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ORIGINAL ARTICLE

Multidrug Efflux Pump In Relation To Antibiotic Resistance Pattern in *Escherichia Coli* Strains Isolated From Benha University Hospital

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ABSTRACT

***Background**: Antimicrobial resistance is one of the most serious public health threats of the twenty-first century, Uropathogenic Escherichia coli (UPEC) are one of the main bacteria causing urinary tract infections (UTIs). The rate of UPEC with high resistance towards antibiotics has increased dramatically in recent years. **Objectives**: This study aimed to assess the antibiotic resistance pattern of UPEC and to detect the relationship of antibiotic resistance with the presence of efflux pump genes (AcrA-AcrB-TolC). **Methodology**: This study included 50 UPEC strains, Identification of E.coli by Gram stain, culture and biochemical reactions was done, Antibiotic susceptibility for isolated E.coli strains by vitek system and detection of AcrA-AcrB-TolC genes by conventional PCR among isolated strains were also performed. **Results**: the prevalence of MDR was 70%,UPEC isolates showed high level of resistance to : ampicillin(94%), nalidixic acid (84%), ticacillin (82%), ciprofloxacin (76%) and trimethoprim/sulfamethoxazole (76%), low level of resistance of UPEC to:gentamicin (34%), amoxicillin/clavulinic acid (28%), ceftazidime (21%), cefoxitin (16%), piperacillin/ tazobactam (8%), tobramycin(2%) and ertapenem (2%) but no resistance to amikacin , imipenem and nitrofurantoin. 50%, 66% and 68% of isolates had genes acrA, acrB and tolC respectively. there was a significant correlation between tol C gene and MDR phenotype. **Conclusion**: the rate of MDR UPEC is rising, efflux pumps play an important role in mediating antibiotic efflux and increase the rate of antibiotic rasistance. The frequency of tol C gene was significantly higher in MDR than non MDR, while the acr A B level showed non significant variation among MDR and non MDR.*

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common types of infections, every year, about 150 million people worldwide are infected with UTI[1], Uropathogenic *Escherichia coli* (UPEC) is the leading causative agent of UTI in both communities and hospitals worldwide, therapeutic management of UTI is particularly problematic because of the increasingly widespread resistance to all classes of antibiotics[2], Efflux pumps are one of the major mechanisms of Multiple Drug Resistance (MDR) in bacteria which effluxes out the drugs accumulated[3], Multidrug resistance to antibiotics is defined as resistance to three or more antibiotics from different classes[4], Clinical experiences have shown a high rate of antibiotic resistance among UPEC[5], The mechanisms responsible for increased antimicrobial resistances include biofilm formation, decreased membrane permeability, alteration of binding sites, enzymes that can inactivate antibiotics and active efflux of antimicrobials[6], *Escherichia coli* posses different efflux pump systems and these efflux pumps are important source of multidrug resistance, which export antibiotics from the cell, increasing their antibiotic resistance. The primary multidrug resistance efflux pump in *E. coli* is AcrAB-TolC from the RND family[7,8]. AcrAB-TolC efflux system is responsible for the extrusion of a broad range of compounds such as lipophilic antimicrobial drugs, i.e., penicillin G, cloxacillin, nafcillin, macrolides, novobiocin, linezolid, and fusidic acid, antibiotics such as fluoroquinolones, cephalosporins, tetracyclines [9], various dyes (eg crystal violet, acridine, acriflavine, ethidium) detergents, organic solvents, steroid hormones (bile acids, estradiol and progesterone) and essential oils[10], AcrAB–TolC is a tripartite transporter that captures substrates from the periplasm and effluxes them across the outer membrane and out of the cell, it is composed of the outer membrane protein TolC, the periplasmic adaptor protein AcrA, and the inner membrane transporter AcrB[11].

METHODOLOGY

This work was carried out in Microbiology and Immunology Department, Benha Faculty of Medicine in the period between January 2019 and October 2019. It included 50 strains of *E. coli* isolated from 80 patients suffering from UTI, the patients included in the study were 37 females and 13 males and their ages ranged from 20-50 years old, this study was approved by Benha University ethical committee and consent was obtained from all patients under study.

Samples and methods

Mid stream urine samples were collected in sterile screw capped containers from patients with UTI. Each collected urine sample was quantified for bacterial count and those $\geq 10^5$ were then centrifuged and the deposit was used for isolation and identification of *E.coli* by routine methods according to Cheesbrough[12] .*E coli* strains were sored at – 60°c in glycerol broth untill used.

Antimicrobial susceptibility

Antimicrobial susceptibility was done by Vitek 2 compact system , biomerieux , france according to manufacture's instructions by making bacterial suspension in 3.0 mL of sterile saline (0.45%) and The turbidity was adjusted accordingly (0.50-0.63) before being used to rehydrate the antimicrobial medium within the card. The card was then filled, sealed and placed into the instrument incubator/reader VITEK® 2 system .The instrument monitored the growth of each well in the card over a defined period of time (up to 18 hours for bacteria). At the completion of the incubation cycle, MIC values (or test results, as appropriate) were determined for each antimicrobial contained on the card (AST-N 233).

PCR

DNA extraction by Quick-DNA™ Miniprep Plus Kit, Zymo Researsh,USA according to manufacture's instructions and Detection of *acrAB tolC* genes by conventional PCR(Master mix Dream Taq.fermentas, life science, Thermo Fisher Scientific) using primers listed in table 1.

Table 1: Sequence of primers that were used in this study

Acr A-F	5'- CTCTCAGGCAGCTTAGCCCTAA
Acr A-R	5'-GCAGAGGTTCAGTTTTGACTGTT
AcrB – F	5'- GGTCGATTCCGTTCTCCGTTA
AcrB – R	5'-CTACCTGGAAGTAAACGTCATTGGT
TolC –F	5' – AAGCCGAAAAACGCAACCT
TolC –R	5'- CAGAGTCGGTAAGTGACCATC

Three PCR reactions were made for each sample. Each reaction contained either *acr A* primer or *acr B* primer or *tol c* primer in afinal reaction volume of 50 µL contained: Green PCR Master Mix: 25µl,forward Primer: 2 µl Reverse Primer: 2 µl, Template DNA: 5 µl.nuclease-free Water: 16µl, Amplification was performed in 40 cycles: 3 minutes of initial denaturation at 95°C, 39 cycles of 30 seconds of denaturation at 95°C, 30seconds of annealing at 52°C, 1 minute of extension at 72°C and a final extension at 72°C for 5 minutes. The PCR products were electrophoresed by a gel agarose (Hopkins and Williams, England)) and visualized by a UV transilluminator (Biometra, Germany).

RESULTS

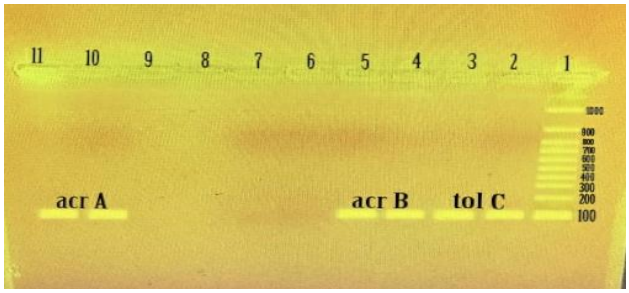

Fig 1: The results of PCR by gel electrophoresis
Lane 1: DNA Ladder 100 bp
Lane 2,3: *tol C* positive (100 bp)
Lane 4,5: *acr B* positive (107 bp)
Lane 10,11: positive *acr A* (107 bp).

Table 2: Antimicrobial susceptipility pattern of *E coli* isolates

Antibiotic	Resistant		Sensitive		intermediate	
	No.	%	No.	%	No.	%
Amp	47	94.0	3	6.0	0	0.0
Amox-clav	14	28.0	28	56.0	8	16.0
Ticarcillin	41	82.0	9	18.0	0	0.0
Pipertaz	4	8.0	46	92.0	0	0.0
Cefalotin	21	42.0	16	32.0	13	26.0
Cefoxitin	8	16.0	41	82.0	1	2.0
Cefoxime	20	40.0	30	60.0	0	0.0
Ceftazidim	21	42.0	29	58.0	0	0.0
Ertapenem	1	2.0	49	98.0	0	0.0
Imipenem	0	0.0	50	100.0	0	0.0
Amikacin	0	0.0	50	100.0	0	0.0
Gentamicin	17	34.0	31	62.0	2	4.0
Tobramycin	1	2.0	6	12.0	43	86.0
NA	42	84.0	8	16.0	0	0.0
Cipro	38	76.0	12	24.0	0	0.0
Ofloxacin	36	72.0	12	24.0	2	4.0
Nitrofurant	0	0.0	43	86.0	7	14.0
Trim/sulfa	38	76.0	12	24.0	0	0.0

Table 2 shows that the highest level of resistance of *E coli* was against ampicillin (94**%)** and the lowest resistance was against imipenem, amikacin and nitrofurantoin (0%).

Table 3: Frequency of MDR among *E coli* isolates

MDR	No.	%
No	15	30
Yes	35	70
Total	50	100

Table 3 shows that the prevalence of MDR among isolates was 70%

Table 4: Frequency of *Acr-A, Acr-B and Tol-C*

Gene	Positive		Negative	
	No.	%	No.	%
Acr-A	25	50.0	25	50.0
Acr-B	33	66.0	17	34.0
Tol-C	34	68.0	16	32.0

Table 4 shows that the prevalence of efflux pump genes among *E coli* isolates was as follows *acr A* 50%, *acr B* 66% and *tol C* 68%.

Table 5: Antibiotic resistance pattern by MDR isolates

Antibiotic	MDR				Z-test	P
	No (no.=15)		Yes (no.=35)			
	No.	%	No.	%		
Ampicillin	12	80.0	35	100.0	2.73	0.006 (S)
Amoxicillin-clavulinic acid	7	46.67	7	20.0	1.92	0.05
Ticarcillin	11	73.33	30	85.71	1.04	0.30
Piperacillin-tazobactam	4	26.67	0	0.0	3.18	0.001 (S)
Cefalotin	11	73.33	10	28.57	2.94	0.003 (S)
Cefoxitin	6	40.0	2	5.71	3.03	0.002 (S)
Cefotaxime	10	66.67	10	28.57	2.52	0.01 (S)
Ceftazidim	11	73.33	10	28.57	2.94	0.003 (S)
Ertapenem	1	6.67	0	0.0	1.54	0.12
Imipenem	0	0.0	0	0.0	-	-
Amikacin	0	0.0	0	0.0	-	-
Gentamicin	0	0.0	17	48.57	3.32	<0.001(HS)
Tobramycin	0	0.0	1	2.86	0.66	0.51
Nalidixic acid	8	53.33	34	97.14	3.87	<0.001(HS)
Ciprofloxacin	5	33.33	33	94.29	4.62	<0.001(HS)
Ofloxacin	5	33.33	31	88.57	3.99	<0.001(HS)
Nitrofurantoin	0	0.0	0	0.0	-	-
trimethoprim/ sulfamethoxazole	3	20.0	35	100.0	6.07	<0.001(HS)

S: Significant difference (P<0.05) HS: Highly Significant difference (P<0.001)

Table 5 shows that there is high significant correlation between resistantance to gentamicin , nalidixic acid , ciprofloxacin , ofloxacin and trimethoprim/sulfamethoxazole and MDR phenotype.

Table 6: Antibiotic resistance pattern and distribution of *acr A* gene among studied isolates

Antibiotic	Acr-A				Z-test	P	Odds ratio (95%CI)
	Positive (no.=25)		Negative (no.=25)				
	No.	%	No.	%			
Ampicillin	25	100.0	22	88.0	1.79	0.07	-
Amoxicillin- clavulinic acid	2	8.0	12	48.0	3.15	0.002 (S)	0.09 (0.01-0.54)
Ticarcillin	22	88.0	19	76.0	1.10	0.27	2.31 (0.42-16.01)
Piperacillin/ tazobactam	0	0.0	4	16.0	2.08	0.037 (S)	0 (0-0.87)
Cefalotin	12	48.0	9	36.0	0.86	0.39	1.64 (0.46-5.94)
Cefoxitin	2	8.0	6	24.0	1.54	0.12	0.27 (0.02-1.81)
Cefotaxime	12	48.0	8	32.0	1.15	0.25	1.96 (0.54-7.28)
Ceftazidim	12	48.0	9	36.0	0.86	0.39	1.64 (0.46-5.94)
Ertapenem	0	0.0	1	4.0	1.01	0.31	0 (0)
Imipenem	0	0.0	0	0.0	-	-	-
Amikacin	0	0.0	0	0.0	-	-	-
Gentamicin	15	60.0	2	8.0	3.88	<0.001 (HS)	17.25 (2.94-171.71)
Tobramycin	1	4.0	0	0.0	1.01	0.31	-
Nalidixic acid	24	96.0	18	72.0	2.31	0.02 (S)	9.33 (1.01-437.69)
Ciprofloxacin	24	96.0	14	56.0	3.31	<0.001 (HS)	18.86 (2.19-844.39)
Ofloxacin	22	88.0	14	56.0	3.31	<0.001 (HS)	5.76 (1.19-36.51)
Nitrofurantoin	0	0.0	0	0.0	-	-	-
Trimethoprim/ sulfamethoxazole	23	92.0	15	60.0	2.65	0.008 (S)	7.67 (1.30-78.36)

Table 6 shows that there is high significant correlation between resistance to gentamicin,ciprofloxacin , ofloxacin and presence of *acr A* gene and significant correlation between resistance to amoxicillin- clavulinic acid , Piperacillin/ tazobactam, NA , trimethoprim/ sulfamethoxazole and *acr A* gene.

Table 7: Antibiotic resistance pattern and distribution of *acr B* gene among studied isolates

Antibiotic	Acr-B				Z-test	P	Odd ratio (95%CI)
	Positive (no.=33)		Negative (no.=17)				
	No.	%	No.	%			
Ampicillin	33	100.0	14	82.35	2.49	0.01 (S)	-
Amoxicillin-clavulinic acid	7	21.21	7	41.18	1.49	0.14	0.38 (0.09-1.68)
Ticarcillin	29	87.88	12	70.59	1.51	0.13	3.02 (0.53-17.68)
Piperacillin- tazobactam	0	0.0	4	23.53	2.90	0.004 (S)	0 (0-0.42)
Cefalotin	12	36.36	9	52.94	1.12	0.26	0.51 (0.13-1.95)
Cefoxitin	0	0.0	8	47.06	4.30	<0.001 (HS)	0 (0-0.15)
Cefotaxime	12	36.36	8	47.06	0.73	0.46	0.64 (0.17-2.5)
Ceftazidim	12	36.36	9	52.94	1.12	0.26	0.51 (0.13-1.95)
Ertapenem	0	0.0	1	5.88	1.41	0.16	0 (0)
Imipenem	0	0.0	0	0.0	-	-	-
Amikacin	0	0.0	0	0.0	-	-	-
Gentamicin	15	45.45	2	11.76	2.38	0.02 (S)	6.25 (1.12-62.87)
Tobramycin	1	3.03	0	0.0	0.72	0.47	-
Nalidixic acid	32	96.97	10	58.82	3.48	<0.001 (HS)	22.4 (2.26-1039.66)
Ciprofloxacin	30	90.91	8	47.06	3.44	<0.001 (HS)	11.25 (2.05-75.24)
Ofloxacin	28	84.85	8	47.06	2.82	0.005(S)	6.3 (1.37-30.4)
Nitrofurantoin	0	0.0	0	0.0	-	-	-
Trimethoprim/ sulfamethoxazole	31	93.94	7	41.18	4.14	<0.001 (HS)	22.14 (3.34-230.72)

Table 7 shows that there is a high significant correlation between resistance to cefoxitin ,NA ,ciprofloxacin , trimethoprim/sulfamethoxazole and presence of *acr B* gene and a significant correlation between resistance to ampcillin, piperacillin/ tazobactam, gentamicin , ofloxacin and presence of *acr B* gene.

Table 8: Antibiotic resistance pattern and distribution of *Tol C* gene among studied isolates

Antibiotic	Tol-C				Z-test	P	Odd ratio (95%CI)
	Positive (no.=34)		Negative (no.=16)				
	No.	%	No.	%			
Ampicillin	33	97.06	14	87.5	1.33	0.18	4.71 (0.22-286.31)
Amoxicillin-clavulinic acid	5	14.71	9	56.25	3.05	0.002 (S)	0.13 (0.03-0.64)
Ticarcillin	30	88.24	11	68.75	1.67	0.09	3.41 (0.59-20.08)
Piperacillin-tazobactam	0	0.0	4	25.0	3.04	0.002 (S)	0 (0-0.37)
Cefalotin	10	29.41	11	68.75	2.63	0.009 (S)	0.19 (0.04-0.8)
Cefoxitin	2	5.88	6	37.5	2.84	0.004 (S)	0.10 (0.01-0.74)
Cefotaxime	10	29.41	10	62.5	2.23	0.02 (S)	0.25 (0.06-1.03)
Ceftazidim	10	29.41	11	68.75	2.63	0.009 (S)	0.19 (0.04-0.80)
Ertapenem	0	0.0	1	6.25	1.47	0.14	0 (0)
Imipenem	0	0.0	0	0.0	-	-	-
Amikacin	0	0.0	0	0.0	-	-	-
Gentamicin	15	44.12	2	12.5	2.20	0.03 (S)	5.53 (0.98-55.86)
Tobramycin	1	2.94	0	0.0	3.17	0.001 (S)	-
Nalidixic acid	33	97.06	9	56.25	3.67	<0.001 (S)	25.67 (2.55-1190.09)
Ciprofloxacin	31	91.18	7	43.75	3.66	<0.001 (HS)	13.28 (2.35-90.12)
Ofloxacin	31	91.18	5	31.25	4.40	<0.001 (HS)	22.73 (3.83-158.37)
Nitrofurantoin	0	0.0	0	0.0	-	-	-
Trimethoprim/ sulfamethoxazole	33	97.06	5	31.25	5.08	<0.001 (HS)	72.6 (6.89-3212.43)

Table 8 shows that there is a high significant correlation between resistance to ciprofloxacin, ofloxacin, trimethoprim/ sulfamethoxazole and presence of *tol C* gene and significant correlation between resistance to amoxicillin-clavulinic acid, Piperacillin/tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime ,gentamicin, tobramycin, NA and presence of *tol C* gene.

Table 9: Prevalence of *acr A, B* and *tol C* among MDR isolates

Gene		MDR				X^2	P	Odd ratio (95%CI)	
		No (no.=15)		Yes (no.=35)					
		No.	%	No.	%				
Acr-A	Positive	10	66.67	15	42.86	2.38	0.12	0.37	(0.08 – 1.55)
	Negative	5	33.33	20	57.14				
Acr-B	Positive	7	46.67	26	74.29	3.57	0.06	3.30	(0.77-14.06)
	Negative	8	53.33	9	25.71				
Tol-C	Positive	1	6.67	33	94.29	FET	<0.001 (HS)	231	(15.88-10020)
	Negative	14	93.33	2	5.71				

Table 9 shows that there is high significant correlation between presence of *tol C* gene and MDR phenoyupe but no significant correlation between the presence of *acr AB* and the MDR phenotype.

DISCUSSION

Urinary Tract Infections represent a major health threat due to the wide spread of antibiotic resistance, the associated high recurrence rate and the emergence of multidrug resistant UPEC clones[13].

The occurrence of MDR in E. coli has been attributed to the AcrAB-TolC complex of efflux pumps[14]. This rise in multidrug resistance of organism is caused mainly by the excessive use of antibiotics by physicians[15].In accordance with global trends, our results revealed higher prevalence of urinary tract infections in female patients than in males[16], this is because females have a shorter wider urethra.

In our study, the prevalence of MDR is 70%, which agrees with Igwe et al[17] who repored the frequency of MDR among UPEC to be 72.5%, Maleki et al[14] reported the prevalence was 78 % and Gawad et al [13] found a percentage of 76% of isolates from Giza, Egypt was MDR.

On the other hand, Kafilzadeh &Farsimadan[18] reported that the prevalence of MDR was 81%, Munkhdelger et al [19] found that 93.9% of isolates were considered MDR.

In this study, the UPEC isolates showed a high level of resistance to: ampicillin (94%), nalidixic acid (84%), ticacillin (82%), ciprofloxacin(76%) and trimethoprim / sulfamethoxazole (76%).this is in agreement with Kazemnia et al [20] who reported high level of resistance of UPEC to nalidixic acid,ampicillin and ciprofloxacin.

Igwe et al[17] found that The isolates were highly resistant to Amoxicillin, Cefotaxime and trimethoprim / sulfamethoxazole.

Elsayed et al[21] reported high level of UPEC resistance to ampicillin, nalidixic acid and trimethoprim / sulfamethoxazole. While Abdel Wahed et al[23] reported high level of resistance to nitrofurantoin, ampicillin and cephalexin.

Our study showed low level of resistance of UPEC to:gentamicin (34%), amoxicillin / clavulinic acid (28%), ceftazidime (21%), cefoxitin (16%), piperacillin / tazobactam (8%),tobramycin(2%) and ertapenem (2%) but no resistance to amikacin , imipenem and nitrofurantoin,this is in agreement with Igwe et al[17] who reported that the isolates were mildly resistant to gentamicin but highly susceptible to imipenem and amikacin (0%) also Ramírez-Castillo et al [16] reported low level of resistance to gentamicin and no resistance to ertapenem and imipenem and Shakhatreh et al [22] reported low level of resistance to gentamycin, amikacin and ertapenem and moderate resistance to cefoxitin , ceftazidime, ceftriaxone , ciprofloxacin and cefotaxime, Abdel Wahed et al [23] reported low level of resistance to gentamycin , amikacin and amoxicillin-clavulanate and no resistance to imipenem.

The variation in the results may be due to regional differences in different parts of the world or even within the same country with different therapeutic response to antimicrobial drugs, the origin of these differences can be attributed to genetic variation in various regions also the susceptibility patterns could be changed over time.

In this study, 50%, 66% and 68% of isolates had genes *acrA*, *acrB* and *tolC* respectively, Such findings are consistent with those reported by Kafilzadeh & Farsimadan[18] who reported that 51.1%, 75.0% and 69.4% of isolates had genes acrA, acrB and tolC respectively, Maleki et al [14] found that the frequency of *acrA* and *acrB* genes was 95.5% and 82.9% respectively, this can be partially explained by that the strains included in our study presumably carry other mechanisms of resistance beside the efflux pump genes. Also, some socioeconomic and behavioral factors can contribute to antibiotic resistance such as misuse of antimicrobial agents by hospital physicians or unskilled practitioners and easy access to antibiotics without a prescription[5] especially in devalopping countries.

In this study, there was significant correlation between *acr A* gene and resistance to amoxicillin/ clavulinic acid, piperacillin/tazobactam, gentamicin, nalidixic acid, ciprofloxacin, ofloxacin and trimethoprim/sulfamethoxazole.

In this study there was significant correlation between *acr B* gene and resistance to ampicillin, piperacillin / tazobactam, cefoxitin, gentamicin,nalidixic acid,ciprofloxacin,ofloxacin and trimethoprim/ sulfamethoxazole.

In this study there was significant correlation between *tol C* gene and resistance to amoxicillin/ clavulinic acid,piperacillin/tazobactam, cafalotin, cefoxitin, cafotaxime, ceftazidime, gentamicin, tobramycin , nalidixic acid , ciprofloxacin,ofloxacin and trimethoprim/sulfamethoxazole.

In our study there was significant correlation between *tol C* gene and MDR phenotype.

Okusu & Nikaido[26] reported that deletion of acrAB resulted in hypersensitivity to some compounds such as tetracycline, nalidixic acid, ampicillin, chloramphenicol and rifampin; this reveals the important role of the efflux pump AcrAB-TolC in determining the intrinsic level of resistance in E.coli, also Sulavik et al[27] found that *E coli* strains lacking *acrAB* genes show increased susceptibility to ampicillin, chloramphenicol, florfenicol, clotrimazole, puromycin, erythromycin, methotrexate, novobiocin, ciprofloxacin and nalidixic acid.

Swick et al [28] found that 30% of fluoroquinolone-resistant isolates overproduced *AcrA.*

Kafilzadeh & Farsimadan[18] reported that there is a significant positive correlation between the presence of efflux pumps and resistance to all antibiotics (excluding carbenicillin, meropenem, chloramphenicol, cefotaxime, rifampin and novobiocin.

Li and Nikaido[25] reported that *acr AB-tol C* significantly contributes to intrinsic resistance in E coli and exhibits an incredibly broad substrate profile. Inactivation of *acr AB* in wild-type strains results in hypersusceptibilities not only to clinically relevant β-lactams, fluoroquinolones, macrolides, tetracyclines, tigecycline, chloramphenicol and novobiocin but also to basic dyes, disinfectants, detergents and organic solvents.

Gawad et al[13] reported a significant correlation between the presence of *tolC* and the MDR phenotype this is because *Tol C* functions independently of *AcrA* and *AcrB*,thus it can contribute to intrinsic resistance with or without AcrA B[27], Chetri et al [29] reported That *AcrAB-TolC* has a role in characteristic intrinsic resistance to antimicrobials as well as dyes and detergents .

Chowdhury et al[30] found that Overexpression of the AcrAB-TolC efflux pump is an intrinsic mechanism of multidrug resistance in Gram-negative bacteria, It can be due to the mutation in AcrR gene which is the repressor of the AcrAB operon system.

CONCLUSIONS

From our study , it can be concluded that our results support the hypothesis that acr A B tol C efflux pump plays an important role in determining UPEC resistance to many antimicrobials, which necessitates the importance of administration of new strategies for treatment of UTI. The increasing rate of MDR prevalence in Egypt is also alarming.
In our work. The antibiotics of choice for the treatment of E. coli associated infections with efflux pump genes were imipenem, amikacin and nitrofurantoin.

Conflict of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Abou Heidar NF, Degheili JA, Yacoubian AA and Khauli RB, Management of urinary tract infection in women: A practical approach for everyday practice;urology annals; 2019;11,4,339-346.
2. Pantel A, Remy C, Essebe C N,Mesureur J, Sotto A, Pagès J M,Chanoine M H, Lavignea J, Modulation of Membrane Influx and Efflux in *Escherichia coli* Sequence Type 131 Has an Impact on Bacterial Motility, Biofilm Formation and Virulence in a Caenorhabditis elegans Model; Antimicrobial Agents and Chemotherapy; 2016; 60,2901-2911
3. Gooyandeh S, Jahromy S H, Noorbakhsh F, Evaluation of Efflux pump activity among Uropathogenic *Escherichia coli* and *Klebsiella pneumonia* multiple- Drug Resistance isolates; International Journal of Molecular and Clinical Microbiology; 2016; 6,708-714.
4. Basak S, Singh P, and Rajurkar M,Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study ;journal of Pathogens; 2016.
5. Dehbanipour R, Rastaghi S, Sedighi M, Maleki N and Faghri J, High prevalence of multidrug-resistance uropathogenic *Escherichia coli* strains, Isfahan, Iran; J Nat Sci Biol Med; 2016;7(1): 22-26.
6. Andersen JL, He G X, Kakarla P, KC R, Kumar S and Lakra WS, Multidrug efflux pumps from Enterobacteriaceae, *Vibrio cholerae* and *Staphylococcus aureus* bacterial food pathogens; Int J Environ Res Public Health; 2015; 12: 1487-547.
7. Helaly GF, Shawky S, Amer R, Abdel-kader O, El-Sawaf G and El Kholy A E, Expression of AcrAB Efflux Pump and Role of Mefloquine as Efflux Pump Inhibitor in MDR *E.coli*; American Journal of Infectious Diseases and microbiology; 2016; 4,(1): 6-13.
8. Wen X, Langevin AM and Dunlo MJ, Antibiotic export by efflux pumps affects growth of neighboring bacteria; Scientific Reports; 2018; 8 (15120).
9. Anes J, McCusker MP, Fanning S, Martins M, The ins and outs of RND efflux pumps in *Escherichia coli*; Front Microbiol; 2015;6:587.
10. Fadli M, Chevalier J, Hassani L, Mezrioui NE, Pagès JM, Natural extracts stimulate membrane-associated mechanisms of resistance in Gram-negative bacteria;Lett Appl Microbiol; 2014; 58(5):472-477.
11. Shi X, Chen M, Yu Z, M. Bell JM, Wang H, Forrester I, Villarreal H, Jakana J, Du D, Luisi B F, Ludtke S J &Wang Z ,In situ structure and assembly of the multidrug efflux pump AcrAB-TolC; Nature Communications; 2019; 10: 2635.

12. Cheesbrough M, District Laboratory Practice in Tropical Countries: Part 2; 2018
13. Gawad WE, Helmy OM, Tawakkol WM and Hashem AM, Antimicrobial Resistance, Biofilm Formation, and Phylogenetic Grouping of Uropathogenic *Escherichia coli* Isolates in Egypt: The Role of Efflux Pump-Mediated Resistance; Jundishapur J Microbiol; 2018; 11(2):14444.
14. Maleki D, Jahromy SH, Karizi SZ and Eslami P, The Prevalence of *acrA* and *acrB* Genes Among Multiple-Drug Resistant Uropathogenic *Escherichia coli* Isolated From Patients With UTI in Milad Hospital, Tehran; Avicenna J Clin Microbiol Infect; 2017; 4(1):e39785.
15. El-Mokhtar M A,Mandour S A,Shahat A A, Colistin resistance among multidrug-resistant *E. coli* isolated from Upper Egypt; Egyptian Journal of Medical Microbiology; 2019;28(2):11-17.
16. Ramírez-Castillo FY, Moreno-Flores A C,Avelar-González FJ, Márquez-Díaz F, Hare J and Guerrero-Barrera AL, An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study; Ann Clin Microbiol Antimicrob; 2018; 17: 34.
17. Igwe JC, Michael G, Bolaji RO, Durowaye MT, Olayinka BO, Ehnimidu JO and Onaolapo JA, Molecular Characterization of Efflux Pump Genes in Clinical Isolates of *E. coli* from Urinary Tract Infection UTI and Diarrheic Patients in Zaria, Nigeria; EC Microbiology ; 2019;295-303.
18. Kafilzadeh F & Farsimadan F. Investigating multidrug efflux pumps in relation to the antibiotic resistance pattern in *Escherichia coli* strains from patients in Iran; Biomedical Research; 2016; 27 (4): 1130-1135
19. Munkhdelger Y, Gunregjav N, Dorjpurev A, Juniichiro N, Sarantuya J, Detection of virulence genes, phylogenetic group and antibiotic resistance of uropathogenic *Escherichia coli* in Mongolia; J Infect Dev Ctries; 2017; 11(1):51-57.
20. Kazemnia A, Ahmadi M, and Dilmaghani M, Antibiotic Resistance Pattern of Different *Escherichia coli* Phylogenetic Groups Isolated from Human Urinary Tract Infection and Avian Colibacillosis; Iran Biomed J; 2014;18(4): 219–224.
21. Elsayed T, Hala AF, Ismail HAF, Elgamal SA and Gad AHA. The Occurrence of Multidrug Resistant *E. Coli* which Produce ESBL and Cause Urinary Tract Infections; J Appl Microbiol Biochem; 2017; 1: 2-8
22. Shakhatreh MAK, Swedan SF, Al-Odat MA & Khabour, OF, Uropathogenic *Escherichia coli* (UPEC) in Jordan: Prevalence of urovirulence genes and antibiotic resistance; Journal of King Saud University – Science; 2018.
23. Abdel Wahed FM, El Sayed M K, Erfan DM, Kamal A, The Prevalence of Biofilm Formation, Antimicrobial Resistance and Adhesive Pap Gene (Pyelonephritis Associated Pili) among *Escherichia Coli* Strains Isolated from Outpatients and Inpatients with Urinary Tract Infection ; Egyptian Journal of Medical Microbiology; 2018;27(3):73-83
24. Oethinger M, Kern WV, Jellen-Ritter AS, McMurry LM, Levy SB, Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump; Antimicrob Agents Chemother; 2000; 44(1):10-13
25. Li X and Nikaido H,Antimicrobial Drug Efflux Pumps in *Escherichia coli*; In book: Efflux-Mediated Antimicrobial Resistance in Bacteria: Mechanisms, Regulation and Clinical Implications, Edition: First, Chapter: Antimicrobial Drug Efflux Pumps in *Escherichia coli* ;2016.
26. Okusu H and Nikaido H, AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multipleantibiotic resistance (Mar) mutants; J Bacteriol; 1996;178(1):306–308.
27. Sulavik MC, Houseweart C, Cramer C, Jiwani N, Murgolo N and Greene J, Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes; Antimicrob Agents Chemother; 2001;45(4):1126–1136.
28. 28.Swick MC, Morgan-Linnell SK, Carlson KM, Zechiedrich L. Expression of multidrug efflux pump genes acrAB-tolC, mdfA, and norE in *Escherichia coli* clinical isolates as a function of fluoroquinolone and multidrug resistance; Antimicrob Agents Chemother; 2011;55:921-924.
29. Chetri S, Bhowmik D, Paul D, Pandey P, Chanda D D, Chakravarty A, Bora D and Bhattacharjee , AcrAB-TolC efflux pump system plays a role in carbapenem non-susceptibility in *Escherichia coli*; BMC Microbiology ; 2019; 19:210.
30. Chowdhury N, Chowdhury N, Suhani S, Purkaystha A, Begum MK, Raihan T, Alam J, Islam K, Azad A, Identification of AcrAB-TolC Efflux Pump Genes and Detection of Mutation in Efflux Repressor AcrR from Omeprazole Responsive Multidrug-resistant *Escherichia coli* Isolates Causing Urinary Tract Infections; Microbiology Insights; 2019;12: 1–10