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Characterization of some antimicrobial genes of *Escherichia coli* recovered from pet animals and human with urinary tract infections

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ABSTRACT

The present study is directed to isolate and identify pathogenic *E. coli* from urine of humans and pet animals (dogs and cats) suffering from urinary tract infections (UTIs) who admitted to different hospitals and veterinary clinics (35 for each), at Kaliobia Governorate and to determine their antimicrobial sensitivity, beside detection of some resistance genes. The results revealed that, that *E. coli* were isolated from 41 from the examined samples where, (15/42.9%) from human; (14/40.0%) and (12/34.3 %) from dogs and cats urine samples, respectively. The isolated *E. coli* were highly sensitive to meropenem followed by nitrofurantoin; norfloxacin and gentamycin. Meanwhile, they were highly resistant to nalidixic acid followed by ampicillin; tetracycline; cefotaxime; azithromycin; co-trimoxazole; streptomycin and ciprofloxacin. Moreover, PCR results showed that, *qnr*S; *aad*A1 and bla_{TEM} antibiotic resistant genes were amplified in all sex studied *E. coli* strains and *mph*A resistant gene was amplified in five studied strains. Therefor, the study concluded that, uropathogenic *E. coli* of humans, dogs and cats affected with UTIs may be the most important pathogen with multiple antibiotic resistances and there were positive correlation between the presence of *qnr*S; *mph*A; *aad*A1 and bla_{TEM} genes in these strains with the phenotypic resistance to the antibiotics of these groups.

1. INTRODUCTION

Escherichia coli is a predominant pathogen causing urinary tract infections in humans and pet animals (Ramirez-Castillo *et al*., 2018 and Mustapha *et al*., 2019). It belongs to the family *Enterobacteriaceae* and is Gram negative, non-sporulating, flagellated, rod-shaped, and facultatively anaerobic (it grows best at 37 ºC but can tolerate 49 ºC. Jang *et al*., 2017). While UTIs are treatable, rising incidence of multi-drug resistance organisms (MDRO) such as *E. coli* cause complications, treatment failure, and a rise in mortality and morbidity (Mustapha *et al*., 2019). UPEC has a wide range of virulence factors, including as adhesins, toxins, host defense avoidance mechanisms, and iron acquisition systems, which they use to create an infection (Ramirez-Castillo *et al*., 2018). Most of the community acquired UTIs and half of nosocomial UTIs are caused by different pathotypes of UPEC. Both chromosomal and plasmid-encoded antibiotic resistance and virulence genes in UPEC have been identified (Momtaz *et al*., 2013; Abbasi and Ranjbar, 2018). The limited number of effective medications available to treat these infections is made more difficult by the rise of multidrug-resistant (MDR) UPEC. The most important factor in the development of antibiotic resistance in UPEC was identified as the presence of genes encoding resistance to quinolones (*qnrS*), macrolide phosphotransferases (*mphA*), streptomycin (*aadA1*), and lactamase (*blaTEM*) by the molecular studies (Momtaz et al., 2013; Rzewuska et al., 2015; Salehi et al., 2021 and Belas et al., 2022). The pathogenicity of bacteria, its global distribution, its capacity to colonize and persist in the hosts for more than 6 months, its capacity for transmission between hosts, and its ability to cause recurrent infections are all linked with the classification as an international MDR high-risk clonal lineage, in addition to the association with various antimicrobial resistance determinants. In addition, the dissemination of qnrS among Gram-negative bacteria like UPEC and the transmission of quinolone resistant genes (*qnr*) that are mediated by plasmids are major concerns for the international health care system (Abbasi and Ranjbar, 2018). There is several potential mechanisms by which *Enterobacteriaceae*, and particularly UPEC, develop resistance to macrolides like erythromycin and azithromycin. methylases, notably ermA and ermB, from the erm gene family displace the target site, inactivating enzymes like esterase from the ere (A) and ere (B) genes or phosphotransferases from the mphA gene family (Salehi *et al*.,2021). As a result of the increased prevalence and global spread of extended- spectrum beta-lactamase (ESBL) genes like CTX-M, TEM enzymes that are associated to multidrug-resistant (MDR) phenotypes of UPEC and other Uropathogens exhibiting resistance to most antibiotics, MDR phenotypes have become

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increasingly common (Windahl et al., 2014; Ramirez-Castillo et al., 2018; Alasmary, 2021; Said et al., 2021 and Belas et al., 2022).

The purpose of this investigation was to extract and identify pathogenic *E. coli* from UTI-affected human and canine (dog and cat) urine, as well as to discover certain gene resistance utilizing standard Polymerase Chain Reaction (PCR).

2. MATERIAL AND METHODS

2.1. Samples:

Total of 105 (10 ml of freely voided middle stream urine samples or by catheterization from the urethra) were collected in sterile screw capped tubes diseased human patients and pet animals (dogs and cats) with urinary tract infections, (35 for each), who admitted to different hospitals and veterinary clinics at Kaliobia Governorate according to Yadav *et al.,* (2020). The urine samples were centrifuged at 3000 Xg and the supernatant were aseptically discarded while the sediments were used for *E. coli* isolation.

2.2. The method of Iso, 2001 & Markey et al. 2013 was applied in Isolation and identification of E. coli from urine samples:

After 12 hours of aerobic incubation at 37ºC, a loopful of urine sediment was inoculated into nutritional broth. To do this, we took a loopful of the nutritional broth and streaked it onto MacConkey agar plates, where it was incubated at 37ºC for 24 hours. Colonies that showed signs of lactose fermentation were picked and streaked onto Eosin methylene blue media (EMB) and TBX agar, where they were incubated for an additional 24-48 hours at 37ºC before being examined. These colonies appeared to be *E. coli*, (colonies with a bright green sheen on EMB and blue colonies on TBX agar B- glucuronidase positive) were.

Suspected *E. coli* colonies were confirmed morphologically by Gram- stain, Gram- negative, straight, non- sporulated, medium size rods arranged singly in pairs or short chain colonies were selected for further identification steps and motility tests, by stabbing the bacterial isolate in the center of 0.5% Semi-solid agar tubes then incubated at 37°C for 24 hrs. (*E. coli* isolates were motile and seen to spread from point of inoculation into the agar as paintbrush). Then, they were identified biochemically by indole; methyl red; Voges-Proskauer; citrate utilization; urease test; Eijkman; catalase; oxidase; Sugar fermentation; nitrate reduction and gelatin hydrolysis tests.

In-Vitro anti-microbial sensitivity test:

In-Vitro sensitivity test was done on each *E. coli* isolates to study their sensitivity for 14 different antimicrobial using the antimicrobial standardized discs (Oxoid), {amoxicilin/clavulanic acid(AMC/30); ampicillin (AM/10); azithromycin (AZM/15); cefotaxim (CTX/30); ciprofloxacin (CIP/5); co-trimoxazole (COT/25); doxycycline (DO/30); gentamicin (GEN/10); Meropenem (MEM/10); Naledixic acid (NA/30); Nitrofurantion (F/300); norfloxacin (NOR/10); streptomycin (S/10) and tetracycline (TE/30)} on Mueller–Hinton agar (Oxoid) plates and following disc method of CLSI (2018).

Antimicrobial standardized discs, concentrations, and interpretation of their effect (CLSI, 2018).

Antimicrobial disks		Disk Concentrations	Zone of inhibition(mm)		
			Resistant ≤ mm (R)	Intermediate mm range (IS)	Sensitive ≥ mm (S)
Amoxicillin/ clavulanic acid	AMC	30 µg	13	14 -17	18
Ampicillin	AM10	10 µg	13	14-16	17
Azithromycin	AZM	15 µg	13	14-17	18
Cefotaxime	CTX/30	30 µg	14	15-22	23
Ciprofloxacin	CIP/5	5 µg	15	16-20	21
Co- Trimoxazole	COT/25	(1.25/23.75) µg	10	11-15	16
Doxycycline	Do 30	30 µg	14	15-19	20
Gentamicin	GEN/10	10 µg	12	13-14	15
Meropenem	MEM	10 µg	14	15-19	20
Nalidixic acid	NA/ 30	30 µg	13	14-18	19
Nitrofurantion	F/300	300 µg	14	15-16	17
Norfloxacin	NOR/10	10 µg	12	13-16	17
Streptomycin	S/10	10 µg	11	12-14	15
Tetracycline	TE/30	30 µg	14	15-18	19

2.3. Molecular detection of antibiotic resistant genes of E. coli:

Genotypic detection of four antibiotic resistant genes, quinolones resistant gene (*qnr*S); macrolide phosphotransferases resistance (*mph*A); streptomycin resistant gene (aadA1) and β-lactamase resistance gene (bla_{TEM}) in sex random *E. coli* (two from each sample of human; dogs and cats urine samples) that showed antibiotic resistant by disk diffusion method to the same studied isolates using conventional polymerase chain reaction (cPCR), Primers sequences, target genes, amplicons sizes, and cycling conditions were determined by running PCR reactions with the specified primers and amplification products on 1.5% agarose gels in accordance with the instructions provided by QIAamp® DNA Mini Kit (Qiagen, Germany, GmbH) using Emerald Amp GT PCR mastermix (Takara, Japan) showed through Table (1).

Table 1 Primers sequences, target genes, amplicons sizes and cycling conditions.

Target gene		Primer sequence (5'-3')	Amplified segment (bp.)	Amplification (35 cycles)				Final extension	References
				Primary denaturation	Secondary denaturation	Annealing	Extension		
*qnr*S	F	ACGACATTCGTCAACT GCAA	516 bp.	94˚C	94˚C	55˚C	72˚C	72˚C	**Robicsek *et al*. (2006)**
	R	TAAATTGGCACCCTGT AGGC		5 min.	30 sec.	40 sec	45sec.	10min.	
*mph*A	F	GTGAGGAGGAGCTTC GCGAG	403 bp.	94˚C	94˚C	58˚C	72˚C	72˚C	**Nagyuen *et al*. (2009)**
	R	TGCCGCAGGACTCGG AGGTC		5 min.	45 sec.	40 sec	40 sec	10min.	
*aad*A1	F	TATCAGAGGTAGTTG GCGTCAT	484bp.	94˚C	94˚C	54˚C	72˚C	72˚C	**Randall *et al*. (2004)**
	R	GTTCCATAGCGTTAAG GTTTCATT		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
bla$_{TE}$M	F	ATCAGCAATAAACCA GC	516bp.	94˚C	94˚C	54˚C	72˚C	72˚C	**Colom *et al*. (2003)**
	R	CCCCGAAGAACGTTTT C		5 min.	30 sec.	40 sec.	45 sec.	10 min.	

3. RESULTS

The results of bacteriological examination of urine samples (Table, 2) cleared that, 41 *E. coli* (39.1%) isolates were recovered from 105 urine samples affected with UTIs; they were isolated from human urine (15/42.9%); dog's urine (14/40.0%) and cat's urine samples (12/34.3%).

Table 2 Prevalence of isolated *Escherichia coli* bacteria from different urine samples.

Urine sample species	Number of sample	Negative samples		Positive samples	
		No.	%	No.	%
Human with UTIs	35	20	57.1	15	42.9
Dogs with UTIs	35	21	60.0	14	40.0
Cats with UTIs	35	23	65.7	12	34.3
Total	105	64	60.9	41	39.1

* Percentage in relation to total No. of each examined urine sample (35 & 105 for total)

Phenotypically, it seems that *E. coli* isolates are Gram-negative, medium- sized rods that are solitary, in pairs, or in small groups, but do not produce spores. On Nutrient agar, their colonies appeared as round, non- pigmented bumps, but on MacConkey agar, their colonies appeared as round, non-mucoid brilliant pink bumps (lactose fermenter). The identical isolates displayed the normal blue colonies on TBX agar, but the EMB agar colonies had a characteristic greenish metallic shine (B- glucuronidase positive). Further, all 41 isolates exhibited positive findings in the indole test, Methyl red test, catalase test, sugar fermentation test, nitrate reduction test, and Eijkman test, indicating that they were all E. coli. They also tested negative for the enzyme oxidase. The gelatin hydrolysis, urease, citrate utilization, and Voges-Proskauer tests.

The results of *in- vitro* sensitivity tests for the isolated *E. coli* are tabulated in *(*Table, 3) as the following:

In table (3) there was a significant decrease ($P< 0.0001$) in AST (69.11 ± 2.71 and 67.35 ± 1.03), ALT (18.49 ± 0.80 and 16.44 ± 0.78) and AKP (16.48 ± 0.84 and 14.63 ± 1.10) levels in the kaempferol treated semen samples mainly those supplemented with 25 and 50 µg/ml, respectively if compared with control group (106.0 ± 2.17, 28.09±1.11 and 25.86 ± 0.52, respectively).

Table 3 In-Vitro anti-microbial sensitivity test for isolated 41 isolated *E. coli*.

Antimicrobial agents		Disk concentrations	Sensitive		Intermediate		Resistant		AA
			No.	%	No.	%	No.	%	
Meropenem	MEM	10 µg	37	90.2	4	9.8	0	0.0	S
Nitrofurantion	F/300	300 µg	31	75.6	8	19.5	2	4.9	S
Norfloxacin	NOR/10	10 µg	30	73.2	8	19.5	3	7.3	S
Gentamicin	GEN/10	10 µg	27	65.8	4	9.8	10	24.4	S
Amoxicillin/clav ulanic acid	AMC	30 µg	7	17.1	24	58.5	10	24.4	IS
Doxycycline	DO	30 µg	8	19.5	22	53.7	11	26.8	IS
Nalidixic acid	NA/30	30 µg	0	0.0	7	17.1	34	82.9	R
Ampicillin	AM10	10 µg	2	4.9	6	14.6	33	80.5	R
Tetracycline	TE/30	30 µg	3	7.3	5	12.2	33	80.5	R
Cefotaxime	CTX/30	30 µg	3	7.3	7	17.1	31	75.6	R
Azithromycin	AZM	15 µg	4	9.8	8	19.5	29	70.7	R
Co- Trimoxazole	COT/25	(1.25/23.75) µg	6	14.6	9	22.0	26	63.4	R
Streptomycin	S/10	10 µg	3	7.3	14	34.2	25	58.5	R
Ciprofloxacin	CIP/5	5 µg	7	17.1	11	26.8	23	56.1	R

No.: Number of isolates AA: Antibiogram activity
%: Percentage in relation to total number of *E. coli* isolates (n=41)

The results of genotyping detection of antibiotic resistant genes, showed that, qnrS; aadA1 and blaTEM antibiotic resistant genes were amplified in all sex studied E. coli strains giving products of 516 bp.; 484 bp. and 516 bp., respectively (Figures, 1 and 2). Meanwhile, mphA resistant gene was amplified in five studied E. coli strains giving product of 403 bp. but not amplified in one E. coli strain of cat urine (Fig. 1).

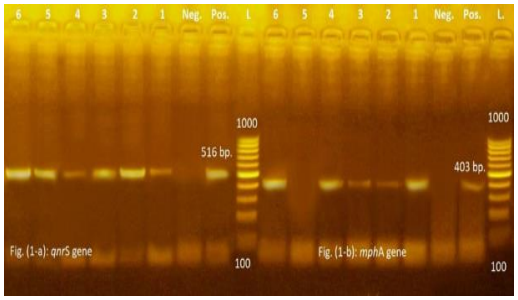

Figure 1 quinolones resistant (*qnr*S) gene. Lane L: 100-1000 bp. DNA Ladder. Neg: Negative control. (*S. aureus* ATCC25923) Pos.: Positive control (*E. coli* form Ahri. at 516 bp.). Lane 1- 6: Positive *E. coli* at 516 bp. (1&2 human urine; 3&4dogs urine and 5&6 cats' urine) Fig. (1-b): Macrolide phosphotransferases resistance (*mph*A) gene. Lane L: 100-1000 bp. DNA.
Neg.: Negative control. (*S. aureus* ATCC25923) Pos.: Positive control (*E. coli* form Ahri. at 403 bp.). Lane1- 4 & 6: Positive *E. coli* at 403 bp. (1&2 human urine; 3&4dogs urine and 6 cats' urine) Lane 5: Negative *E. coli* at 403 bp. (cat urine).

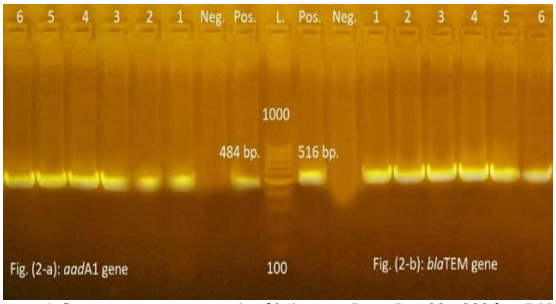

Figure 2 Streptomycin resistant (*aad*A1) gene. Lane L: 100-1000 bp. DNA Ladder. Neg: Negative control. (*S. aureus* ATCC25923) Pos.: Positive control (*E. coli* form Ahri. at 516 bp.). Lane 1- 6: Positive *E. coli* at 484 bp. (1&2 human urine; 3&4dogs urine and 5&6 cats' urine) Fig. (2-b): β-lactamase resistance (*bla*$_{TEM}$) gene. Lane L: 100-1000 bp. DNA. Neg.: Negative control (*S. aureus* ATCC25923) Pos.: Positive control (*E. coli* form Ahri. at 516 bp.)
Lane 1- 6: Positive *E. coli* at 516 bp. (1&2 human urine; 3&4dogs urine and 5&6 cats' urine).

4. DISCUSSION

The present study revealed that, 41 *E. coli* isolates (39.1%) were obtained from 105 urine samples affected with UTIs; they were isolated from human urine (15/42.9%); dog's urine (14/40.0%) and cat's urine samples (12/34.3%). The recorded results for human patients nearly similar to those of Ghavide *et al*. (2020); Mahdi *et* al. (2020) and Said *et* al. (2021). For dog, the results came in constant with chang et *al*. (2015); Liu et *al*. (2017); Moyaert *et* al. (2017); Mustapha et *al*. (2019) and Fonseca et *al*. (2021). Meanwhile, the results of UPEC in cats urine came in harmony with Dorsch *et* al. (2015); Moyaert *et al*. (2017) and Fonseca et *al*. (2021).
Regarding to the colonial appearance *E. coli* isolated, they appeared as round, non-pigmented bumps on Nutrient agar, but on MacConkey agar, their colonies appeared as round, non-mucoid brilliant pink bumps (lactose fermenter). The identical isolates displayed the normal blue colonies on TBX agar, but the EMB agar colonies had a characteristic greenish metallic shine (B- glucuronidase positive). Further, all 41 isolates exhibited positive findings in the indole test, Methyl red test, catalase test, sugar fermentation test, nitrate reduction test, and Eijkman test, indicating that they were all *E. coli*. They also tested negative for the enzyme oxidase. The gelatin hydrolysis, urease, citrate utilization, and Voges-Proskauer tests. Similar results were recorded by Markey et *al*., 2013); Mustapha *et* al. (2019) and Said et *al*. (2021). Antibiotic resistance can arise for a number of reasons, including incorrect dosing, overuse, and failure to finish the full course of treatment for a variety of infections, as well as the acquisition of resistance in low- susceptibility bacteria through selection/spontaneous mutation, the development of resistance in enteric bacteria via R plasmids responsible for multiple drug resistance, and the transmission of resistant strains between humans and animals (Setu et *al*., 2016 and Mustapha et al., 2019). The recorded results in this study for antimicrobial sensitivity of 41 studied uropathogenic *E. coli* isolates (Table 3) cleared that, they were highly resistant for Nalidixic acid followed by ampicillin and tetracycline; cefotaxime; azithromycin; co-trimoxazole; streptomycin and ciprofloxacin. But they were intermediate sensitive to amoxicillin/clavulanic acid and doxycycline, but they were highly sensitive to meropenem; Nitrofurantion; norfloxacin and gentamycin, with lower resistant for these antimicrobial agents. Nearly similar results were obtained by Raeispour and Ranjbar, 2018; Alzahrani et al. (2020); Ghavide *et al. (*2020); Mahdi et al. (2020); Salehi *et al.*,2021; and Said *et al*. (2021) for human UPEC. Meanwhile, these results for dog UPEC strains came in harmony with windahl *et al*. (2014); Chang *et al*. (2015); McMeekin *et al*. (2016); Liu *et al.* (2017); Moyaert *et al*. (2017); Mustapha *et al.* (2019) and Fonseca *et al*. (2021). Moreover, the results agree with those of Harada *et al*.(2012); Moyaert *et al*.(2017) and Fonseca *et al*.(2021) for cats UPEC. The recorded results proved that, phenotypic multiple antibiotic resistances (MDR) are widely spread among 41 studied uropathogenic *E. coli* isolates, drug-resistance of *E. coli* was not related to its source origin and decided the facts of Ramirez-Castillo *et al.* (2018); Mustapha *et al.* (2019) and Salehi *et al.* (2021) who reported that UPEC strains had MDR whatever their sources and they could be transferred between dogs; cats and humans through direct contact.
Bacterial drug resistance may be both inherited and acquired. Hereditary resistance (whether chromosomal or plasmid-based) occurs when a cell's inherent and inherited features impede the actions of antibiotics, leading to the selection of resistant strains from a population of initially susceptible bacteria (Mortazavi- Tabatabaei *et al*., 2019). In Egypt, a few studies on *E. coli* resistance have been described in the literatures, focusing on presence of genes related to the production of quinolones, macrolides, streptomycin and β-lactamase resistant genes. The results of PCR appeared that, *qnr*S; *aad*A1 and *bla*$_{TEM}$ antibiotic resistant genes were amplified in all sex studied *E. coli* strains but *mph*A resistant gene was amplified in five strains, where quinolones resistant gene (*qnr*S) was amplified at 516 bp. (Fig., 1- a); macrolide phosphotransferases resistance (*mph*A)was amplified at 403 bp. (Fig.,1- b); streptomycin resistant gene (*aad*A1) was amplified at 484 bp. (Fig., 2- a) and β- lactamase resistance gene (*bla*$_{TEM}$)was amplified at 516 bp. (Fig., 2- b). Similar detection of these genes in UTIEC strains isolated from urine samples of human; dogs and cats were recorded by Rezazadeh *et al*. (2016); Abbasi and Ranjbar (2018) and Ramirez-Castillo *et al*. (2018) for *qnr*S gene; Nguyen *et al*. (2009); and Salehi *et al*. (2021) for *mph*A gene; Randall *et al*. (2004) and Momtaz *et al*.(2013) for *aad*A1 gene; Ramirez-Castillo *et al*.(2018) and Belas *et al*.(2022) for *bla*$_{TEM}$ gene.

5. CONCLUSION

The results of this study concluded that, *E. coli* isolated from urine of humans, dogs and cats affected with UTIs may be the most important pathogen that had antimicrobial resistance to most antimicrobial drugs (MDR) and they could be transferred between dogs; cats and humans through direct contact. Moreover, there were positive correlation between the presence of *qnr*S; *mph*A); *aad*A1 and *bla*$_{TEM}$ genes in these strains with the phenotypic resistance to the antibiotics of these groups and it would be better to use meropenem; Nitrofurantion; norfloxacin and gentamycin in treating urinary tract infections in human; dogs and cats.

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