doi.org/10.36721/PJPS.2020.33.6.SUP.2759-2766.1

Molecular characterization of multidrug resistant *E. coli* associated to urinary tract infection in Taif, Saudi Arabia

Fethi Ben Abdallah[1,2*], Rihab Lagha[1,2], Badriah Al-Sarhan[1], Imed Mabrouk[1,3], Majid Alhomrani[1,4], Ahmed Gaber[1,5] and Mohamed M Hassan[1,6]

[1]Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif, Saudi Arabia
[2]Unité de Recherche: Virologie, Biotechnologie et stratégies antivirales: UR17ES30, Institut Supérieur de Biotechnologie, University of Monastir. Tunisia
[3]Faculty of medicine, University of Sousse, Tunisia
[4]Department of Clinical Laboratories Science, The Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia
[5]Department of Genetics, Faculty of Agriculture, Cairo University, Cairo, Egypt
[6]Department of Genetics, Faculty of Agriculture, Minufiya University, Shibin El Kom, Egypt

Abstract: *Escherichia coli* account approximately to 85% of the Urinary tract infection. UTI affect the different parts of the urinary tract and is considered as a common bacterial infection. The infection is caused as a consequence of the urinary tract bacterial invasion. *E. coli* were isolated from the urine of UTI patients referred to King Abdulaziz Specialist Hospital, Taif, Saudi Arabia. Antibiotics susceptibility was tested on 25 antibiotics using the disc diffusion method. The prevalence of antibiotics resistance genes was realized by Polymerase Chain Reaction (PCR). Results showed heterogenicity in the percentage of antibiotics resistance from 100% for penicillin to 2% for imipenem. 30% of the isolates appeared as for Extended-Spectrum Beta-Lactamase (ESBL) positive and 74% are multidrug resistance strains. Distribution of antibiotic resistance genes showed that *aac(3)-IV* and *blaSHV* genes were identified in 33.33% of isolates. In addition, *qnrA*, *blaCMY* and *dfrA1* genes were founded in 37.25%, 19.60% and 17.64% of the isolates respectively. In total, 17 different genotypes were detected, and 12 isolates (24%) do not include any genes in their genomes. Multi-drug resistant *E. coli* have antibiotics profiles highly variable and the mechanism of resistance was not correlated to the investigated genes.

Keywords: *Escherichia coli*, urinary tract infection, antibiotic-resistance, resistance genes, PCR.

INTRODUCTION

Urinary tract infections (UTIs) are serious health affecting problems worldwide (Ozturk and Murt, 2020). *E. coli, E. faecalis, K. pneumoniae, S. marcescens, P. aeruginosa, S. saprophyticus, S. aureus*, and *Proteus mirabilis* are the most common bacteria causing UTIs in human beings. The *E. coli* accounts for approximately 85% of community-acquired UTIs and 50% of hospital-acquired UTIs (Fatima *et al.,* 2020). Different factors like age, gender, immuno-suppression, and urological instruments may affect the prevalence of UTIs (Lagha *et al*., 2019). Catheter-associated UTIs are one of the most common healthcare-associated infections (Letica-Kriegel *et al*., 2019).

In Saudi Arabia, UTIs have increased in recent years, and the predominant organisms associated with UTI in this country are Gram-negative bacteria especially *E. coli* and *K. pneumoniae*, which are highly resistant to commonly used oral agents (Alanazi *et al*., 2018).

Discovery of antibiotics was one of the significant advances of modern medicine, but the availability and increased use of antibiotics gradually lead to microbial resistance to them (Hutchings *et al.,* 2019). Antimicrobial resistance is growing around the world, especially in developing countries (Ayukekbong *et al*., 2017). According to Ferri *et al.* (Ferri *et al.,* 2017), antimicrobial resistance became a global threat to public health systems in the world, which is a threat to modern medicine. Due to the widespread use of antibiotics, many bacteria have developed resistance mechanisms to several antibiotics (Li and Webster, 2018). In Europe, the antimicrobial resistance of Gram-negative bacteria is increasing, especially *E. coli*, which accounts for the majority of invasive Gram-negative strains (Lüthje *et al*., 2016). It is now more challenging to treat *E. coli* infections. The shift from TMP-SMX to fluoroquinolones in most of the treatment regimens has increased the resistance to fluoroquinolones (Punjabi *et al*., 2019).

The resistance of uropathogenic *E. coli* to various antibiotics has been reported for beta-lactams, co-trimoxazole, quinolones, gentamicin, amikacin, cefuroxime and nalidixic acid (Nairoukh *et al*., 2018; van Driel *et al*., 2019). However, these antibiotics sensitivity patterns may vary in different geographical locations. UTI caused by multidrug-resistant (MDR) *E. coli* increases the cost of treatment (Mutters *et al*., 2018).

This study aimed to (i) Isolate and identify *E. coli* associated with urinary tract infections (ii) Determine

**Corresponding author:* e-mail: fetyben@tu.edu.sa

antibiotics susceptibility profiles of the isolates and (iii) Screening for antibiotic resistance genes using Polymerase Chain Reaction.

MATERIALS AND METHODS

Sampling

Fifty patients with clinical symptoms of UTI were investigated in King Abdulaziz Specialist Hospital, Taif, Saudi Arabia. There were 35 (70%) females and 15 (30%) males, with age, ranged from two months to 90 years. Clean-Catch midstream urine of the patients was collected in a sterile tube (4-5ml) and immediately transported to the laboratory for analysis. Samples were identified by a label containing information about every patient (name, age, gender) and the date and time of collection (Lagha *et al*., 2019).

Bacterial strains identification

All samples of urine were inoculated on blood agar as well as Mac-Conckey agar and incubated at 37◦C for 24 hours, and for 48 hours in negative cases. A specimen was considered positive for UTI in the light of the number of yielded colonies ($\geq 10^5$ CFU/ml). *E. coli* isolates were identified by standard biochemical tests and were confirmed using Api 20E system (Bio-merieux) (Lagha *et al*., 2019).

Antibiotics susceptibility

Antibiotics susceptibility of *E. coli* was performed according to the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2018) by the disc diffusion method using the Mueller-Hinton (MH) agar. Bacterial isolates were grown on nutrient agar at 37°C for 18-24 h. One or several colonies of the respective bacteria were transferred into saline water (0.85% NaCl) and adjusted to 0.5 McFarland turbidity standards with a DENSIMAT (Bio-merieux). The inoculums of the respective bacteria were streaked onto MH agar plates using a sterile swab, and discs were placed. Antibiotics tested were Piperacicillin-Tazobactam (PRL) 100µg; Norfloxacin (NOR) 10µg; Cefaclor (CEC) 20 µg; Ceftazidime (CAZ) 30µg; Gentamicin (CN) 10µg; Cephradine (CRD) 30µg; Cefuroxime (CXM) 30µg; Aztreonam (ATM) 30µg; Tobramycin (TOB) 10µg; Meropenem (MEM) 10µg; Kanamycin (K) 30µg; Cefotaxime (CTX) 30µg; Amikacin (AK) 30µg; Amoxicillin (AMC) 20µg; Penicillin (P) 10µg; Ceftriaxone (CRO) 30µg; Tetracycline (TE) 30µg; Ciprofloxacin (CIP) 5µg; Imipenem (IPM) 10µg; Levofloxacin (LE) 5µg; Doxycycline (DO) 30µg; Streptomycin (S) 300µg; Ampicillin (AMP) 30µg; Cephalothin (KF) 30 µg and Trimethoprim/ sulfamethoxazole (TMP-SMX).

The CLSI-ESBL phenotypic confirmatory test with ceftazidime, cefotaxime, ceftriaxone, and cefixime was performed for all the isolates by the disc diffusion method on the MH agar plates with and without 10µg of amoxiclav. Susceptibility test results were interpreted according to the criteria established by the Clinical & Laboratory Standard Institute (Clinical and Laboratory Standards institute, 2019). A minimum of 5 mm increase in the zone of the diameter of third generation cephalosporins, tested in combination with amoxiclav versus its zone when tested alone, was considered indicative of ESBL production.

Distribution of antibiotic resistance genes

DNA extraction

Isolates were cultured on nutrient agar for 24h at 37°C and then were cultured in nutrient broth for 24h at 37°C. Bacterial isolates were pelleted from 1.5ml nutrient broth and suspended in 200µl of sterile distilled water. The DNA was extracted by boiling for 5min and centrifugation at 13000 rpm for 8 min. The supernatant was used for amplification by PCR with *E. coli* primers (table 1).

Polymerase chain reaction (PCR)

PCR was performed in 20µL containing 50ng of extracted DNA, 4µL of PCR master mix (Invitrogen) and 1µL of each forward and reversed respective primers (25pM). Amplification was conducted in the Thermocycler PTC 100 (Bio-Rad). The reaction mixtures were heated at 94°C for 5 min and then subjected to 35 cycles of denaturation at 94°C for the 30s, annealing temperature is variable and depends of the gene for the 30s (table 1), and elongation at 72°C for the 30s, followed by 7 min of final extension period at 72°C. PCR products (5µL) were analyzed on 1.5% agarose gel stained with ethidium bromide (0.5mg/mL) at 90V for one h and visualized under ultraviolet transillumination. The amplification products were photographed and their sizes were determined with a 100bp molecular size marker (Life Science).

Ethics approval

Ethical approval was obtained from King Abdulaziz Specialist Hospital and the deanship of College of Sciences at Taif University.

STATISTICAL ANALYSIS

Statistical analysis was carried out using IBM SPSS (v24). Descriptive statistics were performed for all variables. To determine the relationship between age group, sex factor, antibiotic resistance pattern, and antibiotic resistance genes, the Chi-square test was performed. If statistically significant, follow up by either Cramer's V or Phi tests to measure the strength of the association. To measure the association between each resistance gene and the different antibiotic resistance patterns, Fisher's Exact Test was performed and reported.

Table 1: *E. coli* antibiotics resistant genes and primer sequences

Antibiotics	Resistance gene	Sequence	Size (bp)	Annealing temperature	References
Gentamicin	*aac(3)-IV*	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	55	Messele *et al*., 2017
Beta-lactams	*bla*SHV	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768	52	
	*bla*CMY	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462	47	
Trimethoprim	*dfrA1*	(F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC	367	45	Monroy-Pérez *et al*., 2020
Quinolones	*qnrA*	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	50	

RESULTS

Population of the study

This work was conducted on 50 patients with clinical symptoms of UTI in King Abdulaziz Specialist Hospital - Taif. The age of the UTI patients is ranging from two months to 90 years.

Based on sex, we have 35 females (70%) and 15 males (30%) (Lagha *et al*., 2019) (table 2). All the patients were hospitalized, and suffering from one or more of UTI symptoms, UTI diagnoses were established by the hospital medical staff based on clinical symptoms and laboratory investigation.

Antibiotic susceptibility

Antibiogram presented in fig. 1 showed a resistance to all antibiotics except Meropenem. Firstly, we noted that all isolates were resistant to penicillin (100%), 94% of the isolates were resistant to amoxicillin, 88% were resistant to levofloxacin and 80% were resistant to cefuroxime and ampicillin as the highest resistance to these four antibiotics respectively. The isolates also showed resistance of 70% to doxycycline, with 62% of cefotaxime, were resistant to 60% for cefaclor followed resistance 56% for each of Norfloxacin, ceftazidime, ciprofloxacin and trimethoprim/sulfamethoxazole. However, the isolates showed resistance of 50% and 48% to tobramycin and cephalothin respectively. Resistance of 54% to ceftrixone and tetracycline has been demonstrated. However, the weakest resistance was observed in cases of imipenem (2%), amikcacin (4%), streptomycin (18%) and gentamicin (30%). In addition, the highest intermediate resistance was observed in cases of cefaclor (40%), cephradine (36%) and ceftazidime (30%). Furthermore, for Extended Spectrum Beta-Lactamase (ESBL) we noted that 30% of the isolates appeared as ESBL positive.

Antibiotic resistance patterns of *E. coli* isolated from UTI patient are summarized in table 3. Firstly, out of the 50 isolates we noted the existence of 46 profiles. In addition, 37 isolates are considered as multidrug resistance strains. However, only the strain number 21 isolated from child male is resistant to only one antibiotic, which is Penicillin. Whereas, the highest resistance was observed in the strain number 27 isolated from male child and isolates number 44 (adult male sample) with resistance to 21 and 20 antibiotics respectively of the 25 tested antibiotics. In addition, the strains (17, 18), (1, 22), (8, 50) and (5, 20) isolated from different specimen had the same antibiotics resistance pattern. Finally, the reference *E. coli* ATCC 25922 had a profile with resistance to 5 antibiotics.

Table 2: Age category across gender of the patients

		Sex		Total
		Female	Male	
Age category	<10	4	3	7
	11-30	8	0	8
	31-50	14	3	17
	51-70	7	4	11
	>70	2	5	7
Total		35	15	50

Statistical analysis showed significant difference between Trimethoprim-sulfamethoxazole resistance and sex of the patient using Chi-square test; X2 (2, N=50) =4.468, P=0.035. Further no other association between resistance to antibiotics and gender have been detected.

For the chi-square test for independence, the relation between age groups and Doxycycline, Cefotaxime and Tetracycline resistance were statistically significant. In addition, a moderate strength of association has detected between age groups and these antibiotics. For Doxycycline:X2 (4, N=50) =10.092, P=0.039. Phi test for measure of association strength =0.448, P=0.039 which indicates a moderate strength of association. For Cefotaxime:X2 (4, N=50) =10.505, P=0.033. Cramer's V test for measure of association strength =0.458, P=0.035 which indicates a moderate strength of association. For Tetracycline: X2 (4, N=50) =4.468, P=0.042. Cramer's V test for measure of association strength =0.445, P=0.042 which indicates a moderate strength of association. There were no association between age groups and other resistance for any of the rest of antibiotics.

PCR detection of antibiotic resistance genes in E. coli

PCR results showed that *aac(3)-IV* and *blaSHV* genes were identified in 33.33% of isolates. In addition, *qnrA*, *blaCMY* and *dfrA1* genes were founded in 37.25%, 19.60% and 17.64% of the isolates respectively. *aac(3)-IV* and *dfrA1* genes are associated with the resistance to Gentamicin and Trimethoprim respectively. Further, *blaCMY* and *blaSHV* genes are associated with the resistance to cephalosporins and Penicillins respectively. *qnrA* gene is associated with the resistance to Norfloxacin.

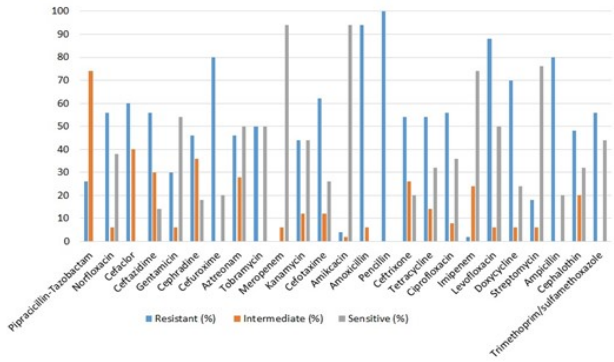

Fig. 1: Antibiotics susceptibility of *E. coli* isolates

According to table 4, we have detected 17 different genotypes. Some isolates harbor only one gene, but other isolates contain two, three and four genes. In addition, 12 isolates (24%) do not include any genes in their genomes. Further, *E. coli* ATCC 25922 harbors *blaSHV*, *blaCMY*, *dfrA1* and *qnrA* genes.

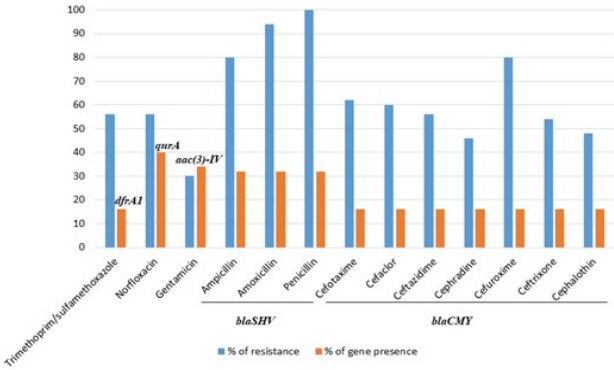

Fig. 2: Correlation between resistance profiles and presence of antibiotics resistance genes in *E. coli* isolates.

Of the 50 *E. coli* isolates tested, 56% were resistant to trimethoprim/sulfamethoxazole, 56% were resistant to norfloxacin and 30% were resistant to gentamicin, in agar disk diffusion assays and these resistance properties were associated with 16%, 40% and 34% prevalence of the *dfrA1*, *qnrA* and *aac(3)-IV* genes, respectively. In addition, 32% of the isolates harbor the *blaSHV* gene and demonstrated a resistance of 80% to ampicillin, 94% to amoxicillin and 100% to penicillin. Furthermore, the *blaCMY* gene was detected in 16% of the isolates who showed a higher resistance to cephalosporins antibiotics (fig. 2).

Fisher's Exact test showed a statistically significant inverse association between Cefuroxime resistance and the presence of *blaCMY* gene, X2 (2, N=50) = 5.357, P=0.041. Further, no other significant associations were found between the investigated resistance genes and other antibiotics.

DISCUSSION

Urinary tract infections are among the most frequent infections in humans (Öztürk and Murt, 2020). At least 80-90% of outpatient and 30-50% of inpatient urinary tract infections are caused by uropathogenic *E. coli* (Abduzaimovic *et al*., 2016). Specific human populations are at increased risk of developing urinary tract infections (UTI). In this work, we have isolated 50 *E. coli* strains from patients with clinical symptoms of UTI referred to King Abdulaziz Specialist Hospital, Taif, Saudi Arabia. The majority of the sample were adult females. The adult females have a higher prevalence of UTI than males. These results are similar to what was reported by Medina et al. (Medina *et al.,* 2019) and Sewify et al. (Sewify *et al.,* 2016). This prevalence is principally owing to anatomic and physical factors.

All the isolates have subjected to antibiotics, and results showed resistance to all antibiotics except meropenem. The percentage of resistance is variable and is ranged from 100% (Penicillin) to 2% (imipenem). The higher rate of resistance was observed in cases of amoxicillin, levofloxacin, cefuroxime, ampicillin, doxycycline. Our results are in agreement with those conducted by Faour-Klingbeil *et al.* (2016) except for gentamicin. They found higher levels of resistance with tetracycline, ampicillin, sulphonamides, gentamicin but lower for cephalothin and streptomycin. According to Singh et al. (Singh *et al*., 2018) the prevalence of the resistance in *E. coli* isolates was observed at high levels with the older antimicrobials (tetracycline, doxycycline, sulphonamides, ampicillin), at low levels with newer antimicrobials (cephalothin, norfloxacin and ofloxacin) and at the intermediate levels with streptomycin, gentamicin and neomycin.

In this study, all *E. coli* isolates were resistant to beta-lactam antibiotics. Similar study showed the same results (Adamus-Białek *et al*., 2018) which is an indication to stop their use as an empiric treatment mainly in developing countries where there are no or little efficient antibiotic stewardship programs (Srinivasan, 2017). Quinolones, mainly ciprofloxacin, were used in the treatment of different *E. coli* infections. Previous research By Malekzadegan et al. (Malekzadegan *et al*., 2019) showed sensitivity to quinolones in UTI treatment. In our study, *E. coli* was resistant to ciprofloxacin (56%).

Table 3: Antibiotics Resistance patterns of *E. coli* isolates

Isolates	Antibiotic Resistance pattern	Antibiotics Number
1,22	NOR, CEC, CAZ, CRD, CXM, ATM, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	15
2	CEC, CXM, K, CTX, AMC, P, TE, DO, AMP	9
3	NOR, CN, TOB, K, AMC, P, CRO, TE, CIP, DO, AMP	11
4	NOR, CRD, CXM, TOB, K, AMC, P, TE, CIP, LE, DO, AMP	12
5,20	NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	18
6	NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AK, AMC, P, CRO, TE, CIP, LE, DO, AMP	19
7	AMC, P, CRO, S, AMP	5
8,50	NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, S, AMP	19
9	CXM, CTX, AMC, P, CRO, CIP	6
10	NOR, CXM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	13
11	AMC, P, TE, DO, S, AMP	6
12	K, AMC, P, S, AMP	5
13	NOR, CXM, AMC, P, DO	5
14	PRL, CEC, CXM, TOB, CTX, AMC, P, AMP	8
15	CEC, AMC, P, CRO, AMP	5
16	NOR, CEC, CAZ, CRD, CXM, ATM, CTX, AMC, P, CRO, CIP, LE, S, AMP	14
17,18	P, DO	2
19	PRL, CEC, CAZ, CN, CRD, CXM, ATM, TOB, CTX, AMC, P, CRO, TE, DO, AMP	15
21	P	1
23	AMC, P, CIP, DO, AMP	5
24	PRL, NOR, CEC, CAZ, CRD, CXM, ATM, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	16
25	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, CIP, LE, AMP	17
26	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, CIP, LE, DO, AMP	18
27	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AK, AMC, P, CRO, TE, CIP, IPM, LE, DO, AMP	21
28	PRL, CEC, CAZ, CN, CXM, K, AMC, P, AMP	9
29	PRL, CEC, CAZ, AMC, P, TE, DO, AMP	8
30	CXM, CTX, AMC, P	4
31	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, R, AMP	19
32	PRL, CXM, AMC, P, CRO, S, AMP	7
33	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	19
34	PRL, NOR, CEC, CAZ, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	18
35	NOR, CN, CXM, TOB, K, AMC, P, CIP, S, AMP	10
36	NOR, CAZ, CXM, AMC, P, TE, CIP	7
37	NOR, CEC, CRD, CXM, TOB, K, AMC, P, TE, CIP, LE, DO, AMP	13
38	NOR, CEC, CXM, AMC, P, CIP, LE, AMP	8
39	NOR, CAZ, CXM, ATM, K, CTX, AMC, P, CIP, LE, DO, AMP	12
40	NOR, CEC, CAZ, CRD, CXM, ATM, CTX, AMC, P, CRO, DO, AMP	12
41	NOR, CEC, CAZ, CRD, CXM, ATM, TOB, CTX, AMC, P, CRO, TE, CIP, LE, DO, AMP	16
42	CEC, CAZ, CRD, CXM, ATM, TOB, CTX, AMC, P, DO, AMP	11
43	CXM, TOB, AMC, P, TE, AMP	6
44	PRL, NOR, CEC, CAZ, CN, CRD, CXM, ATM, TOB, K, CTX, AMC, P, CRO, TE, CIP, LE, DO, S, AMP	20
45	CEC, CAZ, CXM, CTX, AMC, P, TE, DO, AMP	9
46	CXM, CTX, AMC, P, DO	5
47	NOR, CEC, CAZ, CRD, CXM, ATM, TOB, CTX, AMC, P, CRO, TE, CIP, DO	14
48	CAZ, CXM, ATM, TOB, CTX, AMC, P, CRO, DO	9
49	CEC, CAZ, CXM, TOB, K, CTX, AMC, P, TE, DO, AMP	11
E. coli ATCC 25922	NOR, CXM, AMC, P, TE, DO, AMP	7

Table 4: Prevalence of antibiotic resistance genes among *E. coli* isolates

Genotype	Isolate number
aac (3)-IV, blaCMY, dfrA1, qnrA	1
aac (3)-IV, blaSHV, blaCMY, qnrA	6
blaSHV, blaCMY, qnrA	7
blaSHV, dfrA1, qnrA	9,21
aac (3)-IV, blaSHV	13,30
aac (3)-IV, dfrA1	26
aac (3)-IV, qnrA	4,27,29.31,39,42
blaCMY, qnrA	3
blaSHV, qnrA	5,8
blaSHV, dfrA1	25,28
dfrA1, qnrA	2
blaSHV, blaCMY	18, 20
aac (3)-IV	12,14,35,40, 41,46
qnrA	10,34,37
blaSHV	15,16,19, 24
dfrA1	33
blaCMY	17,22
-	11,23,32,36,38,43,44,45,47,48,49,50
blaSHV, blaCMY, dfrA1, qnrA	*E. coli* ATCC 25922

Multidrug drug resistance (MDR) is described as resistant to at least one member from three different antibiotic classes being used for the treatment of *E. coli* (Nairoukh *et al.,* 2018). Thereby, in this work we founded 74% (37 strains) of the isolates are MDR. In this work, 30% of the isolates are ESBL positive. UTIs caused by antibiotic resistant and MDR bacteria have been increased in recent years as reported by Ahmed et al. (Ahmed *et al*., 2019) and we believe that they are responsible for many of the complications in UTIs. Most of the ESBL *E. coli* are resistant to a wide range of beta-lactams and non beta-lactams. This might be due to the fact that the isolates came from different pathogens species isolated from admitted patients in different wards of the hospital.

According to Dehbanipour et al. (Dehbanipour *et al*., 2019), isolates from female patients have higher resistance pattern than those from male patients. In our study, this difference was present but not statistically significant except for the case of Trimethoprim-sulfamethoxazole (TMP/SMX). Our results are consistent with the finding of Sanchez et al. (Sanchez *et al*., 2016) that found a high resistance to TMP/SMX in female patients. Regarding age categories and resistance pattern, our data suggest an increase in the resistance pattern with age. This association was found to be statistically significant with Doxycycline Cefotaxime and Tetracycline. However, we couldn't find a significant association with other antibiotics.

Antibiotics resistance pattern is important to be investigated nowadays due to its high prevalence worldwide. Thus, our study investigated the molecular mechanisms of antibiotic resistance among *E. coli* isolated from UTI patients. Of the 50 isolates tested, 56% were resistant to trimethoprim/sulfamethoxazole, 56% were resistant to norfloxacin and 30% were resistant to gentamicin, in agar disk diffusion assays and these resistance properties were associated with 16%, 40% and 34% prevalence of the *dfrA1*, *qnrA* and *aac(3)-IV* genes, respectively. In addition, 32% of the isolates harbor the *blaSHV* gene and demonstrated a resistance of 80% to ampicillin, 94% to amoxicillin and 100% to penicillin. Furthermore, the *blaCMY* gene was detected in 16% of the isolates who showed a higher resistance to cephalosporins antibiotics. Fisher's exact test showed an inverse association between Cefuroxime resistance and the presence of *blaCMY* gene. Thereby, resistance observed may due to other genetic factors among them SHV enzymes. These enzymes are the predominant plasmid-mediated β-lactamases found in Gram negative Enterobacteria (Liakopoulos *et al*., 2016). Thus, production of the SHV enzymes in *E. coli* is of concern and the detection of in β-lactamase in UTI patient is of great importance. Generally, microorganisms use various mechanisms to develop drug resistance, such as horizontal gene transfer and alteration in genetic material, recombination of foreign DNA in bacterial chromosome and overuse of antibiotics. Multiple mechanisms were involved in the resistance to fluoroquinolones in *E. coli*. In addition to mutations in chromosomal genes encoding DNA gyrase and topoisomerase IV, a plasmid-mediated quinolone resistance (PMQR) mechanism has also been reported, including Qnr-mediated protection of DNA from quinolone binding, expression of a QepA-encoded efflux pump and aac(6')-Ib-cr mediated FQ acetylation (Hooper and Jacoby, 2016).

CONCLUSION

This work concludes that high numbers of UTI are caused by MDR *E. coli*. The antibiotic resistance profiles of uropathogenic *E. coli* are highly variable, and continuous surveillance of trends in resistance patterns of uropathogens is necessary. The mechanism of resistance was not correlated to the inverstigated antibiotic resistance genes. Other factors are certainly involved.

A limitation of our study is the limited antibiotic resistance genes used. Therby, investigation of plasmid encoded carbapenemase genes in addition to virulence genes are of great interest. Furthermore, a phylogentic study in order to monitor the predominance of some strains could be performed.

ACKNOWLEDGMENT

This research was supported by Deanship of Scientific Research, Taif University, Saudi Arabia (Research Group Grant Number 1-440-6148).

REFERENCES

Ozturk R and Murt A (2020). Epidemiology of urological infections: A global burden. *World J. Urol*., **38**(11): 2669-2679

Fatima T, Rafiq S, Iqbal A and Husnain S (2020). Prevalence and antibiogram of MDR *E. coli* strains Isolated from UTI patients-1-year retrospective atudy at Nishtar Medical Hospital, Multan. *SN Compr. Clin. Med*., **2**: 423-431.

Lagha R, Ben Abdallah F, AL-Sarhan BO and Yassin Al-Sodany Y (2019). Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against *Escherichia coli* Isolated from UTI patients. *Molecules,* **23**(6): 24.

Letica-Kriegel AS, Salmasian H, Vawdrey DK, Youngerman BE, Green RA, Furuya EY, Calfee DP and Perotte R (2019). Identifying the risk factors for catheter-associated urinary tract infections: A large cross-sectional study of six hospitals. *BMJ. Open*., **9**(2): e022137.

Alanazi MQ, Alqahtani FY and Aleanizy FS (2018). An evaluation of *E. coli* in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: Retrospective study. *Ann. Clin. Microbiol. Antimicrob*., **17**(1): 3.

Hutchings MI, Truman AW and Wilkinson B (2019). Antibiotics: past, present and future. *Curr. Opin. Microbiol*., **51**: 72-80.

Ayukekbong JA, Ntemgwa M and Atabe AN (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrob. Resist. Infect. Control*, **6**: 47.

Ferri M, Ranucci E, Romagnoli P and Giaccone V (2017). Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food. Sci. Nutr*., **57**(13): 2857-2876.

Li B and Webster TJ (2018). Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *J. Orthop. Res*., **36**(1): 22-32.

Lüthje P and Brauner A (2016). Novel strategies in the prevention and treatment of urinary tract infections. *Pathogens*., **5**(1): 13.

Punjabi C, Tien V, Meng L, Deresinski S and Holubar M (2019). Oral fluoroquinolone or trimethoprim-sulfamethoxazole vs. ß-lactams as Step-Down therapy for Enterobacteriaceae Bacteremia: Systematic review and meta-analysis. *Open. Forum. Infect. Dis*., **6**(10): ofz364.

Nairoukh YR, Mahafzah AM, Irshaid A and Shehabi AA (2018). Molecular characterization of multidrug resistant uropathogenic *E. coli* isolates from Jordanian patients. *Open Microbiol. J*., **12**: 1-7.

van Driel AA, Notermans DW, Meima A, Mulder M, Donker GA, Stobberingh EE and Verbon A (2019). Antibiotic resistance of *Escherichia coli* isolated from uncomplicated UTI in general practice patients over a 10-year period. *Eur. J. Clin. Microbiol. Infect. Dis*., **38**(11): 2151-2158.

Mutters NT, Mampel A, Kropidlowski R, Biehler K, Günther F, Bălu I, Malek V and Frank U (2018). Treating urinary tract infections due to MDR *E. coli* with isothiocyanates - a phytotherapeutic alternative to antibiotics? *Fitoterapia*., **129**: 237-240.

National Committee for Clinical Laboratory Standards (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically 11th ed. J. Approved standard M07. National Committee for Clinical Laboratory Standards, Wayne, PA 19087 USA.

Clinical and Laboratory Standards institute (2019). Performance standards for antimicrobial susceptibility testing 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA 19087 USA.

Messele YE, Abdi RD, Yalew ST, Tegegne DT, Emeru BA and Werid GM (2017). Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. *Ann. Clin. Microbiol. Antimicrob*., **16**(1): 55.

Monroy-Pérez E, Cerón AB, García Cortés LR, Alonso NN, Domínguez-Trejo P, Hernandez-Jaimes T, Bustos-Martínez J, Hamdan-Partida A, Rojas Jiménez EA, Vaca S, Vaca-Paniagua F and Paniagua-Contreras GL (2020). Virulence gene transcription, phylogroups, and antibiotic resistance of cervico-vaginal pathogenic *E. coli* in Mexico. *PLoS. One*, **15**(6): e0234730.

Abduzaimovic A, Aljicevic M, Rebic V, Vranic SM, Abduzaimovic K and Sestic S (2016). Antibiotic resistance in urinary isolates of *Escherichia coli*. *Mater. Sociomed*., **28**(6): 416-419.

Medina M and Castillo-Pino E (2019). An introduction to the epidemiology and burden of urinary tract infections. *Ther. Adv. Urol*., **11**: 1756287219832172.

Sewify M, Nair S, Warsame S, Murad M, Alhubail A, Behbehani K, Al-Refaei F and Tiss A (2016). Prevalence of urinary tract infection and antimicrobial susceptibility among diabetic patients with controlled and uncontrolled glycemia in Kuwait. *J. Diabetes. Res*., pp.6573215.

Faour-Klingbeil D, Kuri V, Fadlallah S and Matar GM (2016). Prevalence of antimicrobial-resistant *Escherichia coli* from raw vegetables in Lebanon. *J. Infect. Dev. Ctries*., **10**(4): 354-62.

Singh AK, Das S, Singh S, Gajamer VR, Pradhan N, Lepcha YD and Tiwari HK (2018). Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India. *PLoS One*., **13**(6): e0199179.

Adamus-Białek W, Baraniak A, Wawszczak M, Głuszek S, Gad B, Wróbel K, Bator P, Majchrzak M and Parniewski P (2018). The genetic background of antibiotic resistance among clinical uropathogenic *Escherichia coli* strains. *Mol. Biol. Rep*., **45**(5): 1055-1065.

Srinivasan A (2017). Antibiotic stewardship: Why we must, how we can. *Cleve. Clin. J. Med*., **84**(9): 673-679.

Malekzadegan Y, Rastegar E, Moradi M, Heidari H and Sedigh Ebrahim-Saraie H (2019). Prevalence of quinolone-resistant uropathogenic *Escherichia coli* in a tertiary care hospital in south Iran. *Infect. Drug. Resist*., **12**: 1683-1689.

Ahmed SS, Shariq A, Alsalloom AA, Babikir IH and Alhomoud BN (2019). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *Int. J. Health. Sci (Qassim).,* **13**(2): 48-55.

Dehbanipour R, Khanahmad H, Sedighi M, Bialvaei AZ and Faghri J (2019). High prevalence of fluoroquinolone-resistant *Escherichia coli* strains isolated from urine clinical samples. *J. Prev. Med. Hyg*., **60**(1): E25-E30.

Sanchez GV, Babiker A, Master RN, Luu T, Mathur A and Bordon J (2016). Antibiotic resistance among urinary isolates from female outpatients in the United States in 2003 and 2012. *Antimicrob. Agents Chemother*., **60**(5): 2680-2683.

Liakopoulos A, Mevius D and Ceccarelli D (2016). A Review of SHV Extended-Spectrum β-Lactamases: Neglected yet ubiquitous. *Front. Microbiol*., **7**: 1374.

Hooper DC and Jacoby GA (2016). Topoisomerase inhibitors: Fluoroquinolone mechanisms of action and resistance. *Cold. Spring. Harb. Perspect. Med*., **6**(9): a025320.