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Detection of Antibiotics Resistance E-Coli Gens Isolates From Urinary Tract Infection

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***Abstract*---**This study was completed in laboratories of Biology Department in Faculty of Science. It explains of antibiotics resistance Escherichia coli that isolated from urinary tract Infection infection patients in the province of Najaf. A total number of (225) samples were collected from patients with from hospitals and laboratories AL-Najaf Governorate, E.coli isolated from urinary tract Infection infection revealed that 80 E.coli isolates against 22 commonly used antibacterial agents by using the disk diffusion method. E. coli isolates has a great resistance to most commonly antibiotics used in treatment of urinary tract Infection, the highest rate of resistance is seen with, Ciprofloxacin, Levofloxacin 60 /80 (75 %) followed by Norfloxacine 57/80 (71.25 %) Trimethoprime-sulfamethaxazol 55/80 (68.75 %), The results showed that the(gyrA) gene was detected in E. coli isolates, from the 30 (100%) all isolates of E. coli were have(gyr A) gene, The results also showed that the (Sul1)gene was detected in E. coli isolates, from the 30 (100%) isolates of E. coli 24 (80%) were have (Sul1) gene, while the results showed four positive isolates of the Ant(2")-1a, from the 30 (100%) isolates of E. coli 4 (13.3%) were have (Ant(2")-1a) gene, Regarding erm(B) gene the result (100%) isolates of E. coli 3 (10 %) were have erm (B) gene, The results also displayed that the bla SHV was detected in E. coli isolates, from the 30 (100%) isolates of E. coli 5 (16.7%) were have bla SHV gene, While The results showed that the qnrA was detected in E. coli isolates, from the 30 (100%) isolates of E. coli 7 (23.3 %) were have qnrA gene.

***Keywords*---**esherichia coli, infection, resistance gens, urinary tract.

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Introduction

Urinary tract infection usually abbreviated (UTIs) are a significant public health problem that consider one of the common sources of infections causing systemic illness in infants and children, if not treated the complication may lead to renal scaring, end stage renal failure and hypertension, UTIs are the third most common infection after respiratory and gastrointestinal infections, It causes a significant morbidity and considerable mortality that affects about 150 million people each year worldwide (1). Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively, bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics, bacteria, when replicates even in the presence of the antibiotics, are called resistant bacteria, antibiotics are usually effective against them, but when the microbes become less sensitive or resistant, it requires a higher than the normal concentration of the same drug to have an effect (2). The development of specific mechanisms of resistance had provoked their therapeutic use, several Enterobacteriaceae strains have been isolated which are resistant to antibiotics, Gram-negative bacteria are intrinsically resistant to several antibiotic classes because of the presence of a second, OM compared to Gram-positive bacteria which these antibiotics cannot penetrate (3).

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Materials and Methods

Samples collection and bacterial identification

A total number of 225 (urine samples) were collected from patients with Urinary tract infection admitted to AL-Zhraa Hospital and AL-Hakim General Hospital in AL-Najaf Governorate, during the period from (November, 2021 to December, 2021). All samples were collected in a way to avoid any potential contamination, Swabs were taken and close it until transported to advanced Microbiology laboratory/ College of Science / University of Kufa and culturing on different media for 24 hours at cultivate 37 °C for bacterial diagnosis.

Molecular Techniques

Extraction of Genomic DNA

Genomic DNA was extracted by using a method of (4).

Molecular Identification

Gel electrophoresis was used to determine of DNA via UV trans illuminator, the primer was planned by Alpha DNA company, Canada as in table (1).

Table 1
Primers used in this study

Primer Type	Primer sequence (5'-3')	Amplicon size (pb)	Reference
sul1	F:5- CGGCGTGGGCTACCTGAACG-3	432	(5)

	R:5-GCCGATCGCGTGAAGTTCCG-3		
blaSHV	F:5- GGGTTATTCTTATTTGTCGC-3	927	
	R:5-TTAGCGTTGCCATTCCTC-3		(5)
erm(B)	F:5- GAAAAAGTACTCAACCAAATA-3	639	
	R:5-AATTTAAGTACCTTTACT-3		(6)
Ant(2")-1a	F:5- ACGCCGTGGGTCGATGTTTGAGTG-3	572	
	R:5-ACGCCGTGGGTCGATGTTTGATGT-3		(7)
qnrA	F:5- AGAGGATTTTCACGCCAGG-3	580	
	R:5-TGCCAGGCACAGATCTTGAC-3		(8)
gyrA	F:5- GGTATACCGTCGCGTACTTT-3	311	(9)
	R:5-CAACGAAATCGACCGTCTCT-3		

PCR Thermo - cycling conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

Table 2
Amplification conditions of genes were used by PCR reactions

Gene Name	Temperature (◦c) / Time					Cycles Number
	Initial	Cycling conditions			Final	
	Denaturation	Denaturation	Annealing	Extention	Extension	
sul1	94/1 min	98/30 sec	55/30 sec	72/30 sec	72/10 min	30
blaSHV	95/5 min	95/45 sec	53/45 sec	72/1min	72/7 min	35
erm(B)	95/3 min	95/30 sec	45/30 sec	72/30 sec	72/10 min	30
Ant(2)"-1a	94/5 min	94/30 sec	55/30 sec	72/1min	72/10 min	30
qnrA	95/10 min	95/1min	54/1min	72/1min	72/10 min	35
gyrA	95/2 min	95/10 sec	57/30 sec	72/1 min	72/7 min	40

Results and Discussion

Detection of genes that responsible for antibiotic resistance in *E. coli*

The results showed that the(*gyrA*) gene was detected in *E. coli* isolates, from the 30 (100%) all isolates of *E. coli* were have*(gyr* A) gene Figure (3-1), The results also showed that the (Sul1)gene was detected in *E. coli* isolates, from the 30 (100%) isolates of *E. coli* 24 (80%) were have *(*Sul1) gene Figure (3-2), while the results showed four positive isolates of the Ant(2")-1a, from the 30 (100%) isolates of *E. coli* 4 (13.3%) were have (Ant(2")-1a) gene Figure (3-3), Regarding erm(B) gene the result presented that the erm(B) gene was detected in *E. coli* isolates, from the 30 (100%) isolates of *E. coli* 3 (10 %) were have erm (B) gene Figure (3-4). The results also displayed that the blaSHV was detected in *E. coli* isolates, from the 30 (100%) isolates of *E. coli* 5 (16.7%) were have bla SHV gene, While The results showed that the *qnrA* was detected in *E. coli* isolates, from the 30 (100%) isolates of *E. coli* 7 (23.3 %) were have *qnrA* gene, Figure (3-5and 3-6).

Fluoroquinolones are a class of synthetic broad spectrum antibiotics that target the tybe ll topoisomerases (DNA gyrase and topoisomerase IV) involved in the maintenance of DNA topology, One of the main mechanisms of Fluoroquinolone resistance is amino acid substiutions in the DNA gyrase and topoisomerase IV

proteins in particular in (gyrA), Certain single mutations in (gyrA) are suffcient to generate high-level resistance to nalidixic acid, a non-fluorinated first generation quinolone. Additional mutations in (gyrA) or other type Il topoisomerase genes are, however, necessary for high-level resistance to later generations of fluoroquinolones, such as ciprofloxacin (10). Both substitutions have been associated with fluoroquinolone resistance for *E. coli* and confer higher levels of resistance than any other substitutions in the QRDR (11).

So all isolates found resistant to Ciprofloxacin were also resistant to Levofloxacin and Norfloxacine were included in our study, In addition Sulfonamides are structural analogues of paraamino benzoic acid (PABA), which competitively inhibit dihydropteroate synthetase activity, Simultaneous prescription Of dihydrofolate reductase (trimethoprim) with sulfonamides creates a synergistic antimicrobial activity in bacterial infections, Combination Of trimethoprim and sulfamethoxazole with the trade name of cotrimoxazole is the first antibiotic that has been used for the treatment of urinary tract infections (12). Dihydropteroate synthetase with low affnity for sulfonamides is encoded on a plasmid, which has highspeed transfer potential to other organisms. Furthermore, (SuL1) gene was known plasmid encoded sulfonamide resistance genes, which produce dihydropteroate synthetase (DHPs) and induce resistance against sulfonamides (13). Based on our results the (suLl) gene has the highest prevalencein *E. coli* strains resistant to cotrimoxazole Existence o (f suL1) genes in different kinds of clinical and environmental isolates indicates that these genes have a universal function Of carrying and spreading sulfonamide resistance in bacteria (14).

The (suLl) gene is part Of class I integrons in many sulfonamide resistant bacteria Class 1 integrons play an important role in antibiotic resistance dissemination in many Multidrug Resistant (MDR) Gram-negative bacteria (15), The (Ant(2")-1a)one of genes are responsible for the resistance in Aminoglycoside antibiotics block protein synthesis by targeting the A site or recognition site located in the 16S rRNA of the bacterial 30S ribosomal subunit where codon— anticodon accuracy is assessed (16). Bacteria have been furnished with various resistance mechanisms to cope with aminoglycosides, the most common beingchemically modifying aminoglycoside _modifying enzymes (AMES) (17). Beside AME genes other mechanisms of resistance include change in the bacterial membrane permeability for aminoglycoside antibiotics and increased efflux of aminoglycosides from bacterial cell to the exterior environment (18). Even though clinical applications of aminoglycosides have not completely halted, the ever-increasing resistance to all major antimicrobial drugs has once again led to an interest in these compounds, particularly their application in the treatment of Severe infections by Gram-negative bacteria (19). Acquired resistance to macrolides may result from a variety of mechanisms of resistance, several of which have already been reported in Enterobacteriaceae (20). These mechanisms include target site modification by methylases encoded by erm genes, in particular erm(A), erm(B), and erm(C). Macrolides may be inactivated by modifying enzymes first reported in Enterobacteriaceae (21), e.g., esterase encoded by ere (A) or ere (B) genes (22).

All of these genes confer full cross-resistance between erythromycin and azithromycin (23). We aimed to assess the prevalence of acquired resistance to

macrolides in commensal and clinical isolates of *E. coli* from various geographic origins and to characterize the mechanisms underlying *E. coli* resistance to macrolides, Beta-lactam antibiotics are it one of the greatest usually prescribed treatment classes with many therapeutic indications, these advent initial from the 30s of the twentieth century drastically different the scenario of the fight in contradiction of bacterial infectious diseases, the mechanism of action for this antibiotics compris, the peptidoglycan or murine is a vital basic of the bacterial cell wall this gives mechanical constancy for it, that is an very conserved constituent of within the gram-positive and gram-negative covers, the beta-lactam antibiotics prevent the latter stage in peptidoglycan creation via acylating the trans peptidase involved in cross-linking peptides to make peptidoglycan (24)(25). The goals for activities of beta-lactam antibiotics it called as penicillin-binding proteins (PBPs), the binding, in turn, interrupts the terminal transpeptidation method and gives failure of viability and lysis, and by autolytic methods with the bacterial cell, β-Lactamases are by far the greatest significant resistant prosess in Gram-negative bacilli, with the popularization of genetic techniques, an increasing number of this enzymes have been categorized different in amino acid series and hydrolytic activity for β-lactam antibiotics (26). Gram-negative bacteria, inducible appearance of β-lactamases is ordinarily originate in chromosomal β-lactamases whereas plasmid-mediated enzymes are usually constitutively expressed improvement appearance for this hydrolytic action is frequently controlled whitin promoters appear in upstream genes (27).

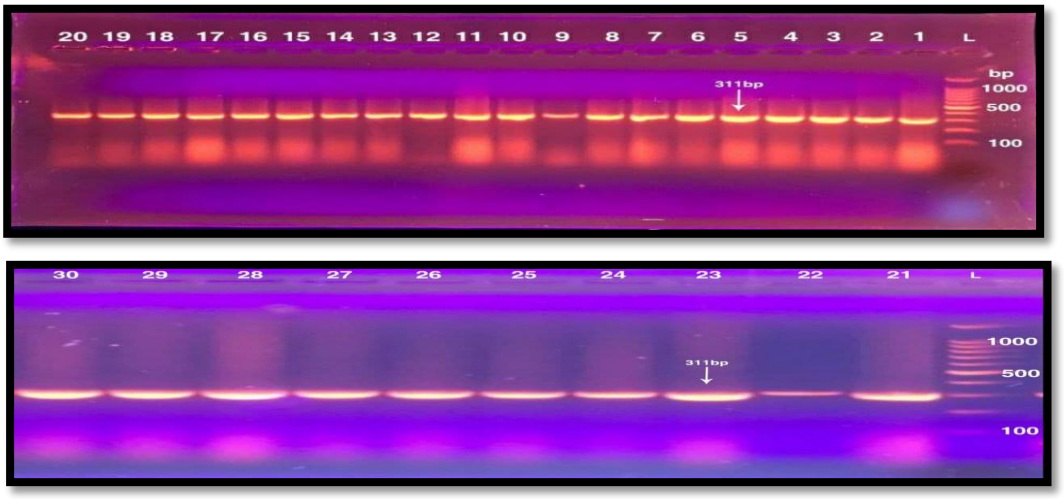

Figure 1. Agarose gel with ethidium bromide stained of mono-plex amplified product from extract DNA of *E.coli* isolates with gyrA gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , all *E. coli* isolates positive results

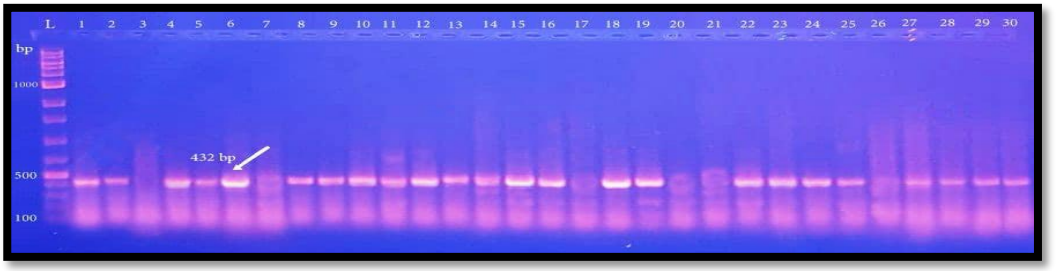

Figure 2. Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *E. coli* isolates with(Sul1) gene primers , Lane (L) DNA molecular size marker (100-bpladder) , Lane (1, 2, 4,5,6, 8,9, 10,11, 12, 13,14, 15,16 ,18,19, 22 , 23, 24, 25,27,28,29,30) show positive results

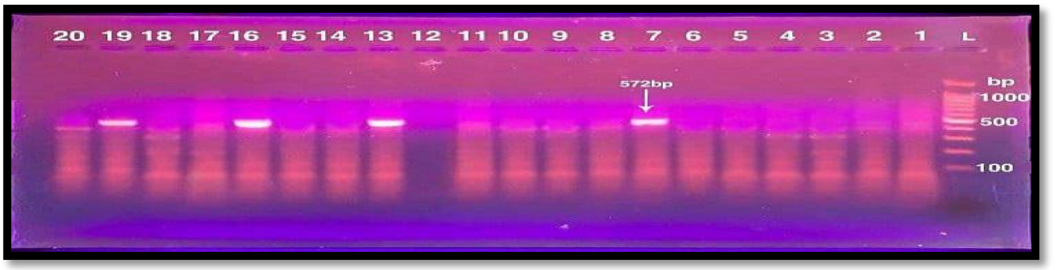

Figure 3. Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *E.coli* isolates with *Ant(2")-1a* gene primers , Lane (L) DNA molecular size marker (100-bpladder) , Lane (7, 13, 16,19) show positive results

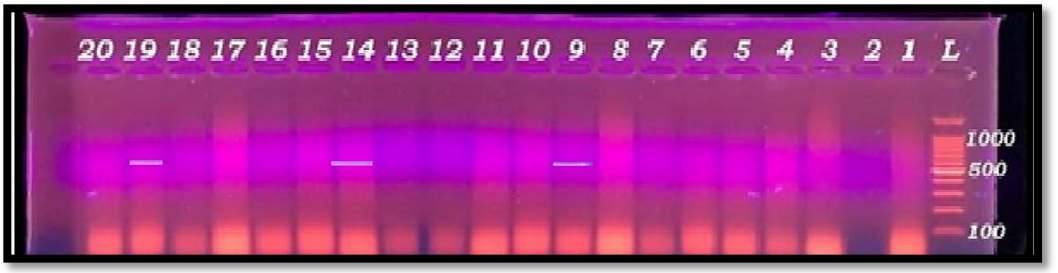

Figure 4. Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *E. coli* isolates with erm(B) gene primers, Lane (L) DNA molecular size marker (100-bpladder) , Lane (9,14,19) show positive results

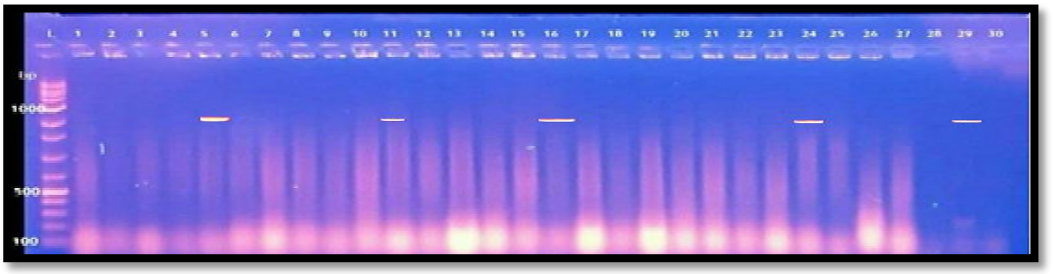

Figure 5. Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *E. coli* isolates with bla SHV, primers , Lane (L) DNA molecular size marker (100-bpladder) , Lane (5, 11,16, 24,29) show positive results

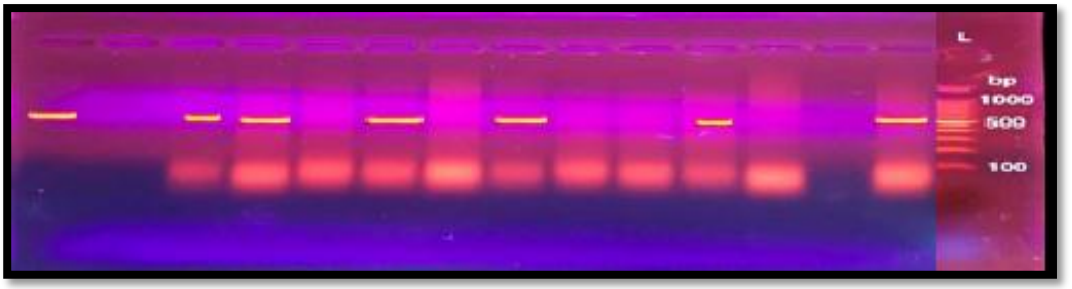

Figure 6. Agarose gel with ethidium bromide stained of mono-plex amplified product from extract DNAof E.coli isolates with *qnrA* gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (1,4,7,9,11,12,14) show positive results

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