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ORIGINAL ARTICLE

Activity of Fosfomycin against Extended-Spectrum-Beta-Lactamase producing *Enterobacteriaceae* isolates from Menoufia University Hospitals

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ESBL, fosfomycin, Enterobacteriaceae, Fos gene

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ABSTRACT

Background: *Fosfomycin is an old antibiotic that is being reconsidered for the treatment of lower urinary tract, due to the global increasing resistance of several bacteria to numerous-antimicrobials.* ***Objectives:*** *To evaluate the activity of fosfomycin and to detect fosfomycin resistant genes (FosA3 and FosC2) in extended-spectrum-beta-lactamase producing Enterobacteriaceae isolates from Menoufia University Hospitals.* ***Methodology:*** *The study included 155 samples collected from patients from different departments and ICUs at Menoufia University Hospitals, detected the ESBL-producing,by combined confirmatory method, fosfomycin resistance was assessed by disk diffusion and the minimal inhibitory concentration (MIC) by E-test method and fosfomycin resistance genes (FosA3 and FosC2) were detected by the conventional PCR.* ***Results:*** *Among the 48 ESBL-producing Enterobacteriaceae isolates analyzed in this study, 21 isolates had fosfomycin resistant genes (FosA3 and FosC2).* ***Conclusion:*** *Fosfomycin showed good antimicrobial activity against multidrug-resistant ESBL-positive Enterobacteriaceae. Resistance genes (FosA3 and FosC2) were detected in some strains, however, other resistance mechanisms to fosfomycin may exist.*

INTRODUCTION

Fosfomycin, which was discovered in 1969, is a low molecular mass (138 Da) derivative of a phosphoric acid isolated from cultures of *Streptomyces* spp. (*Streptomyces fradiae, Streptomyces viridochromogenes*, and *Streptomyces wedomorensis*). It is also produced in a biosynthetic process involving a unique combination of carbon and phosphorous[1,2].

Fosfomycin enters the bacterium through two different membrane transportation systems:L-alpha glycerol-3-phosphate and the glucose-6-phosphate transporter (G6P) (GlpT and UhpT, respectively).Cyclic adenosine monophosphate (cAMP) is also essential for the expression of the genes of both transportation systems [1].The bacterial activity of fosfomycin involves interfering with the initiation reaction in the biosynthesis of the peptidoglycan (PG), and inhibits the enzyme UDP-*N*-acetylglucosamine enolpyruvyl transferase (or MurA). Fosfomycin forms a covalent adduct with the thiol group of a cysteine and inactivates the active side of MurA.Consequently, UDP-N-acetylmuramic acid, the precursor of PG,is not formed, leading to the loss of PG layer integrity, cell lysis and death[3].

Several resistance mechanisms have been described.Inherent resistance due to mutations in the MurA gene confer resistance to fosfomycin[4], and acquired resistance mechanisms involving the fosfomycin transportation. The bacteria may develop resistance to fosfomycin by chromosomal mutations in the structural genes that encode the GlpT and UhpT membrane transporters[5]. MurA mutation has been described in which aspartate substitutes for cysteine in position 115 of the *E. coli* MurA renders susceptible isolates highly resistant to fosfomycin[6]. Moreover, antibiotic modification,a mechanism that was first described for the metalloenzyme glutathioneS-transferase, encoded by *FosA*, that uses Mn^{+2} and K^{+}as cofactors. Glutathione-S-transferase inactivates fosfomycin by opening the antibiotic epoxide group and adding the sulphydryl group of the cysteine of tripeptide glutathione (GSH) to C1 of the epoxide ring of fosfomycin[7,8].

The fosfomycin-modified enzymes (*Fos* genes) in *Enterobacteriaceae*, it have at least 10 kinds of (*Fos* genes) have been described[9,10]. The first, *FosA*, which encodes (Mn^{+2})-dependent glutathione-S-transferase (GST). *FosA* and its subtypes, and *FosC2* were mainly responsible for fosfomycin resistance in *Enterobacteriaceae*[10]. The *FosA3* in plasmids has been the most frequently found *FosA* subtype. A report from Japan involved CTX-M-ESBL-producing *E. coli* and efficaciously modified fosfomycin *via* glutathione-S-transferase (GST) activity. The *FosC2*, is located on plasmids, and it shares 56% amino acid sequences with

FosA. Although different sequences exist between *FosA* and *FosC2*, both modify fosfomycin *via* GST activity[9].

METHODOLOGY

Patients and specimen collection

This study was performed in the Microbiology and Immunology Department, Faculty of Medicine, Menoufia University during the period from March 2018 to July 2019. The study included 155 patients from different departments and ICUs at Menoufia University Hospitals. Their ages ranged from 4 to 73 years (31.41±14.52). Approval of the ethics committee for the research design was obtained from Faculty of Medicine, Menoufia University.

Microbiological culture and counting [11]:

Identification of isolates [12].

Antimicrobial susceptibility screening tests for Enterobacteriaceae bacilli[13].

Detection of ESβL production by *Enterobacteriaceae* isolates

Disk diffusion screening test[13].

Combined disk diffusion confirmatory test[13].

Fosfomycin susceptibility testing: this was done for the following methods

Disk diffusion method [13].

Determination of the minimal inhibitory concentration (MIC) of fosfomycin.

The MIC of fosfomycin was determined by the E-test method[13].

Molecular Methods:

Detection of fosfomycin resistance genes, (*FosA3* and *FosC2*) was carried out using the conventional PCR[14].

Plasmid extraction and purification

Plasmid DNA extraction and purification was carried out using the QIAamp DNA mini kit and microcentrifuge according to the Manufacturer's instructions[15].

Preparation of the primers

Primers were shipped and received in a lyophilized state[15].

FosA3 / F :5′ GCGTCAAGCCTGGCATTT 3′ /*R* : 5′ GCCGTCAGGGTCGAGAAA 3′

Annealing temp (57.5°C) and size (bp) 282

FosC2/ F : 5 ′ TGGAGGCTACTTGGATTTG 3 ′ /*R* : 5′AGGCTACCGCTATGGATTT3′

Annealing temp (50.5°C) and size (bp) 217

A master stock (for each primer) was created by adding RNAse free water as described by the Manufacturer's instructions.

Preparation of PCR mix

The protocol described by Qiagen (Germany) was carried out:

Thawing primer solutions and keeping them on ice after complete thawing, mixing the Taq PCR master mix by vortexing briefly, and dispension of 25 µl into each PCR tube, distributing the appropriate volume of the diluted primer, mixing into the PCR tubes containing the master mix, adding template DNA (≤1 µg/reaction) to the individual PCR tubes, then completing the reaction volume up to 50ul by nuclease free water.

PCR analysis cycle:

PCR initial denaturation was set at 94°C for 3 minutes. Cycling consisted of denaturation step that was set at 94°C for 0.5–1 minutes, and combined annealing/extenstion step that was set at 50–68°C and 1 min 72°C for 0.5–1 min. The cycles were repeated for 25–35 times.

Detection of PCR products:

The agarose gel (1.5%) was prepared by adding 1.5 gm agarose to 100 ml of Ix Tris borate EDTA (TBE) buffer. The agarose was dissolved by boiling and allowed to cool to 50°C, and then 10 µl of ethidium bromide (10 mg/ml) were added for later visualization of the bands[16]. After cooling, it was poured into the cast. Then a comb was placed in the cast to create wells for loading samples, and the tray was flooded with I x TBE[17]. Ten microliters of the amplification products were slowly loaded into the slits of the submerged gel using a micropipette. A DNA molecular weight marker was run in parallel. The electrophoresis was carried out at 120 Volts[16]. DNA bands were visualized on UV trans-illuminator and photographed. The *FosA3* (282 bp) and *FosC2* (217 bp) genes were determined by the site of the amplified product in comparison with the known ladder.

RESULTS

The relation between demographic and clinical data of the studied patients and the results of urine culture. Significant higher rates of UTIs (55.3%) were found in patients with duration of hospital stay more than 14 days. Patients whose catheter is inserted in general ward had higher rate of UTI (66.7%) than ICU (24.2%) and operation room (9.10%) (P<0.001) and in, diabetic patients (P<0.001) than non diabetics. The highest rate of positive cultures was found in ICUs while the lowest rate was found in the Pediatrics department (no growth among 10 specimens) with a high statistically significant difference (P<0.001) were shown in table 1 , 2.

The antibiotic resistance of *E. coli*, *Proteus* spp. and *K. pneumoniae* isolates by the disk diffusion method. They were highly resistant to amoxacillin/clavulanic acid (100%), ofloxacin (67.6%), ciprofloxacin (94.2%), gentamycin (82.4%), tobramycin (73.5%), cefotaxime (73.5%) and ceftazidime (76.5%). Carbepenem, amikacin, fosfomycin and levofloxacin were the most active agents against the isolated *Enterobacteriaceae.* 87.8% of *E. coli* isolates were MDR, 61.2% were XDR and 4.10 % were PDR. Regarding *Proteus spp*., 87.5%

were MDR and 62.5% were XDR while there was no PDR srrains. On the other hand, 82.41%, 58.8% and 8.8% of *K. pneumoniae* strains were MDR, XDR, and PDR respectively, were shown in, table 3, figure 1.

In our study, showed that all the isolated *Enterobacteriaceae* were screened for ESβL production by simple disk diffusion method and confirmed by combined disk method. Most of the isolates (39/48) were ESβL producers by using CAZ/CAC combination. Three isolates were ESβL- producers by using CTX/CTC combination. Six isolates were ESβL-producers by using both CAZ/CAC and CTX/CTC combinations. Fosfomycin susceptible and resistant strain of *E. coli* respectively, by the E-test, were shown in table 4, figure 2 (A , B).

Show the amplified products of fosfomycin resistance gene (*FosC2* and *FosA3*) from the *E. coli.* Lanes: 3,4,5 were positive for the *FosC2* (217 bp) gene and lanes: 5,6,7 were positive for *FosA3* (282 bp) gene. 25/26 of *E. coli* were susceptible to fosfomycin by the E-test (1/26) was resistant, (17/18) of *K. pneumonia* were susceptible to fosfomycin, (1/18) was resistant and the *Proteus* spp. (4/4) susceptibile to fosfomycin, (13/26, 50.0%), (7/18, 38.8%) and (1/4, 25.0%) positive fosfomycin resistance genes (*FosA3* and *FosC2*), respectively. Fosfomycin-resistant gene among ESBL–producing *Enterobacteriaceae* species in relation to the demographic and clinical data of the studied patients, 21/48 positive genes (*FosA3* and *FosC2)* were detected in our isolates. On relating the presence of positive genes to the different factors, they were more common (13/21, 61.9%) among the age group > 50 years, (15/21, 71.4%), among patients with duration of hospital stay more than 14 days, and among ICU patients (11/21, 52.4%), figure 3, 4 and table 5 , 6.

Table 1: Results of urine culture in relation to the different demographic characters of the studied patients (N = 155).

Socio demographic characters		Urine culture				Total (N=155)		X^2	P value
		Positive (N = 94)		Negative (N=61)					
		No.	%	No.	%	No.	%		
Age / years	<20	8	8.50	18	29.5	26	16.8		
	20 – 50	30	31.9	28	45.9	58	37.4	21.5	<0.001**
	>50	56	59.6	15	24.6	71	45.8		
Gender	Male	42	44.7	32	52.5	74	47.7		
	Female	52	55.3	29	47.5	81	52.3	0.89	0.344
Marital status	Married	70	74.5	41	67.2	111	71.6	0.95	0.328
	Single	24	25.5	20	32.8	44	28.4		
Duration of hospital stay	<7	18	19.1	25	41.0	43	27.7		
	7 – 14	24	25.5	14	23.0	38	24.5	9.33	0.009**
	>14	52	55.3	22	36.1	74	47.7		
Antibiotic adminstration	Used	84	89.4	49	80.3	133	85.8	2.47	0.115
	Not used	10	10.6	12	19.7	22	14.2		
The main invasive procedures was urinary cathetiziation	Present	66	70.2	38	62.3	104	67.1	1.05	0.305
	Absent	28	29.8	23	37.7	51	32.9		
Place of catheter insertion		N=66		N=38		N=104			
	Operating room	6	9.10	21	55.3	27	26.0	34.9	<0.001**
	ICUs	16	24.2	12	31.6	28	26.9		
	General ward	44	66.7	5	13.2	49	47.1		
Duration of hospital stay		N=66		N =38		N=104			
	<3days	7	10.6	9	23.7	16	15.4	11.6	0.009**
	3 – 7 days	26	39.4	22	57.9	48	46.2		
	>7 days	33	50.0	7	18.4	40	38.4		
Comorbidities Diabetes	Diabetics	59	62.8	26	42.6	85	54.8	6.06	0.014*
	Non diabetics	35	37.2	35	57.4	70	45.2		

Significant level at p< 0.05 *Significant **Highly significant

Table 2: Results of urine culture in different departments

Departments	Total studied patients		Positive cultures				Negative cultures		X^2	P value
			Single culture		Mixed culture					
	No.	%	No.	%	No.	%	No.	%		
Chest	8	5.20	4	4.5	0	0.00	4	6.60	0.60	0.742
Obestetrics & Gynecology	10	6.50	6	6.7	0	0.00	4	6.60	0.36	0.835
Internal Medicine	25	16.1	16	18.0	0	0.00	9	14.8	127	0.529
ICUs	40	25.8	30	33.7	3	60.0	7	11.5	12.5	0.001**
Orthopedics	12	7.70	6	6.70	0	0.00	6	9.80	0.56	0.756
Pediatrics	10	6.50	0	0.00	0.00	0.00	10	16.4	16.4	0.001**
Surgery	18	11.6	10	11.2	0	0.00	8	13.1	0.80	0.669
Urology	32	20.6	17	19.1	2	40.0	13	21.3	1.29	0.524
Total	155	100	89		5		61			

X^2 test was done at 5% level of significance **highly significant ICU: Intensive care unit

Table 3: Antibiogram of *E. coli* , *Proteus* spp. *and K. pnemoniae* isolates by disk diffusion method

Antibiotics Disk content (mg)	*E. coli*						*Proteus* spp.						*K. pneumoniae*					
	S		I		R		S		I		R		S		I		R	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin/ clavulanic acid (20/10)	0	0.00	0	0.00	49	100	0	0.00	0	0.00	8	100	0	0.00	0	0.00	34	100
Ceftazidime (30)	17	34.7	1	2.00	31	63.3	0	0.00	2	25.0	6	75.0	7	20.6	1	2.90	26	76.5
Cefotaxime (30)	18	36.7	1	2.00	30	61.2	0	0.00	1	12.5	7	87.5	7	20.6	2	5.90	25	73.5
Imipenem (10)	31	63.3	0	0.00	18	36.7	4	50.0	0	0.00	4	50.0	19	55.9	0	0.00	15	44.1
Meropenem (10)	29	59.2	1	2.00	19	38.8	6	75.0	0	0.00	2	25.0	19	55.9	0	0.00	15	44.1
Amikacin (30)	30	61.2	0	0.00	19	38.8	4	50.0	1	12.5	3	37.5	17	50.0	2	5.90	15	44.1
Gentamycin (10)	18	36.7	2	4.10	29	59.2	1	12.5	1	12.5	6	75.0	5	14.7	1	2.90	28	82.4
Tobramycin (10)	21	42.9	0	0.00	28	57.1	1	12.5	0	0.00	7	87.5	9	26.5	0	0.00	25	73.5
Ciprofloxacin (5)	7	14.3	1	2.00	41	83.7	0	0.00	2	25.0	6	75.0	2	5.90	2	5.90	30	88.2
Levofloxacin (5)	23	46.9	1	2.00	25	51.0	2	25.0	0	0.00	6	75.0	19	55.9	0	0.00	15	44.1
Ofloxacin (5)	20	40.8	0	0.00	29	59.2	1	12.5	1	12.5	6	75.0	11	32.4	0	0.00	23	67.6
Fosfomycin (200)	25	51.0	0	0.00	1	2.00	4	50.0	0	0.00	0	0.00	17	50.0	0	0.00	1	2.90

S:Sensitive R: Resistant I: Intermediate R+I (Non susceptible)

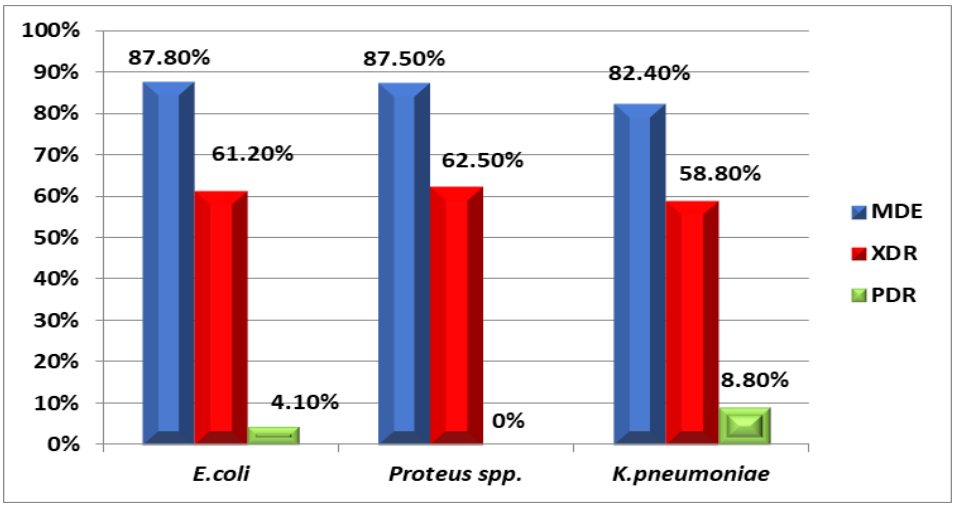

Fig. 1: Percentage of multi-drug, extreme–drug and pan-drug resistance among *E. coli, Proteus spp and K. pneumoniae* isolates.

*MDR: non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories.

** XDR: non-susceptible to ≥ 1 agent in all but ≤ 2 categories.

*** PDR: non-susceptible to all antimicrobial agents listed.

Table 4: ESBL production in the studied bacterial isolates by the combined confirmatory method.

ESBL-producing *Enterobacteriaceae*	**CAZ/CAC**	**CTX/CTC**	**CAZ/CAC and CTX/CTC**
E. coli (n=26)	21	2	3
K. pneumoniae (n=18)	14	1	3
Proteus (n=4)	4	0	0
X^2	1.07	0.37	0.88
P value	0.585	0.829	0.644

CAZ: Ceftazidime CAC: Ceftazidime / Clavulanate CTX: Cefotaxime CTC: Cefotaxime / Clavulanate

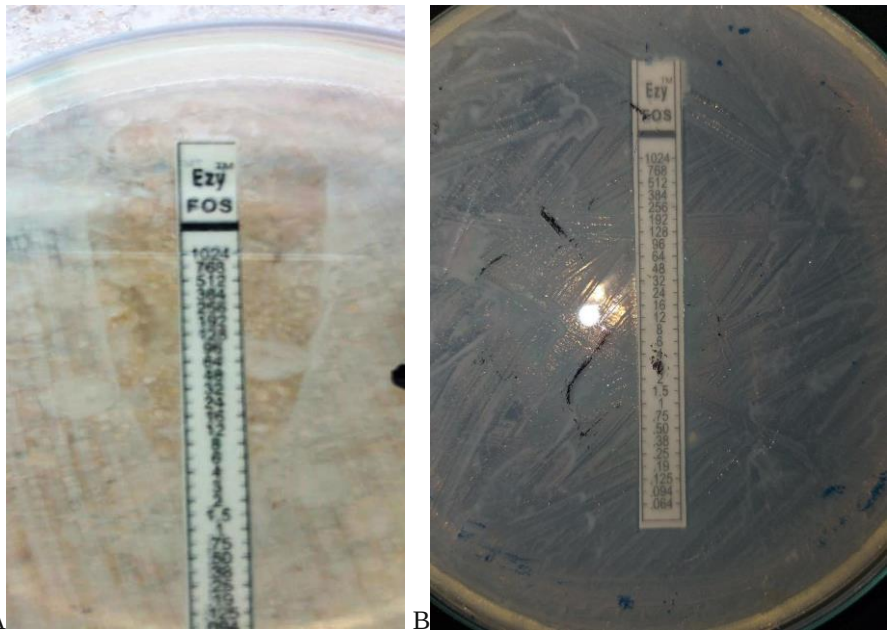

Fig. 2: *E. coli* susceptibility to fosfomycin by the E-test and resistance. (A): MIC ≤ 64 µg/ml; (B): MIC ≥256 µg/ml

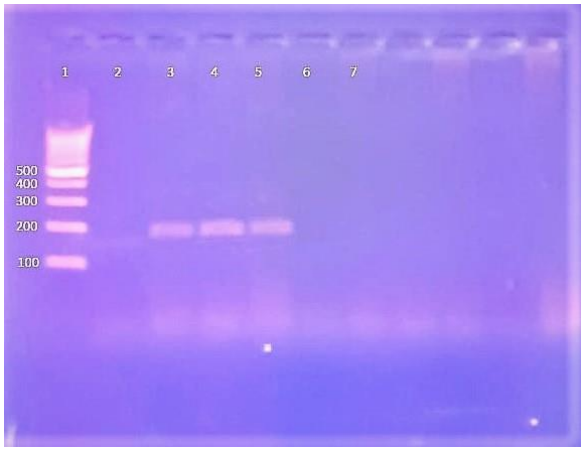

Fig. 3: Agarose gel electrophoresis, showing the conventional PCR amplified products of fosfomycin resistance gene (*FosC2)* in *E. coli*
Lane 1: DNA molecular size marker (100-500 bp).
Lanes : 3,4,5 were positive for the *FosC2* (217 bp) gene
Lanes : 2,6,7 were negative for the *FosC2* gene

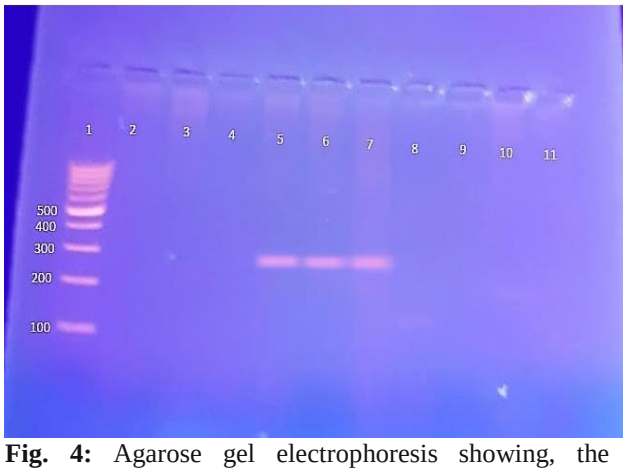

Fig. 4: Agarose gel electrophoresis showing, the conventional PCR amplified products of fosfomycin resistance gene (*FosA3)* in *E. coli*
Lane 1: DNA molecular size marker (100-500 bp).
Lanes: 5.6,7 were positive for *FosA3* (282 bp) gene
Lanes: 2,3,4,8,9,10,11 were negative for the *FosA3* gene.

Table 5: Fosfomycin susceptibility patterns in relation to presence of fosfomycin resistant genes (FosA3 and FosC2) Enterobacteriaceae in the studied.

Fosfomycin resistance genes (*FosA3*and *FosC2*)	Fosfomycin susceptibility patterns by MIC (E-test)														
	E. coli				FE P value	*K. pneumoniae*				FE P value	*Proteus* spp.				FE P value
	S		R			S		R			S		R		
	N	%	N	%		N	%	N	%		N	%	N	%	
Positive (n=21)	12	48.0	1	100	1.04	6	35.3	1	100	1.66	1	25.0	0	0.00	0.00
Negative (n=27)	13	52.0	0	0.00	0.30	11	64.7	0	0.00	0.19	3	75.0	0	0.00	1.00
Total 48	25		1			17		1			4		0		

FE:Fisher exact test

Shows that, 13/21 *E. coli*, 7/21 *K. pneumonia* and 1/21 *Proteus* spp. positive fosfomycin resistance genes (*FosA3* and *FosC2*).

Table 6: Fosfomycin-resistant genes among ESBL–producing *Enterobacteriaceae* species in relation to demographic and clinical data of the studied patients.

Demographic and clinical characteristics	Fosfomycin resistance genes *(FosA3* and *FosC2)*				X^2	P value
	Positive (N=21)		Negative (N=27)			
	No.	%	No.	%		
Age / years						
<20	2	9.50	3	11.1		
20 – 50	6	28.6	8	29.6	0.047	0.977
>50	13	61.9	16	59.3		
Gender						
Male	11	52.4	10	37.0	1.13	0.288
Female	10	47.6	17	63.0		
Marital state						
Married	16	76.2	22	81.5	FE=	0.654
Single	5	23.8	5	18.5	0.201	
Duration of hospital stay					3.91	0.141
<7	3	14.3	5	18.5		
7 – 14	3	14.3	10	37.0		
>14	15	71.4	12	44.4		
Antimicrobial administration						
Used	19	90.5	24	88.9	0.032	0.858
Not used	2	9.50	3	11.1		
The main invasive procedures was urinary catheteriziation						
Present	17	81.0	18	66.7		
Absent	4	19.0	9	33.3	1.22	0.269
Comorbidities						
Present						
Diabetes only	1	4.80	2	7.40		
DM + Other comorbidities	11	52.4	14	51.9	0.349	0.951
Other comorbidities only	4	19.0	6	22.2		
Absent	5	23.8	5	18.5		
Departments						
Chest	0	0.00	1	3.70		
Internal Medicine	1	4.80	8	29.6		
ICUs	11	52.4	8	29.6	11.4	0.043*
Orthopedics	0	0.00	2	4.70		
Surgery	1	4.80	8	14.8		
Urology	8	38.1	4	14.8		

Significant level at p< 0.05 *significant

DISCUSSION

In the present study, UTI was more common among old patients (> 50 years old) (59.6%) than other age groups with a high statistically significant difference (P< 0.001) between different age groups. This finding agrees with that reported by Amine, et al[18] and El Lawindi, et al[19]. In our study, there was a high statistically significant difference between the rate of UTI and length of hospital stay. This observation agrees with that found by Hammad, et al[20] and Al-Hazmi, et al [21] in Saudi Arabia. Diabetic patients had statistically (P<0.001) higher rate of UTI than non diabetics, a finding which has been previously found by Lee, et al[22]. Interestingly, our study showed that higher rates of UTI were found in patients with urinary catheters (70.2%) compared to those having no urinary catheter (29.8%). Also, the length of time of catheterization was statistically proportional to the development of infection. Tasbakan, et al[23] and Malled, et al[24] found that there was a direct relationship between the duration of catheterization and nosocomial UTI. With short term catheterization (up to 7 days), 10-50%, of patients develop infections, whereas in long-term catheterization (>28 days), usually all patients develop UTI. Patients whose catheter is inserted in general ward had higher rate of UTI (66.7%) as compared to ICU (24.2%) and operation room (9.10%) with a high statistically significant difference. This observation agrees with Tasbakan, et al [23] who demonstrated that occurrence of UTI was related to the place of catheter insertion 52.7% in general ward, 29.4% in ICU, 14.1% in the emergency ward and 3.6% in operating room).

The percent of positive cultures in the different departments was highest in ICU followed by Urology, Internal Medicine and Surgery Departments (P<0.001). This result matched with other studies done by Hassaneen et al.[25] in Zagazig, Egypt (45%). On the other hand, the Pediatrics department showed no growth in, 16.4% of case.

About 99 isolates (mainly Gram-negative bacteria) were obtained from the 155 studied patients. Among Gram-negative organisms the most frequent isolates were *E. coli* (50.5%), followed by *Proteus spp.* (8.20%), *K. pneumoniae* (35.1%), *P. aureginosa* (3.10%). On the other hand, the only isolated Gram-positive organism was *S. aureus* (3.10%). This result correlates with other studies conducted across our country and around the world. Shaaban, et al[26], Abd El–Mongy and Reyad,[27] found that the most predominant isolated of *Enterobacteriaceae* was *E. coli* followed by *K. pneumoniae.* About 89 specimens resulted in single microbial growth while 5 specimens showed mixed cultures (2 isolates for each).

In our study, antibiogram of the isolated *E. coli*, *Proteus spp.* and *K. pneumoniae* demonstratesd that all isolates were resistant to amoxicillin/clavulanic acid. They were highly resistant to ofloxacin (67.6%), ciprofloxacin (94.2%), gentamycin (82.4%), tobramycin (73.5%), cefotaxime (73.5%) and ceftazidime (76.5%). Carbepenem, amikacin, fosfomycin and levofloxacin were the most active agents against the isolated *Enterobacteriaceae*.

The most alarming finding in our study is that 87.8% of *E. coli* isolates were MDR, 61.2% were XDR and 4.10 % were PDR. Regarding *Proteus* spp. 87.5% were MDR and 62.5% were XDR and no PDR srrains. On the other hand, 82.41%, 58.8% of *K. pneumoniae* strains 58.8% were MDR, XDR, and PDR respectively. This alarming result is nearer to that reported by Abd El–Mongy and Reyad,[27] in Saudi Arabia who found that most of the isolated uropathogens were highly resistant to most of the antibiotics except, levofloxacin and carbapenem. The authors explained the efficiency of levofloxacin and carbapenem antibiotics against all UTI pathogenic strains by their multiple mechanisms of action which enabled them to retain potent activity against pathogens. In agreement with this, Vasudevan[28], El-Esawi and Mostafa[29], confirmed the altered antimicrobial pattern among the UTI-causing pathogens like *E. coli*, *Klebsiella* spp. and *Proteus* spp. where they showed high resistance to multiple antibiotics in theis studies.

In the present study, all the isolated *Enterobacteriaceae* were screened for ESβL production by simple disk diffusion method and confirmed by combined disk method as recommended by the CLSI,[15] for *E. coli*, *K. pneumoniae* and *Proteus* spp. of *Enterobacteriaceae* isolated by Garrec, et al.[30] and Dortet, et al[31] for other *Enterobacteriaceae*. 52.7% showed, ESBL-production by the combined confirmatory method. This result agrees with that of Yesmin, et al [32] who reported that (87%) were potential ESβL-producers by CLSI screening method while confirmed ESβLs by combined disk test were (71%) with statistically significant difference, a finding which was also found in a recent study performed in Benha university, Khater and Sherif, [33] and Rashed, et al[34]. In our study, the fosfomycin sensitivity was screened by using a 200-µg disk with a diameter cut-off of ≥16 mm and the MIC was determined by the E-test method which confirmed susceptibility to fosfomycin (MIC ≤ 64 µg/ml) as previously reported by Yahia, et al[35] .

In the present study, we looked for the fosfomycin resistance genes (*FosA3* and *FosC2*) among our isolated strains. Our results that showed, that 25/26, 17/18 and 4/4 of the ESBL-producing *E. coli*, *K. pneumoniae* and *Proteus* spp respectively, were susceptible to fosfomycin. Among the 48 strains, 46 (95.8%) were sensitive. The 2 resistant strains included one *E. coli* and one *K. pneumoniae.* Strain PCR results indicated

that (43.75%) harbored the (*FosA3* and *FosC2)* genes. This result agrees with that reported, in a Korean study by Lee, et al[36] who showed that, 92.9% (157/165) of ESBL-producing *E. coli* isolates were susceptible to fosfomycin and 7 isolates harbored the *FosA3* gene. Other researchers have reported that *FosC2* was accompanied by other resistant genes in class 1 integrons Wachino, et al[9] and Wang, et al[37]. Relating the fosfomycin--resistant gene among ESBL–producing *Enterobacteriaceae* species to demographic and clinical data of the studied patients showed that 21/48 strains were positive for genes *FosA3* and *FosC2*, (13/21), were among age group > 50 years, (15/21), were found in patients with duration of hospital stay more than 14 days, and the among ICU patients (11/21).

CONCLUSION

Fosfomycin had higher effect than the other antibiotics against the ESBL-producing isolates of the most common *Enterobacteriaceae*. Therefore, fosfomycin may be deemed a promising antibiotic for the treatment of urinary tract infections caused by ESBL-producing *Enterobacteriaceae*.

Conflicts 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.

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