

ORIGINAL ARTICLE

Effect of some Efflux Pump Inhibitors on the Resistance of some *Escherichia coli* Strains to some Antimicrobial Agents

¹Wegdan A. Mohamed*, ¹Enas A. Daef, ¹Nahla M. Elsherbiny, ¹Bahaa Eldin A. Abdel-Raady, ²Ahmad T. Abdelaal

¹Department of Microbiology & Immunology, Faculty of Medicine, Assiut University

²Department of Microbiology & Immunology, Faculty of Pharmacy, Alazhar Assiut University

ABSTRACT

Key words:

E.coli, EPI, CPZ, OMP, MIC, PCR

Background: The efflux pumps are one of the main mechanisms of the antibiotic resistance in *Escherichia coli*. The efflux pump inhibitors (chlorpromazine and omeprazole) were tested for their effect on the antibiotic resistance by inhibiting efflux pump activity. **Objective:** The present study aims to estimate the effect of some efflux pump inhibitors on the antibiotic resistance of some *Escherichia coli* isolates. **Methodology:** A total of 100 isolates of *Escherichia coli* were studied for antibacterial susceptibility pattern by disk diffusion method with and without efflux pump inhibitors chlorpromazine (25 µg) and omeprazole (100 µg), determination of the MIC of amikacin and gentamicin on 60 *E.coli* resistant isolates, the effect of the efflux pump inhibitors on the MIC of amikacin and gentamicin and PCR amplification of the efflux pump genes *AcrD* and *MdfA* genes. **Results:** The difference between all tested antibiotics in the change of resistance to totally sensitive *E.coli* isolates after addition of CPZ and OMP by disk diffusion method were statistically highly significant (p value <0.001), in which the highest percentage value were reported for aminoglycoside antibiotics (amikacin and gentamicin). The highest reduction in the MIC of amikacin and gentamicin was observed with chlorpromazine than omeprazole ($p < 0.05$). The proportion of isolates with greater than two-fold reductions in MIC in the presence of CPZ were 69.2% and 50.9% for amikacin and gentamicin respectively ($p > 0.05$) while in the presence of OMP were 46.2% and 30.9% for amikacin and gentamicin respectively, ($p > 0.05$). PCR detection of efflux pump genes detected a high level of *AcrD* gene detection than *MdfA* gene (p value < 0.05). The percentage of *AcrD* detection in amikacin and gentamicin resistant isolates were 77% and 87.3% respectively, while for *MdfA* gene detection in amikacin and gentamicin resistant isolates were 59% and 71% respectively. **Conclusion:** Antibacterial efflux pumps are involved in establishment of resistance among the tested isolates. Chlorpromazine and Omeprazole were capable of inhibiting the efflux pump with higher activity on aminoglycoside antibiotics. Chlorpromazine was more effective than Omeprazole as EPI. PCR results showed that *AcrD* and *MdfA* efflux pump genes contributed to the resistance of the tested aminoglycosides.

INTRODUCTION

Escherichia coli is an important member of the intestinal microbiota of humans and other mammals, it is a common pathogen linked with community-associated as well as nosocomial infections¹.

The mechanisms responsible for increased antimicrobial resistances include alteration of binding sites, enzymes that can inactivate antibiotics, biofilm formation, decreased membrane permeability and active efflux of antimicrobials².

***Corresponding Author:**

Wegdan Abdelhamid Mohamad
Department of Microbiology & Immunology, Faculty of Medicine,
Assiut University
Email: wegdan.elagan@yahoo.com; Tel.: 01090442688

Active efflux is now recognized as an important component of bacterial resistance to most classes of antibiotics. This mechanism is mediated by efflux pumps, which are membrane-associated active transporters promoting the extrusion of the compounds, including antibiotics, from the cells³.

The efflux pumps of Gram-negative bacteria, which are involved in the extrusion of a variety of non-related antibiotics, obtain their energy from the proton-motive force (PMF). The PMF is maintained by the metabolic activity of the bacterium and is the result of protons generated from the hydrolysis of ATP that are transported via channels to the surface of the bacterium⁴.

E. coli has been shown to have at least nine different major proton-dependent efflux pump systems that confer resistance to two or more antibiotics. They belong to one of three genetically and structurally defined families: the major facilitator superfamily (MFS) (*emrD*, *mdfA*, *emrB*); the resistance–nodulation–division (RND) family (*acrB*, *acrF*, *acrD*, *yhiV*); and the small multidrug resistance (SMR) family (*emrE*, *tehA*)⁵.

AcrD is an aminoglycoside efflux pump, which belong to the resistance-nodulation division (RND) family in *E. coli*. AcrD form a tripartite complex with the periplasmic membrane fusion protein (AcrA), and the outer membrane channel protein (Tol-C)⁶.

MdfA is a multidrug transporter belonging to major facilitator superfamily (MFS) in *E. coli* which was initially described as a membrane-associated efflux pump for chloramphenicol. However, detailed analysis of the substrate spectrum revealed that MdfA confers resistance to aminoglycosides, erythromycin and fluoroquinolones⁷.

Inhibition of efflux pumps appears to be an attractive approach to overcome the problem of drug resistance. Efflux pump inhibitors can be utilized for increasing the antibiotic concentration inside a pathogenic cell making these drugs more effective⁸.

CPZ inhibits access of calcium to Ca²⁺-dependent ATPases and therefore limits the production of protons required for the maintenance of the PMF. It is under these conditions that phenothiazine is expected to indirectly express its effects on the activity of the efflux pump and hence render the bacterium increasingly susceptible to the antibiotic to which it was initially resistant as a consequence of an overexpressed efflux pump⁹.

Omeprazole, a proton pump inhibitor used as an antiulcer agent, has been demonstrated to act as EPI on NorA of Gram-positive bacteria¹⁰.

METHODOLOGY

Bacterial isolates:

A total of 100 hospital acquired *Escherichia coli* isolates were collected from the infection control laboratory unit according to the site of infection. Out of the 100 collected isolates, The most common source of isolates are sputum isolates which represents (35%) followed by Endotracheal isolates (33%), Blood isolates (12%), urine isolates (10%) and wound isolates (10%).

Antibiotic susceptibility patterns of *E. coli* Isolates:

All isolates were screened for susceptibility to eight antimicrobial discs namely; Amikacin (AK, 30µg), Ceftriaxone (CRO, 30µg), Chloramphenicol (C, 30µg), Doxycycline (DO, 30µg), gentamicin (CN, 10µg), Imipenem (IMP, 10µg), levofloxacin (LEV, 5µg),

nitrofurantoin (F, 300 µg) using the standard disc diffusion method¹¹. All discs were supplied from Bioanalyse Company.

Effect of chlorpromazine (25 µg) and omeprazole (100 µg) on the antibiotic susceptibility patterns of *E. coli* Isolates:

The same technique of antibiotic sensitivity was performed after addition of the efflux pump inhibitors chlorpromazine (25µg/ml) and omeprazole (100 µg/ml). The zone diameters in the presence of EPI were measured and compared with the zone diameters in absence of EPI to determine the effect of efflux pump inhibitors on antibiotic sensitivity¹².

Determination of Minimum Inhibitory Concentration (MICs) of amikacin and gentamicin antibiotics against the resistant strains of *E. coli* isolates:

MIC of the antibacterial agents was carried out by twofold serial dilution in muller-hinton broth with an inoculum of 1.0 x 10⁶ cfu/ml. Growth was scored after an overnight incubation at 37°C¹³.

Effect of chlorpromazine and omeprazole on the MIC of amikacin and gentamicin antibiotics against the resistant strains of *E. coli* :

The MIC of the tested antibacterial agents in the presence of chlorpromazine (25 µg) and omeprazole (100 µg) were determined by the same technique. Each of these compounds is considered as effective efflux pump inhibitors if it reduces the MIC of the tested antibacterial agents by ≥ 4 –folds¹⁴.

Genotypic detection of efflux pump genes by polymerase chain reaction:

Extraction of Genomic DNA from culture:

Genomic DNA was extracted using Quick DNA universal kit according to the protocol provided by manufacturer instructions (Zymo research, American, catalog No. D 4068).

Amplification of DNA by PCR:

Primer sequence for efflux pump genes is shown in table 1. PCR amplification procedure for AcrD and MdfA efflux pump genes was carried out in 20µl reaction volumes: 1 µl of extracted DNA, 1µl of each primer, 10 µl master mix 2x and 7 µl of steril Distilled Water.

The following conditions were used for amplification of AcrD gene: Initial denaturation at 95°C for 15min, denaturation at 94°C for 60 sec, annealing at 48°C for 60 sec, extension at 72°C for 60 sec and final extension at 72°C for 7 min. Number of cycles were 35 cycles. While the conditions for the amplification of MdfA gene were, Initial denaturation at 95°C for 5 min, denaturation at 94°C for 60 sec, annealing at 50°C for 60 sec, extension at 72°C for 60 sec and final extension at 72°C for 7 min. Number of cycles were 40 cycles.

Table 1: Oligonucleotide primers used for amplification of selected efflux pump genes:

Gene	Primer sequence	Size (bp)	Reference
AcrD	F: 5` GATTATCTTAGCCGCTTCAA 3`	187	[15]
	R: 5` CAATGGAGGCTTTAACAAAC 3`		
mdfA	F: 5` CATTGGCAGCGATCTCCTTT 3`	103	[16]
	R: 5` TTATAGTCACGACCGACTTCTTTCA 3`		

Agarose gel electrophoresis and identification of PCR products:

The PCR products were visualized using agarose (2%) gel electrophoresis, stained with ethidium bromide for 45 minutes under 80V in tris borate EDTA buffer and visualized by ultraviolet trans illuminator.

Statistical analysis:

The collected data were statistically analyzed using the Statistics Package for Social Sciences (SPSS) version, 21 and the difference was considered to be statistically significant when $P < 0.05$.

RESULTS

Results of antibacterial susceptibility testing by disk diffusion method:

The susceptibility testing to eight antimicrobial agents was performed by disk diffusion method for the 100 *E. coli* isolates. Percentage of different antibiotic resistance among *E. coli* isolates was shown in table 2 and chart 1.

Table 2: Frequency of antibiotic resistance pattern of *E. coli* isolates:

	R		S	
	No.	%	No.	%
Amikacin	39	39.0	61	61.0
Gentamicin	55	55.0	45	45.0
Chloramhnicol	46	46.0	54	54.0
Ceftriaxone	76	76.0	24	24.0
Doxycycline	68	68.0	32	32.0
Levofloxacin	77	77.0	23	23.0
Imipenem	15	15.0	85	85.0
Nitrofurantion	13	13.0	87	87.0

From the results of table 2, it is obvious that *E. coli* isolates exhibited maximal resistance against levofloxacin (77%), and minimal resistance against nitrofurantion (13%).

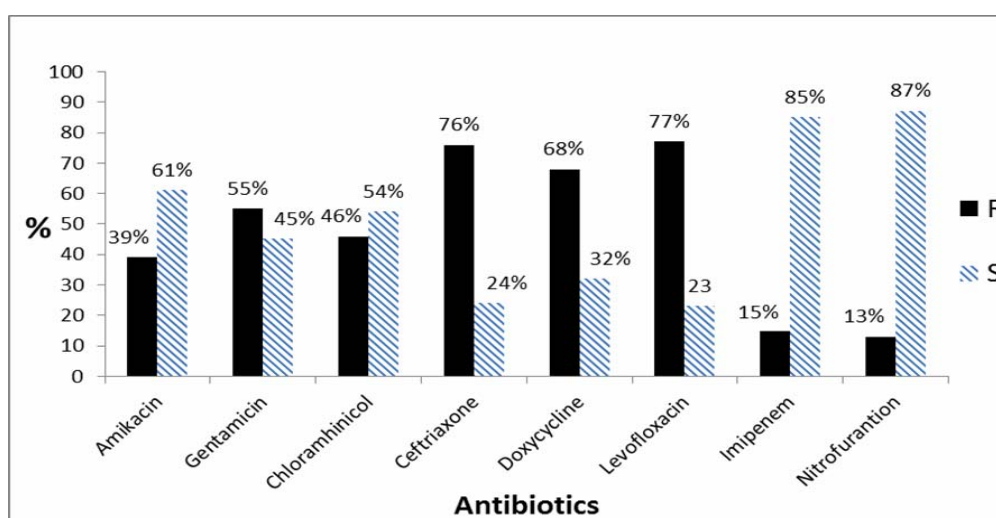


Chart 1: Frequency of antibiotic resistance pattern of *E. coli* isolates.

Results of the effect of CPZ (25 µg/ml) and OMP (100 µg/ml) on the restoration of antibiotic susceptibilities against the resistant strains of *E.coli* by disk diffusion method.

Table 3: Effect of CPZ on the restoration of drug susceptibility against resistant strains of *E.coli*:

<i>Antibiotics</i>	<i>Resistant isolates</i>	<i>Change of R to S by CPZ</i>		<i>P. value</i>
	<i>No.</i>	<i>No.</i>	<i>%</i>	
Amikacin	39	24	61.5	<0.001**
Gentamicin	55	26	47.3	
Imipenem	15	4	26.7	
Doxycycline	68	18	26.5	
Nitrofurantion	13	3	23.1	
Chloramhnicol	46	9	19.6	
Ceftraixone	76	11	14.5	
Levofloxacin	77	3	3.9	

Table 3 showed that the maximum effect of CPZ on the restoration of antibiotic susceptibility against aminoglycoside antibiotics, amikacin and gentamicin were 61.5% and 47.3%, respectively.

Table 4: Effect of OMP on the restoration of drug susceptibility against resistant strains of *E.coli*:

	<i>Resistant isolates</i>	<i>Change of R to S by OMP</i>		<i>P. value</i>
	<i>No.</i>	<i>No.</i>	<i>%</i>	
Amikacin	39	15	38.5	<0.001**
Gentamicin	55	16	29.1	
Imipenem	15	3	20.0	
Doxycycline	68	11	16.2	
Nitrofurantion	13	2	15.4	
Chloramhnicol	46	5	10.9	
Ceftraixone	76	4	5.3	
Levofloxacin	77	2	2.6	

Table 4 showed that the maximum effect of OMP on the restoration of antibiotic susceptibility against aminoglycoside antibiotics amikacin and gentamicin were 38.5% and 29.1%, respectively.

Table 5: comparison between the effect of CPZ and OMP on the restoration of drug susceptibility against resistant strains of *E.coli*.

<i>Antibiotics</i>	<i>Change of R by CPZ</i>			<i>Change of R by OMP</i>		<i>P. value</i>
	<i>R</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	
Amikacin	39	24	61.5	15	38.5	0.035*
Gentamicin	55	26	47.3	16	29.1	0.038*
Imipenem	15	4	26.7	3	20.0	0.889
Doxycycline	68	18	26.5	11	16.2	0.137
Nitrofurantion	13	3	23.1	2	15.4	1.000
Chloramhnicol	46	9	19.6	5	10.9	0.383
Ceftraixone	76	11	14.4	4	5.3	0.0570
Levofloxacin	77	3	3.9	2	2.6	0.999

The difference between the effect of CPZ and OMP on aminoglycoside resistance was highly significant (p value <0.05) but not statistically significant on other antibiotics.

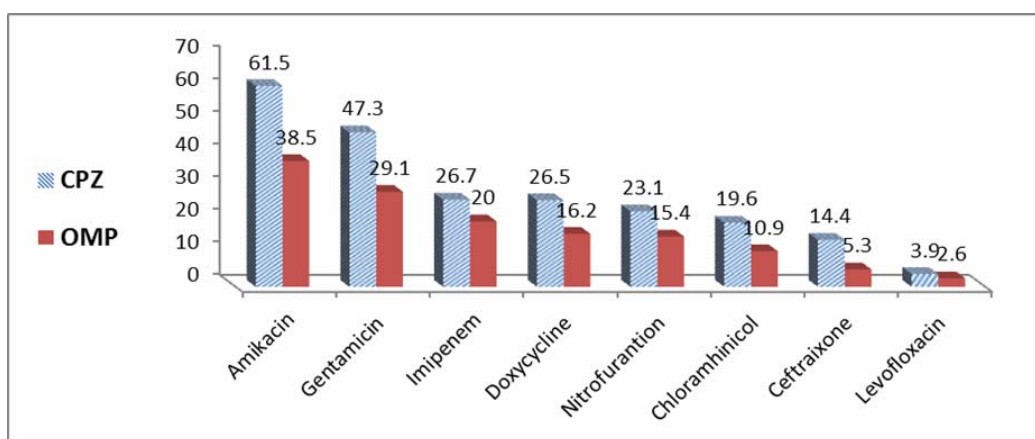


Chart 2: Effect of CPZ and OMP on the restoration of drug susceptibility against resistant strains of *E. coli*.

Results of Minimum Inhibitory Concentration (MICs) of amikacin and gentamicin against the resistant strains of *E. coli*:

MIC was performed on only *E. coli* Isolates which were resistant to amikacin and gentamicin antibiotics by disc diffusion method. Susceptibility break points for amikacin ($\leq 16\mu\text{g/ml}$) and gentamicin ($\leq 4\mu\text{g/ml}$) (Table 6).

Table 6: MIC of amikacin and gentamicin against the resistant strains of *E. coli*.

Antibiotic	No of Resistant isolates	MIC									
		64 $\mu\text{g/ml}$		128 $\mu\text{g/ml}$		256 $\mu\text{g/ml}$		512 $\mu\text{g/ml}$		1024 $\mu\text{g/ml}$	
		N	%	N	%	N	%	N	%	N	%
Amikacin	39	2	5.1	5	12.8	7	17.9	15	38.5	10	25.6
Gentamicin	55	0	0	6	9	9	16.3	17	30.9	24	43.6

Table 6 showed that MIC range of amikacin was from 64 to 1024 $\mu\text{g/ml}$ while gentamicin was from 128 to 1024 $\mu\text{g/ml}$.

Results of the effect of CPZ and OMP on the MICs of amikacin and gentamicin against the resistant strains of *E. coli*.

Table 7: Effect of CPZ and OMP on the MICs of amikacin against the resistant strains of *E. coli*:

Efflux pump inhibitor	No of resistant isolates	<i>X-fold decrease in MIC of amikacin after addition of CPZ and OMP</i>									
		2 fold		4 fold		8 fold		32fold			
		n	%	n	%	n	%	n	%		
CPZ	39	5	12.8	17	43.6	8	20.5	2	5.1		
OMP	39	7	17.9	15	38.5	3	7.7	0	0		

From the results of table 8 it was found that the fold decrease in MIC by CPZ ranged from 2-32 fold while by OMP it was from 2-8 fold.

Table 8: Effect of CPZ and OMP on the MICs of gentamicin against the resistant strains of *E. coli*:

Efflux pump inhibitors	No of resistant isolates	<i>X-fold decrease in MIC of Gentamicin after addition of CPZ and OMP</i>							
		2 fold		4 fold		8 fold		16fold	
		n	%	n	%	n	%	n	%
CPZ	55	7	12.7	17	30.9	7	12.7	4	7.3
OMP	55	10	18.8	15	27.3	2	3.6	0	0

From the results of table 8 it was found that the fold decrease in MIC by CPZ ranged from 2-32 fold while by OMP it was from 2-8 fold.

Results of the significant effect of CPZ and OMP on the MICs of amikacin and gentamicin antibiotics against the resistant strains of *E. coli*

Each of selected agents was considered effective EPI if its combination with the tested aminoglycoside showed 4-folds or more reduction in the MIC.

Table 9 : The significant Effect of CPZ on the MIC of amikacin and gentamicin:

Antibiotic	No of resistant isolates	≥4 fold decrease in MIC by CPZ		P. value
		No.	%	
Amikacin	39	27	69.2	0.0757
Gentamicin	55	28	50.9	

From the results of table 9 it was found that ≥4fold decrease in MIC by CPZ for amikacin and gentamicin was(69.2% and 50.9%), respectively.(p-value >0.05).

Table 10: The significant Effect of OMP on the MIC of amikacin and gentamicin:

Antibiotic	No of resistant isolates	≥4 fold decrease in MIC by OMP		P. value
		No.	%	
Amikacin	39	18	46.2	0.1320
Gentamicin	55	17	30.9	

It was found that ≥4 fold decrease in MIC by OMP for amikacin and gentamicin (46.2 % and 30.9 %) respectively.(p-value >0.05).

Table 11: Comparison between the significant Effect of OMP and CPZ on the MIC of amikacin and gentamicin:

Antibiotic	No of resistant isolates	≥4 fold Change in MIC by CPZ		≥4 fold Change in MIC by OMP		P. value
		No.	%	No.	%	
		Amikacin	39	27	69.2	
Gentamicin	55	28	50.9	17	30.9	0.0329*

The difference between the significant effect of chlorpromazine and omeprazole on the MIC of amikacin and gentamicin is statistically significant (p < 0.05).

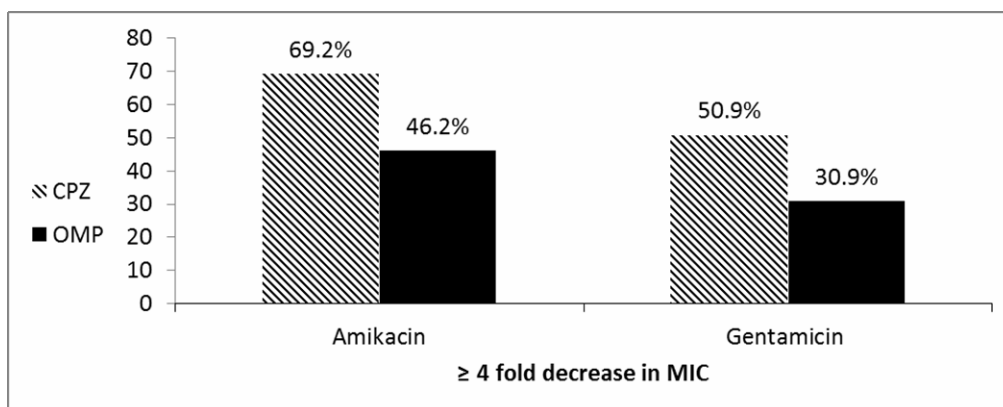


Chart 3: The significant Effect of OMP and CPZ on the MIC of amikacin and gentamicin.

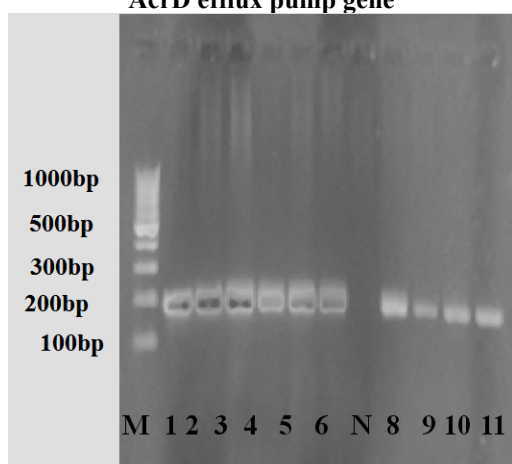
Results of PCR for the amplification of efflux pump genes:

The AcrD gene amplicone was detected at 187 bp while MdfA amplicon was detected at 103 bp (photo 1) .The prevalence of AcrD and MdfA efflux pump genes among amikacin and gentamicin resistant isolates were shown in table 12.

Table 12: Prevalence of AcrD gene and MdfA gene among amikacin and gentamicin resistant *E.coli*.

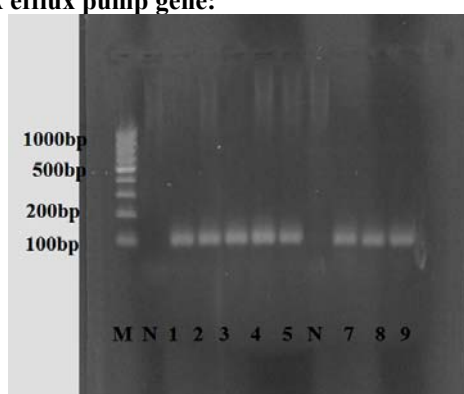
Antibiotic	No of resistant isolates	AcrD gene				MdfA gene				P- value
		Positive		Negative		Positive		Negative		
		N	%	N	%	N	%	N	%	
Amikacin	39	30	77	9	23	23	59	17	43.6	0.019*
Gentamicin	55	48	87.3	7	12.7	39	71	16	29	

Fig. 1: Agarose gel electrophoresis of PCR amplified AcrD efflux pump gene



Lanes: M, 100 bp DNA Ladder; (1- 11) PCR products of *AcrD* gene (187bp); N: negative control

Fig. 2: Agarose gel electrophoresis of PCR amplified MdfA efflux pump gene:



Lanes: M, 100 bp DNA Ladder; (1- 9) PCR products of *MdfA* gene (103bp); N: negative control

DISCUSSION

Many investigations have been performed on antibiotic resistance in *Escherichia coli* with different results depending on time and region. The results of this study showed the rates of antibiotic resistance to

levofloxacin as 77%, ceftriaxone as 76%, doxycycline as 68%, chloramphenicol as 46%, gentamicin as 55%, amikacin 39 % ,imipenem 15% and nitrofurantion 13%.

Our percentages of resistance were higher than that reported by ¹⁷ who found that resistance to levofloxacin (63.3%), ceftriaxone (68.9%), gentamicin (52.2%), amikacin (5.6%) and imipenem (0%).

A previous Egyptian study by ¹⁸, found that all the isolated *Escherichia coli* were sensitive to imipenem and the resistant rates for nitrofurantion ,gentamicin, levofloxacin, amikacin and chloramphenicol are 63.3%,63.3%, 60% ,53.3% and 40% respectively, in which the resistant rates for nitrofurantion, gentamicin and amikacin are higher than our study.

In another study performed by ¹⁹, the resistant rates for chloramphenicol, ceftriaxone, gentamicin and doxycycline were 65.5%, 62%, 51.7% and 44.8% respectively, in which the resistant rate for chloramphenicol was higher than our study.

An Egyptian study performed by ²⁰, the resistant rates for levofloxacin, ceftriaxone, gentamicin and amikacin were 100% ,100% ,100% and 55% respectively, in which all the resistant rates were higher than our study.

The antibiotic resistant rates of *E.coli* isolates to amikacin has been reported as 16% by ²¹ ,58,3% by ²² and 44,8% by ²³ , in which the resistant rates were higher than our study except that reported by ²¹.

The antibiotic resistant rates of *E.coli* to gentamicin has been reported as 95,8% by ²² , 0% by ²⁴ and 68,7% by ²³ , in which the resistant rates were higher than our study except that reported by ²⁴.

The antibiotic resistant rates of *E.coli* to nitrofurantion has been reported as 5.5% by ²⁵ ,17.9 % by ²⁶ and 68,7% by ²⁷ , in which the resistant rates were higher than our study except ²⁵.

The antibiotic resistant rates of *E.coli* isolates for ceftriaxone has been reported as 38,8% by ²³ 45.6 % by²⁸ , in which the resistant rates were lower than our study .

The antibiotic resistant rates of *E.coli* to imipenem has been reported as 0% by ²⁴ , 0 % by ²³ and 14.1% by ²⁸ , in which the resistant rates were lower than our study.

The antibiotic resistant rates of *E.coli* to Chloramphenicol has been reported as 92.4% by ²⁹, 65.5% by ¹⁹ and 0% by ³⁰, in which the resistant rates were higher than our study.

The antibiotic resistant rates of *E.coli* to levofloxacin has been reported as 59 % by ³¹, 13.6% by ³² and 65 %by ³³, in which the resistant rates were lower than our study.

The antibiotic resistant rates of *E.coli* to doxycycline has been reported as 44.8% by ¹⁹, 79.3% by ³⁴ and 66.7% by ³¹, in which the resistant rates were lower than our study except ³⁴.

The determination of the effect of EPI on the antibiotic susceptibility by disk diffusion method was one of the methods used for screening of efflux pump activity in bacteria ¹². In our study, Two EPIs (CPZ and OMP) were used to determine the efflux pump contribution to antibiotic resistance.

In Our study, the difference between all tested antibiotics in the change of resistance after addition of EPI were statistically highly significant (p value <0.001), in which the highest percentage value were reported for aminoglycoside antibiotics .

From Table 5 and chart 2 , it was shown that that the difference between the effect of CPZ and OMP on aminoglycoside resistance were statistically significant (p value <0.05), but not significant in the resistance of other tested antibiotics.

Nguyen *et al.*,³⁵ , performed disc diffusion system to determine the effect of the efflux pump inhibitor, PAβN on the antibiotic susceptibility against *E. coli* isolates .After treatment with PAβN, *E. coli* isolates increased their inhibition zone diameters in the disc diffusion test to all investigated antimicrobials .The percent of strains that increased their zone diameter by at least one mm was greatest for ciprofloxacin (39.3%), followed by chloramphenicol (27.1%), gentamicin (25.2%), ampicillin (16.8%), trimethoprim/sulphamethoxazole (16.8%), and tetracycline (7.7%) . The treatment resulted in reductions in absolute levels of resistance prevalence of 3.3%, 2.6%, 2.6%, and 0.7% for chloramphenicol, ciprofloxacin, ampicillin, and gentamicin, respectively. No changes in the prevalence of resistance for trimethoprim/sulphamethoxazole and tetracycline were observed..The prevalence values for gentamicin and chloramphenicol were lower than our study.

Jeyaseeli *et al.*,³⁶ performed the disk diffusion method for the determination of the effect of EPI flupenthixol dihydrochloride (Fp) (closely related to CPZ) on the antibiotics resistance against *E. coli* isolates and reported that the EPI changed the resistance of gentamicin, streptomycin and penicillin to fully sensitive and no effect with chloramphenicol and tetracycline.

Minimal Inhibitory Concentrations (MICs) of aminoglycoside against selected *E. coli* isolates ranged

from 64 to 1024 µg/ml for amikacin and from 128 to 1024 µg/ml for gentamicin. These results of MIC range were lower than that obtained by ²⁹ who reported that MIC range of amikacin against *E.coli* isolates >1024 µg/ml and MIC range of gentamicin 512->1024µg/ml.

Also these results of MIC range were lower than that obtained by³⁷ who reported that MIC range of amikacin and gentamicin against *E.coli* isolates ≥512 µg/ml .

By measuring the ability of chlorpromazine and omeprazole to decrease the MICs of amikacin, it was found that chlorpromazine decreased amikacin MIC by ≥ 4 folds against 69.2% of isolates while omeprazole decreased amikacin MIC by ≥4 folds against 46.2 % of isolates. Regardless of gentamicin it was found that chlorpromazine decreased gentamicin MIC by ≥ 4 folds against 50.9% of isolates while omeprazole decreased gentamicin MIC by ≥ 4 folds against 30.9 % of isolates.

Coutinho *et al.*,³⁸ reported that CPZ decreased amikacin MIC by fourfold decrease from 64 to 16 µg/ml . Rodrigues *et al.*,³⁹ reported that chlorpromazine decreased amikacin MIC by ≥ 4 folds against 83.3% of isolates , this rate was higher than our study.

Jayshree *et al.*,³⁶ reported that chlorpromazine decreased gentamicin MIC by ≥ 4 folds against 90% of isolates , this rate was higher than our study.

El-Naggar *et al.*,¹⁸ reported that omeprazole had the lowest effect on MIC of the gentamicin as it decreased MIC of gentamicin, by 4 folds or more against 50%, of *E. coli*, this relatively agreed with our results.

The concentration of CPZ selected as EPI always less than half the MIC of CPZ . The MIC of CPZ was recorded at (60 µg/ml) ⁴¹ . CPZ exerts bactericidal effect at concentration greater than it is MIC, this effect may be due to the agent reaches the DNA and via intercalation binds to sites of the DNA that are rich in guanosine and cytosine bases . When intercalation takes place, it is irreversible and therefore inhibits all DNA based processes ⁴² .In the other, omeprazole not showed any affect on the growth of *E.coli* strains up to concentration (2500 µg/ml) as reported by ¹⁵.

The results of PCR amplification, illustrated the presence of the AcrD efflux pump gene in 51 isolates (85%), while the MdfA efflux pump gene was reported in 40 isolates (66.7%). The prevalence of AcrD gene among amikacin and gentamicin resistant isolates were 77% and 87.3% respectively, while The prevalence of MdfA gene among amikacin and gentamicin resistant isolates were 59% and 71% respectively.

In a previous study by reported by ⁶ that AcrD of *Escherichia coli* is an aminoglycoside efflux Pump and deletion of the *acrD* gene decreased the MICs of amikacin, gentamicin, neomycin, kanamycin, and tobramycin by a factor of two to eight.

REFERENCES

1. Drago L, Nicola L, Mattina R, De Vecchi E.: In vitro selection of resistance in Escherichia coli and Klebsiella spp. at in vivo fluoroquinolone concentrations. *BMC. Microbiol.*, 2010; 10, 119.
2. Andersen JL, He GX, Kakarla P, K CR, Kumar S, Lakra WS, et al: Multidrug efflux pumps from Enterobacteriaceae, Vibrio cholerae and Staphylococcus aureus bacterial food pathogens. *Int J Environ Res Public Health.*, 2015; 12, 1487-547.
3. Webber MA & Piddock LJ: The importance of efflux pumps in antibiotic resistance. *J Antimicrob Chemother.*, 2003; 51, 9-11.
4. Martins A, Machado L, Costa S, Cerca P, Spengler G, Viveiros M & Amaral L: Role of calcium in the efflux system of Escherichia coli. *International journal of antimicrobial agents*, 2011; 37(5), 410-414.
5. Viveiros M, Jesus A, Brito M, Leandro C, Martins M, Ordway & Amaral L: Inducement and reversal of tetracycline resistance in Escherichia coli K-12 and expression of proton gradient-dependent multidrug efflux pump genes. *Antimicrobial agents and chemotherapy*, 2005; 49(8), 3578-3582.
6. Rosenberg EY, Ma D & Nikaido H: AcrD of Escherichia coli is an aminoglycoside efflux pump. *Journal of bacteriology*, 2000; 182(6), 1754-1756.
7. Putman M, van Veen HW & Konings WN: Molecular properties of bacterial multidrug transporters. *Microbiology and Molecular Biology Reviews*, 2000; 64(4), 672-693.
8. Bhardwaj AK, Mohanty P: Bacterial efflux pumps involved in multidrug resistance and their inhibitors: rejuvenating the antimicrobial chemotherapy. *Recent. Pat. Anti. Infects. Drug. Discov.*, 2012; 7, 73-8.
9. Martins A, Machado L, Costa S, Cerca P, Spengler G, Viveiros M & Amaral L: Role of calcium in the efflux system of Escherichia coli. *International journal of antimicrobial agents*, 2011; 37(5), 410-414.
10. Zechini B & Versace I: Inhibitors of Multidrug Resistant Efflux Systems in Bacteria. *Recent Patents on Anti-Infective Drug Discovery*, 2009; 4, 37-50.
11. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
12. Coban AY, Guney AK, Cayci YT & Durupinar B: Effect of 1-(1-Naphtylmethyl)-piperazine, an efflux pump inhibitor, on antimicrobial drug susceptibilities of clinical Acinetobacter baumannii isolates. *Current microbiology*, 2011' 62(2), 508-511.
13. Ngwai YB, Shekwodza CA, Istifanus H, Nkene IH & Owuna GE: Mutant Prevention Concentrations of Some Aminoglycoside Antibiotics for Fecal Isolates of Escherichia coli under different Growth Temperatures. *J Natural Sci Res*, 2013; 3, 147-155.
14. Yang Y & Chua KL: Assessment of the effect of efflux pump inhibitors on in vitro antimicrobial susceptibility of multidrug-resistant Acinetobacter baumannii. *International journal of antimicrobial agents*, 2013; 42(3), 283-284.
15. Viveiros M, Jesus A, Brito M, Leandro C, Martins M, Ordway & Amaral L: Inducement and reversal of tetracycline resistance in Escherichia coli K-12 and expression of proton gradient-dependent multidrug efflux pump genes. *Antimicrobial agents and chemotherapy*, 2005; 49(8), 3578-3582.
16. Swick MC, Morgan-Linnell SK, Carlson KM, & Zechiedrich, L: Expression of multidrug efflux pump genes acrAB-tolC, mdxA, and norE in Escherichia coli clinical isolates as a function of fluoroquinolone and multidrug resistance. *Antimicrobial agents and chemotherapy*, 2011; 55(2), 921-924.
17. Wang S, Zhao S-Y, Xiao S-Z, Gu F-F, Liu Q-Z, Tang J, et al.: Antimicrobial Resistance and Molecular Epidemiology of Escherichia coli Causing Bloodstream Infections in Three Hospitals in Shanghai, China. *PLoS*, 2016; ONE 11(1): e0147740
18. El-Naggar W, El-Sokkary MA, Barwa R, Abd El Galil K, Shokralla S, and AbdelRhman H, SH: Phenotypic and Genotypic Characteristics in Relation to Some Efflux Pump Systems in Escherichia coli and Klebsiella pneumoniae Clinical Isolates.
19. Mama M, Abdissa A & Sewunet T.: Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. *Annals of clinical microbiology and antimicrobials*, 2014; 13(1), 1.
20. Ghada F. Helaly¹, Sherine Shawky, Rania Amer, OlaAbdel- kader, Gamal El-Sawaf¹, Mohammed A. El Kholy: Expression of AcrAB Efflux Pump and Role of Mefloquine (as Efflux Pump Inhibitor in MDR E.coli), 2016; 4(1): 6-13
21. Gad GF, Mohamed HA & Ashour HM: Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. *PLoS One*, 2011; 6(2), e17224.
22. Rama Sikka, Jk Mann, Deep, Mg Vashist, Uma Chaudhary, Antriksh Deep: Prevalence and Antibiotic Sensitivity Pattern of Bacteria isolated from Nosocomial Infections in a Surgical Ward, *Indian Journal of Clinical Practice*, 2012; 22(10).
23. Moini AS, Soltani B, Ardakani AT, Moravveji A, Erami M, Rezaei MH & Namazi M: Multidrug-

- Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolated From Patients in Kashan, Iran. *Jundishapur journal of microbiology*, 2015; 8(10).
24. Hammuel C, Jatau ED & Whong CM: Prevalence and Antibiogram Pattern of Some Nosocomial Pathogens Isolated from Hospital Environment in Zaria, Nigeria. *Aceh International Journal of Science and Technology*, 2014; 3(3).
 25. Sharma AR, Bhatta DR, Shrestha J & Banjara MR: Antimicrobial susceptibility pattern of *Escherichia coli* isolated from urinary tract infected patients attending Bir Hospital. *Nepal Journal of Science and Technology*, 2013; 14(1), 177-184.
 26. Niranjana V & Malini A: Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. *Indian J Med Res*, 2013; p 945-948
 27. R Kadhar Nivas; Abdel-Ghaffer, Mamdouh H1; Al-Harbi, Abdulkarim Samir, Al-Ghonaim, Mohammed, Enas SH Khater: Antimicrobial Susceptibility Pattern of *Escherichia coli* isolated from urinary tract infections at Al-Quwayiyah General Hospital, Al-Quwayiyah, Kingdom of Saudi Arabia. *International Research Journal of Medical Sciences.*, 2014; 2(2), 18-21.
 28. Ayatollahi, Shahcheraghi & Akhondi: Antibiotic Resistance Patterns of *Escherichia coli* Isolated from Children in Shahid Sadoughi Hospital of Yazd, Iranian Journal of Pediatric Hematology Oncology. 2012; 13(2).
 29. Deng Y, Zeng Z, Tian W, Yang T & Liu JH: Prevalence and characteristics of *rmtB* and *qepA* in *Escherichia coli* isolated from diseased animals in China. *Frontiers in microbiology*, 2013; 4, 198.
 30. Mulu W, Kibru G, Beyene G & Damtie, M: Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiopian journal of health sciences*, 2012; 22(1), 7-18.
 31. Sangeeth, Rajesh, Indrapriyadharsini: Antibiotic resistance pattern of *Escherichia coli* causing urinary tract infection with an emphasis on fluoroquinolone resistance. *global journal of medicine and public health*, 2014 .
 32. Zayed AAF, Essam TM, Hashem AGM & El-Tayeb OM: 'Supermutators' found amongst highly levofloxacin-resistant *E. coli* isolates: a rapid protocol for the detection of mutation sites. *Emerging microbes & infections*, 2015; 4(1), e4.
 33. Huang, Y, Ogutu JO, Gu J, Ding F, You Y, Huo Y & Chen X: Comparative analysis of quinolone resistance in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* from Chinese children and adults. *BioMed research international*, 2015
 34. Ullah F, Malik S & Ahmed J: Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. *African Journal of Biotechnology*, 2009; 8(16).
 35. Nhung NT, Thuy CT, Trung NV, Campbell J, Baker S, Thwaites G & Carrique-Mas J: Induction of Antimicrobial Resistance in *Escherichia coli* and Non-Typhoidal *Salmonella* Strains after Adaptation to Disinfectant Commonly Used on Farms in Vietnam. *Antibiotics*, 2015; 4(4), 480-494.
 36. Jeyaseeli L, Dasgupta A, Dastidar SG, Molnar J & Amaral L: Evidence of significant synergism between antibiotics and the antipsychotic, antimicrobial drug flupenthixol. *European journal of clinical microbiology & infectious diseases*, 2012; 31(6), 1243-1250.
 37. Sun H, Li S, Xie Z, Yang F, Sun Y, Zhu Y & Jiang S: A novel multidrug resistance plasmid isolated from an *Escherichia coli* strain resistant to aminoglycosides. *Journal of antimicrobial chemotherapy*, 2012; 67(7), 1635-1638.
 38. Coutinho HD, Costa JG, Lima EO, Falcão-Silva VS & Siqueira-Júnior JP: Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. *Chemotherapy*, 2008; 54(4), 328-330.
 39. Liliana Rodrigues, Jorge Ramos, Isabel Couto1, Leonard Amaral & Miguel Viveiros: Ethidium bromide transport across *Mycobacterium smegmatis* cell-wall: correlation with antibiotic resistance, *BMC Microbiology*, 2011; 11:35
 40. Rajyaguru JM & Muszynski MJ: Enhancement of *Burkholderia cepacia* antimicrobial susceptibility by cationic compounds. *Journal of Antimicrobial Chemotherapy*, 1997; 40(3), 345-351.
 41. Viveiros M, Martins A, Paixão L, Rodrigues L, Martins M, Couto I, & Amaral L.: Demonstration of intrinsic efflux activity of *Escherichia coli* K-12 AG100 by an automated ethidium bromide method. *International journal of antimicrobial agents*, 2008; 31(5), 458-462.
 42. Amaral L, Martins M & Viveiros M.: Phenothiazines as anti-multi-drug resistant tubercular agents. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, 2007; 7(3), 257-265.