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MAPPING OF ANTIMICROBIAL RESISTANCE GENES AND POINT MUTATIONS EXISTENCE WITHIN URO-PATHOGENIC *ESCHERICHIA COLI*

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Abstract

Bacteria cause numerous dangerous disorders with multidrug resistance (MDR). These pathogens can live in the gastrointestinal tracts and exchange mobile genetic material. *E. coli* is an example of concern encountered in clinical laboratories either as an intestinal pathogenic strain or extraintestinal pathogenic bacteria (ExPEC), specifically in urinary-tract infection (UTI) as a global public infection worldwide. Within 30 samples, women (66.66%) were a major source of Uro-Pathogenic *E. coli*. Routine disc diffusion test exposes resistance against Trimethoprim+Sulphamethoxazole (17) at 56.66%, then Ciprofloxacin and Gentamicin (14) at 46.66%. However, (9) 30.00% of Ampicillin/Sulbactam and Cefixime witnessed resistance in 16 antibiotics of different classes. Whole genome sequencing (WGS) as an instrument for investigating the resistance genes within five highly resistant strains, including 19 genes manifest β-Lactamase (bla_{TEM-1B}, $bla_{CTX-M-15}$, and bla_{OXA-1}) withstand to Cephalosporins in assorted generations. *sul1*, *sul2*, and *dfrA17, dfrA14* contribute to Trimethoprim and Sulphamethoxazole resistance. Aminoglycosides, Tetracyclines, Chloramphenicol, Fluoroquinolones and Macrolides sustain *aph(3")-Ib*, *aph(6)-Id*, *aadA5*, *tet(B)*, *catA1, qnrS1* and *mph(A)* respectively. Fluoroquinolones are facing mutations inwards *gyrA*, *parC*, and *parE*. Carbapenems are stable in each of these categories. This study's WGS identifies chromosomal point mutations and expansion of Uro-pathogenic *E. coli* resistance traits.

Keywords: Antibiotics resistance, *E. coli*, WGS, Urinary tract infection, *β*-Lactamase.

Introduction

The consequences of Bacterial antibiotic resistance are far-reaching with a global health disaster. By 2050, millions of deaths could result from Drug-resistant infections; developing countries brew approximately 90% of oracle deaths to occur (Islam *et al*., 2019; Safain *et al*., 2020). More excellent knowledge of the formation and transmission of drug-resistant bacteria and improved diagnostic techniques that assist clinicians in selecting the best antimicrobial medicines are necessary for combating this issue (Andersson *et al*., 2019). As a result of antibiotics, excessive use of strains of *Escherichia coli* (*E. coli*)become resistible to many groups; additionally, this bacteria developed Pan resistant strains which means overcoming all recorded antibiotics and widely prevising (Walsh & Toleman, 2012). Two significant reasons make gut-inhabitant *E. coli*

necessary in the Antimicrobial resistance (AMR) model. Firstly, it is the leading cause of Extra intestinal contagion (ExPEC); these strains colonize the patients' gut and act as a reservoir at the same time; secondly, the lions share AMR transferring horizontally through plasmids whether they maintain and spreading of resistance genes within species (van Schaik, 2015; Wright, 2007).

Gram-hostile members, chiefly *E. coli*, caused Urinary-tract (UTIs) and blood infections. Multiple virulence factors imply by pathogenic and commensal strains such as polysaccharides coats, iron acquisition systems, toxins, and adhesins (Sannes *et al*., 2004). Bacilli enteric *E. coli* are the primary pathogens isolated from women's genital tract. They can colonize the vagina and endocervical cavity and cause disorders for pregnant women and newborns, counting early and late neonatal sepsis and intra-amniotic and puerperal infection (Guiral *et al*., 2011).

Beta-lactamase frequency in *E. coli* strains responsible for healthcare-related UTIs and intraabdominal infections are significantly rising. Due to the excessive and broad clinical antibiotics consumption, the incidence of these resistant strains surpasses 50% in low-hygiene nations (Wang *et al.*, 2002). Trimethoprim+Sulfamethoxazole, quinolones, cephalosporins, and semisynthetic penicillins with/without beta-lactamase suppressors, are the most popular antibiotics applied to treat UTIs (Arslan *et al*., 2005). Several surveillance studies conducted in Europe, North America, and South America during the 2000s revealed that between 20 and 45 percent of Uro-Pathogenic *E. coli* (UPEC), when introduced to antibiotics of the first group, including cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, was resistance. In contrast, UPEC was relatively susceptible to these antibiotics until the late 1990s (Foxman, 2010). Multidrug resistance (MDR; resistance to at least three different antimicrobial categories) is a common indication of strains. (Magiorakos *et al*., 2012). Severe strains develop Extended Spectrum β-lactamase (ESBL), carbapenem, or fluoroquinolone resistance (WHO, 2014). In addition, it is feasible for antimicrobial-resistant genomes to evolve due to the ongoing accumulation of numerous mutations (Herring *et al*., 2006). As a result, the emergence of microorganisms' Whole Genome Sequencing (WGS) as a method for detecting the resistance of antibiotics and assessing the frequency of mutations and the functionality of the affected genes (Zeng *et al*., 2019; Ben Zakour *et al*., 2016). Moreover, WGS has significant promise for creating novel antibiotics, reducing antimicrobial resistance, improving diagnostics, and promoting public health microbiology (Hasman *et al*., 2014; Niemann *et al*., 2009).

The current study focuses on mapping antimicrobial resistance genes and point mutations within the Uro-Pathogenic *E. coli* genome using whole-genome sequencing and a web-based database for data analysis.

Materials and methods

Samples identification

In sterile containers, patients' midstream urine specimens were taken at Atatürk university research hospital (Erzurum-Türkiye) between January 2021 and April 2021 and were suspected of having UTIs. The samples were then promptly sent to the lab for microbiological analysis. Thirty samples on MacConkey and blood agar were cultured for one night of incubation at 37C°. *E. coli* selective medium (Eosin methylene blue EMB) courage our isolates to grow after incubation at 37C° within

22-24 hours. Specified colonies of our accurate identification are green metallic. The biochemical test confirmed the culture media appearance.

Antibiotics sensitivity test.

Since different *E. coli* strains have varying susceptibilities to antibiotics, we selected 16 types (Oxoid Ltd., Basingstoke, UK) from several groups (Piperacillin/tazobactam (100/10)µg , Ertapenem(10) µg, Amikacin(30) µg ,Ceftazidime(30) µg ,Ciprofloxacin(5) µg ,Gentamicin(10) µg , ceftriaxone (30) µg, Cefotaxime(30) µg , Cefuroxime (30) µg, Cefepime(30) µg, Imipenem(10) µg, Trimethoprim+Sulfamethoxazole (1.25/23.75) µg ,Ampicillin/Sulbactam(10/10) µg ,Cefixime(5) µg ,Meropenem(10) ,Levofloxacin(5) µg). Popular disk diffusion methodology Kirby Bauer was used with medium Muller Hinton agar based on the strategies of Clinical and Laboratory Standard Institute (Dolinsky, 2020). Clinicians frequently utilize antibiograms to determine susceptibility averages for antibiotic therapy as a practical tool for choice and to figure out variations in resistance inside institutions with time. Furthermore, it could compare organizational resistance rates and screen resistance trends (Hulscher *et al*., 2010).

Whole genome sequencing (WGS).

Five isolates were chosen for whole genome sequencing at the BM laboratory in Ankara, Turkey, using the criteria of antibiotic resistance (isolates 1, 2, 3, and 4,5). It was performed by the Illumina Miseq platform (San Diego, CA, USA). Reads are delivered in Fastq file format, and the FastQC analysis verifies their quality. The NCBI's blast tool determined that NZ-CP017669.1 would be a good reference strain through December 2021. The OmicsBox platform tools (via ABySS) assembler as a *de novo* assembly under company instructions.

Genomic mediated resistance

According to phenotypic resistance, we chose the five isolates that showed significant overcoming of the previously mentioned antibiotics to determine the genetic traits (genotypically) that hold resistance traits. *E. coli* ResFinder 4.1 database at Center for Genomic Epidemiology (CGE) http://genomicepidemiology.org/services/ free web tools used for finding the resistance genes and point chromosomal mutations by a prediction which includes many parameters to many groups of antibiotics. ResFinder 4.1 accepted sequence reads as assembled genomes (control strains) or raw reads (evaluation strains) through a threshold of 80% identity and 60% minimum length.

Data availability

Our data were submitted successfully in the NCBI database under Bio projects (PRJNA835456 & sequence read archive SRR19553197 for isolate (1) and PRJNA846072 & SRR 19543626, 19543627, 19543628, and 19543629 for isolates 2,3,4 and 5 respectively) for more details about them.

6- statistical analysis.

All samples go through ANOVA was used to assess statistical parameters. Critical limits were established at P<0.05when utilizing Turkey's HSD test to evaluate significant levels. For each measured variable, values are reported as means of the standard deviation (SD).

Results

Patients' Characteristics.

After receiving informed consent and meeting the criteria, 30 midstream urine samples were accumulated from patients across four months who were hospitalized at Erzurum Teaching Hospital and were highly suspected of having UTIs. The patients in table (1) ranged in age from 15 to 56 years; 20/30 (66.66%) of the study participants were females, while the remaining percentages of men were located; statistical differences were significant at p<0.05. Age group 56≥ (33.33%) exemplifies the elevated prevalence of bacterial UTI in the total samples.

Table (1): illustrates how infections are distributed among age groups.

Age group	Frequency
≤ 15	7
16	3
26	4
36	2
46	4
56≥	10
Total	**30**

Phenotypic Antibiotic Resistance.

All 30 isolates were liable to antibiotic susceptibility tests against 16 known antibiotics. In Table 2, it is noteworthy that Trimethoprim+Sulphamethoxazole (17) exhibited the highest rate of resistance, followed by Ciprofloxacin and Gentamicin (14) and Ampicillin/Sulbactam and Cefixime (9) with mild resistance percentages of 56.66 and 46.66 respectively. Piperacillin/tazobactam, Ertapenem, Imipenem, and Meropenem all demonstrated no or very little resistance from the bacterium.

Table (2): Phenotypic resistance in *E. coli*.

Antibiotic	Sensitive (%)	Moderate (%)	Resistance (%)
Piperacillin/tazobactam	(28) 93.33	(1) 3.33	(1) 3.33
Ertapenem	(29) 96.66	-	(1) 3.33
Meropenem	(30) 100.00	-	-
Amikacin	(23) 76.66	-	(7) 23.33
Ceftazidime	(21) 70.00	(2) 6.66	(7) 23.33
Ciprofloxacin	(16) 53.33	-	(14) 46.66
Gentamicin	(16) 53.33	-	(14) 46.66
Ceftriaxone	(22) 73.33	(1) 3.33	(7) 23.33
Cefotaxime	(23) 76.66	(1) 3.33	(6) 20.00
Cefuroxime	(22) 73.33	-	(8) 26.66

Cefepime	(24) 80.00	-	(6) 20.00
Imipenem	(30) 100.00	-	-
Trimethoprim+Sulphamethoxazole	(13) 43.33	-	(17) 56.66
Ampicillin/Sulbactam	(21) 70.00	-	(9) 30.00
Cefixime	(21) 70.00	-	(9) 30.00
Levofloxacin	(21) 70.00	(2) 6.66	(7) 23.33

Whole genome sequencing

The output of the data from our five isolates was in Fataq format, which went one step further and was entirely forward with the bioinformatics tools. Fastqc quality checking shows that the output files from Illumine passed with flying colors and received good marks for most of the quality parameters. Table (3) explicate the quality scores.

Table (3): Quality score of *E. coli* isolates

Isolate no.	Total sequence (bp)	Seq. flagged as poor quality	Sequence length (bp)	GC %
1	4036936	0	150	50
2	3716627	0	150	50
3	4072225	0	150	50
4	3696919	0	150	50
5	4730241	0	150	50

Resistance genes.

Nowadays, there are many databases and platforms that could aid in prediction with a high level of insurance about what the genome has or haven't within its million bases, the information that talks to us about what is coming. In this study, CGE provides the exact purpose that we are looking for (Zhao *et al*., 2021). These samples had several resistance genes, according to analysis, which is consistent with the phenotypic data. Nineteen resistance genes were identified and mapped, while cephalosporines, sulfonamides, and aminoglycosides were recorded in the most incidence.

Since all of the isolates possessed resistance genes to many groups of antibiotics, a significant number of resistance genes were found in our data; for instance, *E. coli* has a multidrug resistance ability in the event of β-Lactamase development (*bla*$_{TEM-1b}$, *bla*$_{CTX-M-15}$, and *bla*$_{OXA-1}$) which are plasmid-mediated resistance of Extended-spectrum β-Lactamases across 1st, 2nd, third and fourth generations of Cephalosporins (table 4,5,6,7,8). In a similar vein with Metabolic pathway inhibitors (sulfonamides) Aminoglycosides, Tetracyclines, Chloramphenicol, Fluoroquinolones, and Macrolides (intrinsic resistance), these groups of antimicrobials implicate genes.

A substantial percentage of *E. coli* 5 strains displayed resistance genotypically to trimethoprim (*dfrA17, drfA14*) and sulfamethoxazole (*sul1*, *sul2*), which are both members of the competitive

metabolic group and come with phenotypic resistance as a high percent included in sensitivity test (56.66%). In tables (2,3,4), the presence of proteins aminoglycoside O-phosphotransferase, ANT(3")-Ia Family Aminoglycoside Nucleotidyltransferase, aminoglycoside N-acetyltransferase, and AAC(6')-Ib Family Aminoglycoside 6'-N-acetyltransferase can cause interruption to Aminoglycosides derivatives like Streptomycin, Gentamicin, and Amikacin; Kanamycin; Tobramycin respectively. Tetracyclines share the total genome through enzyme tetracycline efflux MFS transporter activity that leads to eliminating the effect of doxycycline, tetracycline, and minocycline. Viewing the table (1,2,3,5).

Four strains introduce Mph(A), family, macrolide 2'-phosphotransferase as an important protein against Macrolides antibiotics erythromycin, azithromycin, spiramycin, and telithromycin while isolates 1 and 2 present type A-1 chloramphenicol O-acetyltransferase which encoded by *catA1* as the factor of resistance towards chloramphenicol doses. Finally, with ciprofloxacin, the agent could lose activity in the occurrence of the *qnrS1* table (5) and with *aac(6')-Ib-cr* genes, which translated to quinolone resistance pentapeptide repeat protein. Carbapenems (Ertapenem, Meropenem, and Imipenem) are the only antibiotics still facing hydrolyze or other defense mechanisms employed by Uro-pathogenic *E. coli*.

Table (4): Resistance genes occurrence in isolate 1

Resistance gene	Identity	Start-end	Alignment leg. / Gene leg.	Strand	Contig or depth	Phenotype	Notes
sul2	100.0	1709 - 2524	816/816	+	155.63	sulfamethoxazole	Sulfonamide-resistant dihydropteroate synthase
dfrA17	100.0	1013 - 1486	474/474	-	151.96	trimethoprim	Trimethoprim-resistant dihydrofolate reductase
tet(B)	100.0	4152 - 5357	1206/1206	+	158.24	doxycycline, tetracycline, minocycline	Tetracycline efflux MFS transporter

***bla*$_{TEM-1B}$**	100.0	81-941	861/861	+	141.39	amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	Class A broad spectrum β-lactamase
catA1	99.85	4199-4858	660/660	–	153.0	chloramphenicol	type A-1 chloramphenicol O-acetyltransferase
aph(3")-Ib	100.0	2561-3388	828/828	+	85.493	Streptomycin	Aminoglycoside O-phosphotransferase
Aph(6)-Id	100.0	3388-4224	837/837	+	85.4937	Streptomycin	Aminoglycoside O-phosphotransferase
aadA5	100.0	94-882	789/789	-	3472	Streptomycin	ANT(3'')-Ia family aminoglycoside nucleotidyltransferase

Table (5): Resistance genes occurrence in isolate 2

Resistance gene	**Identity**	**Start-end**	**Alignment leg. / Gene leg.**	**strand**	**Contig or depth**	**Phenotype**	**Notes**
mph(A)	100.0	147-1068	906/906	+	114.08	erythromycin, azithromycin, spiramycin, telithromycin	Mph(A) Family macrolide 2'-phosphotransferase
dfrA17	100.0		474/474	-	111.41	trimethoprim	trimethoprim-resistant

		8668-9141					dihydrofolate reductase
sul2	100.0	1938-2753	816/816	+	115.56	sulfamethoxazole	Sulfonamide-resistant dihydropteroate synthase
sul1	99.88	6363-7202	839/840	-	108.45	sulfamethoxazole	Sulfonamide-resistant dihydropteroate synthase
tet(B)	100.0	970-2175	1206/1206	+	108.45	doxycycline, tetracycline, minocycline	Tetracycline efflux MFS transporter
bla$_{TEM-1B}$	100.0	598-1458	861/861	+	106.17	amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	class A broad-spectrum beta-lactamase
catA1	99.85	105-764	660/660	+	134.88	chloramphenicol	Type A-1 chloramphenicol O-acetyltransferase
aadA5	100.0	7749-8537	789/789	-	64.023	Streptomycin	ANT(3")-Ia family aminoglycoside nucleotidyltransferase
aac(3)IId	100.0	6540-7400	861/861	-	65.042	Gentamicin	Aminoglycoside N-acetyltransferase
aph(3")-Ib	100.0	2790-3617	828/828	+	64.877	Streptomycin	Aminoglycoside O-phosphotransferase
aph(6)-Id	100.0	3617-4453	837/837	+	64.87760	Streptomycin	Aminoglycoside O-phosphotransferase

Table (6): Resistance genes occurrence in isolate 3

Resistance gene	Identity	Start-end	Alignment leg. / Gene leg.	Strand	Contig or depth	Phenotype	Notes
Aac(6')-Ib-cr	100.0	169-723	600/600	+	85.83	Amikacin; Kanamycin; Tobramycin	AAC(6')-Ib family aminoglycoside 6'-N-acetyltransferase
aac(3)-IIe	100.0	121-981	861/861	+	70.83	Gentamicin	Aminoglycoside N-acetyltransferase
aadA5	100.0	1780-2568	789/789	-	57.088	Streptomycin	ANT(3")-Ia family aminoglycoside nucleotidyltransferase
Mph(A)	100.0	146-1067	906/906	+	152.35	Erythromycin, Azithromycin, Spiramycin, Telithromycin	Mph(A) Family macrolide 2'-phosphotransferase
Sul1	99.64	394-1233	837/840	-	91.67	Sulfamethoxazole	Sulfonamide-resistant dihydropteroate synthase
dfrA17	100.0	2699-3172	474/474	-	92.96	Trimethoprim	Trimethoprim-resistance dihydrofolate reductase
tet(B)	100.0	2077-3282	1206/1206	+	144.52	Doxycycline, Tetracycline, Minocycline	Tetracycline efflux MFS transporter
bla$_{OXA-1}$	100.0	854-1684	831/831	+	91.99	Amoxicillin, Amoxicillin+clavulanic acid,	Oxacillin-hydrolyzing class D β-lactamase

						Ampicillin, Ampicillin+clavulanic acid, Cefepime, Piperacillin, Piperacillin+tazobactam	
***bla*$_{CTX-M-15}$**	100.0	2713-3588	876/876	-	106.36	Amoxicillin, Ampicillin, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Piperacillin, Ticarcillin	Class A extended-spectrum β-lactamase

Table (7): Resistance genes occurrence in isolate 4

Resistance gene	**Identity**	**Start-end**	**Alignment leg. / Gene leg.**	**Strand**	**Contig or depth**	**Phenotype**	**Notes**
mph(A)	100.0	5798-6719	906/906	-	95.39	erythromycin, azithromycin, spiramycin, telithromycin	Mph(A) family macrolide 2'-phosphotransferase
sul2	100.0	1785-2600	816/816	-	131.5	sulfamethoxazole	sulfonamide-resistant dihydropteroate synthase
dfrA14	100.0	1635-2108	474/474	+	96.85	Trimethoprim	trimethoprim-resistant dihydrofolate reductase
***bla*$_{TEM-1B}$**	100.0	177-1037	861/861	+	233.99	amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	class A broad-spectrum beta-lactamase

***bla*$_{CTX-M-15}$**	100.0	64573 - 65448	876/876	-	162.8	amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, ticarcillin	class A extended-spectrum beta-lactamase
aph(3')-Ia	100.0	164-979	816/816	+	83.711	Kanamycin	aminoglycoside O-phosphotransferase
aph(6)-Id	100.0	85-921	837/837	-	79.1575	Streptomycin	aminoglycoside O-phosphotransferase
aph(3'')-Ib	100.0	921-1748	828/828	-	79.157	Streptomycin	aminoglycoside O-phosphotransferase

Table (8): Resistance genes occurrence in isolate 5

Resistance gene	**Identity**	**Start-end**	**Alignment leg. / Gene leg.**	**Strand**	**Contig or depth**	**Phenotype**	**Notes**
mph(A)	100.0	146-1067	906/906	+	115.43	erythromycin, azithromycin, spiramycin, telithromycin	Mph(A) family macrolide 2'-phosphotransferase
qnrS1	100.0	9738-10394	657/657	+	155.7	ciprofloxacin	quinolone resistance pentapeptide repeat protein
sul1	100.0	20991 - 21830	840/840	-	118.14	sulfamethoxazole	sulfonamide-resistant

							dihydropteroate synthase
sul2	100.0	16029-16844	816/816	+	227.92	sulfamethoxazole	sulfonamide-resistant dihydropteroate synthase
dfrA17	100.0	22401-22874	474/474	-	126.98	Trimethoprim	trimethoprim-resistant dihydrofolate reductase
tet(A)	99.84	9747-10946	1275/1275	-	107.5	doxycycline, tetracycline	tetracycline efflux MFS transporter
bla$_{TEM-1B}$	100.0	1-769	861/861	-	155.31	amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	class A broad-spectrum beta-lactamase
bla$_{CTX-M-15}$	100.0	4222-5097	876/876	+	142.81	amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, ticarcillin	class A extended-spectrum beta-lactamase

A point mutation (table 9) from the ResFinder database established the presence of mutations in gyrase-encoding genes to overcome the activity of Quinolones. The occurrence of a single mutation in the *gyrA* gene could confer quinolones resistance, but further mutations within gyrA and/or parC are required for fluoroquinolones (Hopkins *et al*., 2005; Poriel *et al*., 2018). This is consistent with isolates 1, 2, 3, and 4 demonstrating nalidixic acid and ciprofloxacin resistance. *gyrA* (Ser ->Leu, Asp -> Asn) ascends the ratio of occurrence than *parE* (Ser -> Ala, Ile -> Leu) and *parC* (Ser -> Ile).

Table (9): Mutations and consequent changes in *E. coli* isolates

Mutation	Nucleotide change	Amino acid change	Phenotype
Isolate 1			
parC:p.S80I	agc->atc	Ser->Ile	Nalidixic acid, ciprofloxacin
gyrA:p.S83L	tcg->ttg	Ser->Leu	Nalidixic acid, ciprofloxacin
isolate 2			
gyrA:p.D87N	gac->aac	Asp->Asn	Nalidixic acid, ciprofloxacin
gyrA:p.D87N	gac->aac	Asp->Asn	Nalidixic acid, ciprofloxacin
parC:p.S80I	agc->atc	Ser->Ile	Nalidixic acid, ciprofloxacin
gyrA:p.S83L	tcg->ttg	Ser->Leu	Nalidixic acid, ciprofloxacin
parE:p.S458A	tcg->gcg	Ser->Ala	Nalidixic acid, ciprofloxacin
Isolate 3			
gyrA:p.D87N	gac->aac	Ser->Asn	Nalidixic acid, ciprofloxacin
parE:p.S458A	tcg->gcg	Ser->Ala	Nalidixic acid, ciprofloxacin
parC:p.S80I	agc->atc	Ser->Ile	Nalidixic acid, ciprofloxacin
gyrA:p.S83L	tcg->ttg	Ser->Leu	Nalidixic acid, ciprofloxacin
Isolate 4			
parE:p.I529L	att->ctt	Ile->Leu	Nalidixic acid, ciprofloxacin

Note: Ser: Serine, Ile: Isoleucine, Leu: Leucine, Asp: Aspartic acid, Asn: Asparagine, Ala: Alanine.

Discussion.

The cost of sequencing the entire genome of bacterial pathogens has significantly decreased due to the development of high-throughput sequencing technology, making sequencing commonplace in many nations (Hossain *et al*., 2020). So, we target the mapping of genes responsible for resistance. One of the most prevalent bacterial illnesses nowadays, urinary tract infections (UTIs), is thought to be caused by *E. coli* in 80–90% of cases (Ejrnæs, 2011). 73.3% of 30 samples had

resistance to two up to thirteenth antibiotics of many classes (even though belonging to the same class) that were investigated in this research. The majority of specimens were taken from females (66.66 %). Half of the Women undergo UTI throughout their lives, which may be caused by anatomical variances and physical causes (Al-Badr & Al-Shaikh, 2013; Fazly Bazzaz *et al*., 2021). The drug-resistant strains have increased, consequently, unnecessary antibiotic utilization (Xu *et al*., 2011). Additionally, known as one of the primary contributors to nosocomial, gastrointestinal, and extraintestinal disorders (Kaper *et al*., 2004), also over the world, maintains mortality and morbidity. A report in 2015 from the world health organization (WHO) declared *E. coli* sickens 550 million people yearly, 230,000 of whom go on to die (Mehlhorn, 2015). (17) 56.66% of UPEC isolates exhibited resistance to Trimethoprim+Sulphamethoxazole. It was manifested in 18 European nations in 2018; isolates' resistance to Trimethoprim+Sulfamethoxazole touched 32.7% in the range between 23.1-56.2% to *E. coli* collected from urinal threats (Critchley *et al*., 2020). In addition, Ciprofloxacin and Gentamicin have close to 46.66% of which nearby the findings of Alabsi *et al*., 2014 and López-Banda *et al*., 2014 and Giray *et al*., 2012 who stated that *E. coli* resist ciprofloxacin settled in 62.3% and 47% respectively.

The prevalence of β-Lactamase (*bla*$_{TEM-1b}$, *bla*$_{CTX-M-15}$, and *bla*$_{OXA-1}$) towards the item's Amoxicillin, Ampicillin, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Piperacillin, and Ticarcillin via WGS in the current study comes with Tadesse *et al*., 2012 cases indicated the β-lactam resistance rises massively to many classes and more drugs.

Quinolone structure contains a piperazine ring, targeted by *aac(6')-Ib-cr* gene activity and acetylation of an amino group. On the occasion of one exception in Levofloxacin that plugged the amino group (Cattoir & Nordmann, 2009), a mechanism resistance mediated via plasmid against quinolones eventually explained the attendance of resistance phenotypically in the excessive manner (46.66%). Moreover, the peptide coded by *the qnr* gene family can preserve DNA gyrase or DNA topoisomerase IV complexes to restricted through quinolones (Fàbrega *et al*., 2009), and studies in turkey conferred that there is a relation between *qrnS* and *aac(6')-Ib-cr* which slightly going here (Nazik *et al.,* 2011). Furthermore, current research aligned with Azeez *et al.,* 2018 when they concluded within ESBL *E. coli* that *aac(6')Ib-cr* has more incidence in Türkiye than *qrnS* genes and their family as a standard gene causing quinolones resistance, but in the case of much-isolated number. Antimicrobial medication's importance for healing human infections involves Fluoroquinolones and Quinolones. All bacteria could eradicate via them. The acquired resistance in susceptible bacteria against quinolones generally consists of single-stage spontaneous chromosome mutations. Chromosome mutations are generally revealed in two forms; the first is a modification in subunits of DNA gyrase and DNA topoisomerase IV a target enzymes of quinolones, and the second is the degradation of membrane permeability (Heeb *et al*., 2011). Gyrase enzyme, built from 2 subunits gyrA and two subunits gyrB. Topoisomerase IV includes two parC and two parE subunits already attacked by GNB (Hopkins *et al*., 2005). Point mutation recorded Ser83Leu of *gyrA,* Ser80Ile *parC*, and Ser458Ala *parE* in Spain with high prevalence and increased MICs for quinolones (Sorlozano *et al*., 2007). Now it is clear that the recorded

mutations (table9) support the ability of *E. coli* strains to block any practical ways of DNA targeting antimicrobials.

Imipenem, Meropenem, and Ertapenem (0.00–3.3 % resistance) of the 16 antibiotics examined here may be deemed to continue tough; nonetheless, for no more extended period before prolonged exposure to Imipenem shall start resistance this last effective antibiotic. Enterobacteriaceae has by now developed carbapenem resistance, according to recent reports (Li *et al*., 2018).

Conclusion

This paper affirms that phenotypic and genotypic resistance mapping data enables Uro-Pathogenic *E. coli* to resist various antibiotics (sulfonamides, quinolones, and cephalosporins). Point chromosomal mutations increased the accuracy resistances and decreased the treatment of choice. Once new drugs are developed, mutations limit these efficacies and effectiveness.

Statement and declaration

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References

Al-Badr, A., & Al-Shaikh, G. (2013). Recurrent urinary tract infections management in women: A review. *Sultan Qaboos University Medical Journal*, *13*(3), 359–367. https://doi.org/10.12816/0003256

Alabsi, M. S., Ghazal, A., Sabry, S. A., & Alasaly, M. M. (2014). Association of some virulence genes with antibiotic resistance among uropathogenic Escherichia coli isolated from urinary tract infection patients in Alexandria, Egypt: A hospital-based study. *Journal of Global Antimicrobial Resistance*, *2*(2), 83–86. https://doi.org/10.1016/j.jgar.2014.01.003

Andersson, D. I., Nicoloff, H., & Hjort, K. (2019). Mechanisms and clinical relevance of bacterial heteroresistance. *Nature Reviews Microbiology*, *17*(8), 479–496. https://doi.org/10.1038/s41579-019-0218-1

Arslan, H., Azap, Ö. K., Ergönül, Ö., Timurkaynak, F., Aydin, K., Bakir, M., Balaban, N., Baykam, N., Çağir, U., Çaylan, R., Elaldi, N., Erdoğan, H., Fincanci, M., Hasimoğlu, R., Izat, A., Karabay, O., Karaman, S., Mutlu, S., Özakim, C., … Willke-Topçu, A. (2005). Risk factors for ciprofloxacin resistance among Escherichia coli strains isolated from community-acquired urinary tract infections in Turkey. *Journal of Antimicrobial Chemotherapy*, *56*(5), 914–918. https://doi.org/10.1093/jac/dki344

Azeez, D. A., Fındık, D., Türk Dağı, H., & Arslan, U. (2018). Konya, Türkiye'de Escherichia coli klinik izolatlarında plazmid aracılı fluorokinolon direnci. *Cukurova Medical Journal*, *43*(2), 295–300. https://doi.org/10.17826/cumj.341637

Ben Zakour, N. L., Alsheikh-Hussain, A. S., Ashcroft, M. M., Khanh Nhu, N. T., Roberts, L. W., Stanton-Cook, M., Schembri, M. A., & Beatson, S. A. (2016). Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of Escherichia coli ST131. *MBio*, *7*(2). https://doi.org/10.1128/mBio.00347-16

Cattoir, V., & Nordmann, P. (2009). Plasmid-Mediated Quinolone Resistance in Gram-Negative Bacterial Species: An Update. *Current Medicinal Chemistry*, *16*(8), 1028–1046. https://doi.org/10.2174/092986709787581879

Critchley, I. A., Cotroneo, N., Pucci, M. J., Jain, A., & Mendes, R. E. (2020). Resistance among urinary tract pathogens collected in Europe during 2018. *Journal of Global Antimicrobial Resistance*, *23*, 439–444. https://doi.org/10.1016/j.jgar.2020.10.020

Dolinsky, A. L. (2020). M100 Performance Standards for Antimicrobial Susceptibility Testing. In *Journal of Services Marketing* (Vol. 8, Issue 3). http://www.emeraldinsight.com/doi/10.1108/08876049410065598

Ejrnæs, K. (2011). Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. *Danish Medical Bulletin*, *58*(4), B4187. http://www.ncbi.nlm.nih.gov/pubmed/21466767

Fàbrega, A., Madurga, S., Giralt, E., & Vila, J. (2009). Mechanism of action of and resistance to quinolones. *Microbial Biotechnology*, *2*(1), 40–61. https://doi.org/10.1111/j.1751-7915.2008.00063.x

Fazly Bazzaz, B. S., Darvishi Fork, S., Ahmadi, R., & Khameneh, B. (2021). Deep insights into urinary tract infections and effective natural remedies. *African Journal of Urology*, *27*(1). https://doi.org/10.1186/s12301-020-00111-z

Foxman, B. (2010). The epidemiology of urinary tract infection. *Nature Reviews Urology*, *7*(12), 653–660. https://doi.org/10.1038/nrurol.2010.190

Giray, B., Uçar, F. B., & Aydemir, S. Ş. (2012). Characterization of uropathogenic Escherichia coli strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir. *Turkish Journal of Medical Sciences*, *42*(SUPPL.1), 1328–1337. https://doi.org/10.3906/sag-1201-31

Guiral, E., Bosch, J., Vila, J., & Soto, S. M. (2011). Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: Relationship with virulence. *FEMS Microbiology Letters*, *314*(2), 170–173. https://doi.org/10.1111/j.1574-6968.2010.02160.x

Hasman, H., Saputra, D., Sicheritz-Ponten, T., Lund, O., Svendsen, C. A., Frimodt-Moller, N., & Aarestrup, F. M. (2014). Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *Journal of Clinical Microbiology*, *52*(1), 139–146. https://doi.org/10.1128/JCM.02452-13

Heeb, S., Fletcher, M. P., Chhabra, S. R., Diggle, S. P., Williams, P., & Cámara, M. (2011). Quinolones: From antibiotics to autoinducers. *FEMS Microbiology Reviews*, *35*(2), 247–274.

https://doi.org/10.1111/j.1574-6976.2010.00247.x

Herring, C. D., Raghunathan, A., Honisch, C., Patel, T., Applebee, M. K., Joyce, A. R., Albert, T. J., Blattner, F. R., Boom, D. Van Den, Cantor, C. R., & Palsson, B. Ø. (2006). *Comparative genome sequencing of Escherichia coli allows observation of bacterial evolution on a laboratory timescale*. *38*(12), 1406–1412. https://doi.org/10.1038/ng1906

Hopkins, K. L., Davies, R. H., & Threlfall, E. J. (2005). Mechanisms of quinolone resistance in Escherichia coli and Salmonella: Recent developments. *International Journal of Antimicrobial Agents*, *25*(5), 358–373. https://doi.org/10.1016/j.ijantimicag.2005.02.006

Hossain, M., Tabassum, T., Rahman, A., Hossain, A., Afroze, T., Momen, A. M. I., Sadique, A., Sarker, M., Shams, F., Ishtiaque, A., Khaleque, A., Alam, M., Huq, A., Ahsan, G. U., & Colwell, R. R. (2020). Genotype-phenotype correlation of β-lactamase-producing uropathogenic Escherichia coli (UPEC) strains from Bangladesh. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-71213-5

Hulscher, M. E. J. L., Grol, R. P. T. M., & Meer, J. W. M. Van Der. (2010). *Antibiotic prescribing in hospitals : a social and behavioural scientific approach*. *10*(March). https://doi.org/10.1016/S1473-3099(10)70027-X

Islam, S., Aldstadt, J., & Aga, D. (2019). Global antimicrobial resistance: a complex and dire threat with few definite answers. *Tropical Medicine and International Health*, *24*(6), 658–662. https://doi.org/10.1111/tmi.13230

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology*, *2*(2), 123–140. https://doi.org/10.1038/nrmicro818

López-Banda, D. A., Carrillo-Casas, E. M., Leyva-Leyva, M., Orozco-Hoyuela, G., Manjarrez-Hernández, Á. H., Arroyo-Escalante, S., Moncada-Barrón, D., Villanueva-Recillas, S., Xicohtencatl-Cortes, J., & Hernández-Castro, R. (2014). Identification of virulence factors genes in escherichia coli isolates from women with urinary tract infection in Mexico. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/959206

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mehlhorn, H. (2015). Food-Borne Disease Burden Epidemiology Reference Group. *Encyclopedia of Parasitology*, 1–1. https://doi.org/10.1007/978-3-642-27769-6_3884-1

Nazik, H., Bektöre, B., Öngen, B., Ilktaç, M., Özyurt, M., Kuvat, N., Baylan, O., Keküllüoğlu, H., Haznedaroğlu, T., & Keleşoğlu, F. M. (2011). Plasmid-mediated quinolone resistance genes in Escherichia coli urinary isolates from two teaching hospitals in Turkey: Coexistence of TEM, SHV, CTX-M and VEB-1 type β-lactamases. *Tropical Journal of Pharmaceutical Research*, *10*(3), 325–333. https://doi.org/10.4314/tjpr.v10i3.9

Niemann, S., Köser, C. U., Gagneux, S., Plinke, C., Homolka, S., Bignell, H., Carter, R. J., Cheetham, R. K., Cox, A., Gormley, N. A., Kokko-Gonzales, P., Murray, L. J., Rigatti, R., Smith, V. P., Arends, F. P. M., Cox, H. S., Smith, G., & Archer, J. A. C. (2009). Genomic diversity among drug sensitive and multidrug resistant isolates of Mycobacterium tuberculosis with identical DNA fingerprints. *PLoS ONE*, *4*(10). https://doi.org/10.1371/journal.pone.0007407

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). *Antimicrobial Resistance in Escherichia coli*. 1–27. https://doi.org/10.1128/microbiolspec.ARBA-0026-2017.Correspondence

Safain, K. S., Bhuyan, G. S., Tasnim, S., Hasib, S. H., Sultana, R., Islam, M. S., Mahmud-Un-Nabi, M. Al, Sarker, S. K., Noor, F. A., Rahat, A., Bhuiyan, M. A. M., Islam, M. T., Manzoor, F., Anwar, S., Leung, D., Qadri, S. S., Qadri, F., & Mannoor, K. (2020). Situation of antibiotic resistance in Bangladesh and its association with resistance genes for horizontal transfer. *BioRxiv*, 2020.04.06.027391. https://doi.org/10.1101/2020.04.06.027391

Sannes, M. R., Kuskowski, M. A., Owens, K., Gajewski, A., & Johnson, J. R. (2004). Virulence factor profiles and phylogenetic background of Escherichia coli isolates from veterans with bacteremia and uninfected control subjects. *Journal of Infectious Diseases*, *190*(12), 2121–2128. https://doi.org/10.1086/425984

Sorlozano, A., Gutierrez, J., Jimenez, A., Luna, J. D. D., & Martínez, J. L. (2007). Contribution of a new mutation in parE to quinolone resistance in extended-spectrum-β-lactamase-producing Escherichia coli isolates. *Journal of Clinical Microbiology*, *45*(8), 2740–2742. https://doi.org/10.1128/JCM.01093-07

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases*, *18*(5), 741–749. https://doi.org/10.3201/eid1805.111153

van Schaik, W. (2015). The human gut resistome. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670). https://doi.org/10.1098/rstb.2014.0087

Walsh, T. R., & Toleman, M. A. (2012). The emergence of pan-resistant gram-negative pathogens merits a rapid global political response. *Journal of Antimicrobial Chemotherapy*, *67*(1), 1–3. https://doi.org/10.1093/jac/dkr378

Wang, G., Clark, C. G., Rodgers, F. G., & Al, W. E. T. (2002). *Detection in Escherichia coli of the Genes Encoding the Major Virulence Factors , the Genes Defining the O157 : H7 Serotype , and Components of the Type 2 Shiga Toxin Family by Multiplex PCR*. *40*(10), 3613–3619. https://doi.org/10.1128/JCM.40.10.3613

WHO. (2014). Global Report on Surveillance 2014. *WHO 2014 AMR Report*, 1–8. http://www.who.int/drugresistance/documents/AMR_report_Web_slide_set.pdf

Wright, G. D. (2007). The antibiotic resistome: The nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, *5*(3), 175–186. https://doi.org/10.1038/nrmicro1614

Xu, Z., Li, L., Shirtliff, M. E., Peters, B. M., Li, B., Peng, Y., Alam, M. J., Yamasaki, S., & Shi,

L. (2011). Resistance class 1 integron in clinical methicillin-resistant Staphylococcus aureus strains in southern China, 2001-2006. *Clinical Microbiology and Infection*, *17*(5), 714–718. https://doi.org/10.1111/j.1469-0691.2010.03379.x

Zhao, Q., Shen, Y., Chen, G., Luo, Y., Cui, S., & Tian, Y. (2021). Prevalence and Molecular Characterization of Fluoroquinolone-Resistant Escherichia coli in Healthy Children. *Frontiers in Cellular and Infection Microbiology*, *11*(December), 1–8. https://doi.org/10.3389/fcimb.2021.743390