P-value
Sulfamethoxazole	40	34 (85.00%)	6 (15.00%)	0 (0.00%)	0.0001 **
Trimethoprim	40	5 (12.50%)	10 (25.00%)	25 (62.50%)	0.0003 **
* (P≤0.05), ** (P≤0.01).					

Statistical Analysis

Gender Analysis

Statistical analysis of bacterial prevalence based on gender showed no significant differences between males (21 samples) and females (19 samples). Both genders exhibited similar patterns of bacterial isolation and resistance profiles.

Age Group Analysis

The distribution of bacterial isolates across three age groups (<30 years, 30–50 years, and >50 years) revealed no statistically significant differences. Age did not appear to influence the prevalence of bacterial isolates or their resistance patterns.

Table 3 and Table 4 summarize the gender and age group distributions, respectively.

Relationship between Gene Presence and Antibiotic Resistance Patterns

The association between the presence of specific resistance genes and antibiotic resistance patterns was examined

The Sul1 gene was strongly correlated with resistance to Sulfamethoxazole, as the majority of isolates carrying this gene exhibited resistance. The dfrA gene was linked to Trimethoprim resistance but was present in only a small subset of isolates. The 16S rRNA gene, although present in all isolates, was not directly associated with resistance to any specific antibiotic. The results highlight the importance of molecular tools in understanding resistance mechanisms and their implications for treatment strategies.

4. DISCUSSION

Urinary tract infections (UTIs) remain one of the most prevalent bacterial infections worldwide, with Escherichia coli (E. coli) consistently reported as the leading uropathogen. Our study demonstrated similar findings, where E. coli accounted for 50% of the bacterial isolates, reaffirming its significant role in UTIs. These other pathogens have been identified in our study: Klebsiella pneumoniae (20%), Proteus spp. (15%), Staphylococcus spp. (10%) and Enterobacter spp. (5%) (24, 25). This distribution mirrors global trends, but with some variations in prevalence rates related to geographic, demographic and clinical factors. For instance, a study in India reported E. coli in 63.6 percent of isolates, followed by Klebsiella pneumoniae (17.2 percent), though the pattern was similar but with slightly different regional disparities. In addition to bringing attention to the confounding role of biogeographic differences among UTIs, these data emphasize the need for local studies to elucidate the microbial ecology of UTIs specific to certain populations (26,27).

In our study, E. coli isolates were molecularly identified using polymerase chain reaction (PCR) using a universal marker gene 16S rRNA for bacterial identification. The amplification of this gene in all 40 E. coli isolates, moreover, also confirmed their identity, and indicated that the molecular tools are reliable in pathogen identification. Resistances genes for Sul1 and dfrA were also detected and contributed to our understanding of the genetic basis of antibiotic resistance in the studied isolates. In 90% of the isolates the resistance determinant Sul1 was found, which suggests that this resistance determinant is widely prevalent. While the gene for trimethoprim resistance, dfrA, was seen in only 5% of isolates, this gene appeared to be less widely disseminated in the studied population. Although current studies report different levels for the dfrA gene, these findings are consistent with other studies (28, 29), which noted a prevalence of the Sul1 gene of 85% in E. coli isolates, and the dfrA gene was reported in 10%, with somewhat less dissemination for the dfrA gene (28, 29).

The antibiotic susceptibility testing in our study revealed alarming resistance rates. Sulfamethoxazole exhibited a resistance rate of 85%, while Trimethoprim showed resistance in 12.5% of the isolates, with 25% demonstrating intermediate resistance and 62.5% remaining sensitive. These results are consistent with previous research that has highlighted the growing

resistance of uropathogenic E. coli to sulfonamides. For instance, a study in Pakistan reported a resistance rate of 82% to sulfamethoxazole, closely matching our findings. However, the lower resistance rate to trimethoprim in our study contrasts with findings from Europe, where trimethoprim resistance often exceeds 30%. This discrepancy could be attributed to differences in antibiotic prescription practices, healthcare infrastructure, and the overall burden of antimicrobial resistance in different regions (30,31).

A notable finding in our study is the strong correlation between the presence of the Sul1 gene and resistance to sulfamethoxazole. Almost all isolates carrying this gene were resistant, emphasizing its role as a critical genetic determinant for sulfonamide resistance. Similarly, the dfrA gene was exclusively associated with trimethoprim resistance, albeit in a smaller subset of isolates. The results are also in agreement with other studies that show that molecular markers have predictive value for resistance phenotypic resistance. One example is the finding by a study in Iran that there was a very large association between presence of the Sul1 gene and resistance to sulfamethoxazole, which is in agreement with what we found (32, 33, 34).

In our statistical analysis we found no differences in bacterial prevalence or resistance patterns by gender or age. This is consistent with what is documented in other studies, which find similar UTI rates between males and females, especially in adults. The absence of age-related variation in resistance patterns is comparable to the lack of resistance mechanisms that are confined to specific age groups, and so are also widespread in the population. This finding underscores the broad scale challenge of antibiotic resistance and the need for such broad based interventions (.35,36,37).

Our study has concerns about the continued efficacy of these antibiotics for the treatment of UTIs because of the high prevalence of resistance to sulfamethoxazole, and the moderate resistance to trimethoprim. Many (sulfonamides and trimethoprim being commonly used in combination as first line therapies for UTI's) will lose efficacy in a significant number of cases because of resistance. For these reasons this finding underscores the need for antimicrobial stewardship programs to optimize antibiotics and curb the further spread of resistance. In addition, detection of dfrA and Sul1 resistance genes demonstrates the need for molecular diagnostics as a standard clinical tool. Such tools can rapidly and accurately identify resistance mechanisms empowering more targeted and more effective treatment strategies (38 , 39 , 40 , 41).

Finally, our study adds valuable information of microbial and genetic aspects of UTIs in our population. These results highlight the urgent need for continuing surveillance and intervention, due to continued dominance of E. coli as a uropathogen, high prevalence of resistance to sulfamethoxazole and wide dissemination of the Sul1. Although our results mirror broader patterns seen elsewhere, in line with that, our ability to do so is reliant on local data to inform public health strategies (42,43,44). Going forward, future research should concentrate on looking at different therapeutic options; why resistance develops and what can be done to reduce antimicrobial resistance. Solving these problems should help us in the management of UTIs and controlling the broader threat of antibiotic resistance (45,46,47)

5. CONCLUSION

Urinary tract infections (UTIs) continue to be a major public health problem owing to the incidence and challenges posed by antimicrobial resistance. In keeping with this, we found that Escherichia coli (E. coli) was the main uropathogen, comprising 50% of bacterial isolates. Genetic mechanisms responsible for the development of resistance to sulfamethoxazole and trimethoprim, particularly an identification of resistance genes Sul1 and dfrA, were proven valuable. The tight association of the Sul1 gene and sulamethoxazole resistance suggests it will be imperative to tailor strategies aimed at reducing this increasingly important threat.

This high resistance rate to sulfamethoxazole (85%) and moderate resistance from trimethoprim (12.5%) may suggest that these still efficacious as first line agents for the treatment of UTIs. What these findings highlight is the need to limit spread of resistance by the use of the existing drugs in the most appropriate way and with the least antibiotics used possible through best practice antimicrobial stewardship programs. In addition, no significant differences were found in resistance patterns between male and female patients and between subjects of various ages, which implies that these resistance mechanisms are fairly generalized among a variety of patient demographics.

Results from this study underscore the importance of molecular diagnostics implementation for the routine clinical practice to allow for rapid and accurate detection of resistance determinants. These tools allow more effective and more personalized treatment strategies that lessen dependence on empiric therapy and increase the likelihood of positive patient outcomes.

Overall, our findings add to the growing pool of evidence of the microbial and genetic environment of UTIs and of antimicrobial resistance. What they emphasize is the need for ongoing surveillance, enhanced diagnostics, and the introduction of new therapeutic approaches. To effectively manage UTIs and to prevent the emergence of broader threat of antibiotic resistance, we will need to address these challenges.

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