ORIGINAL ARTICLE

Isolation of Gram-Negative Organisms Causing Nosocomial Catheter Associated Urinary Tract Infection and Detection of Fosfomycin Effect on Multi-Drug Resistant Strains in Sohag University Hospital

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Key words:
CAUTI, MDR GN bacilli, ESBL, MBL, fosA, fosA2, fosA3, fosC2

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ABSTRACT

Background: *CAUTIs are the commonest HAIs and frequently caused by MDR Gram negative organisms, fosfomycin could be potentially used for this infection.* ***Objectives:*** *To isolate, identify Gram negative organisms causing CAUTIs, their antibiotic resistance pattern and to investigate their sensitivity to fosfomycin at Sohag University Hospitals.* ***Methodology****: Cross-sectional study included patients with catheter associated urinary tract infections (CAUTIs) admitted in the different Departments of Sohag University Hospitals from December 2019 to December 2022. The study included 310 urine samples from CAUTIs. Urine samples were subjected to routine culture, complete biochemical identification and antibiotic sensitivity testing were done by the BD Phoenix™ automated identification and susceptibility testing system. ESBL producing organisms were detected by double-disk synergy test (DDST), Carbapenemase production by the modified Hodge test (MHT) and MBL production by EDTA combined disk synergy test (CDST). Fosfomycin sensitivity was detected by the disc diffusion method and minimum inhibitory concentration (MIC) by E-test. Conventional PCR was used to detect* ***FosA, fosA2, fosA3, fosC2*** *genes.* ***Results:*** *The study group included (180) patients from them Gram negative bacilli were isolated. Their mean age was 52.8±11.7 years. Males were 99 (55%) of all studied patients. Most of them were from ICU. The most common isolated organism was E. coli 75 (41.7%), followed by Klebsiella pneumoniae 39 (21.7%), and Enterobacter cloacae 24 (13.3), 156 (86.6%) were MDR, 67 (43.3%) were ESBL producers by DDST, 49 (27%) Carbapenemase producers by MHT and 40 (22%) were MBL producers by EDTA CDST. One hundred and fifteen strains (66%) were sensitive to fosfomycin by E- test and FosA, A2, A3, C2 genes were detected in 47 (72.3%) of fosfomycin resistant strains.* ***Conclusion:*** *The study revealed that 86% of CAUTI were caused by MDR Gram negative organisms and 59% of them were susceptible to fosfomycin.*

INTRODUCTION

Catheter associated urinary tract infections (CAUTIs) are the most common nosocomial infection ranging from 30- 40% of all HAI. It may increase morbidity and mortality, extended the time of hospitalization, and cost[1–3]. The antibiotic therapy are three times higher in catheterized patients[4].

CAUTI are frequently caused by *E. coli*, *Klebsiella spp*, *Proteus spp*, *Pseudomonas spp* and other GN organisms[4–7]. There is alarming increase of multidrug-resistant (MDR) bacteria and the World Health Organization (WHO) has identified antibacterial drug resistance as a major threat to public health[8]. Fosfomycin is a broad-spectrum bactericidal antibiotic, with activity against various bacteria, including Gram negative bacteria, by irreversibly inhibiting cell wall synthesis[9]. Fosfomycin has been approved as an oral single-dose treatment for acute uncomplicated UTI[10]. Many studies reported efficacy of fosfomycin against MDR, ESBL Gram negative bacilli isolated from UTI[11–13].

The increase in the clinical use of fosfomycin has led to the development of resistance. fosfomycin resistance is caused by several mechanisms that differ with the geographic locality and the studied bacteria. Fosfomycin resistance can result from reduced permeability, mutations in the MurA target and the production of fosfomycin inactivating enzymes (*fos* genes) which include, fosA and its subtypes, fosC2, and the fos L1-L2[9]. FosA, is a metalloenzyme transferred through plasmids in Gram negative bacilli and the subtype FosA3 enzyme which is the most

frequently identified in Gram negative bacilli as reported by several studies[1,9,14,15].

This is the first study in our hospital to determine the sensitivity of fosfomycin as a potential treatment for MDR Gram negative bacilli causing CAUTI. We aimed to isolate and identify Gram negative organisms causing CAUTI, their antibiotic sensitivity pattern and to detect their sensitivity to fosfomycin with detection of fosfomycin resistance genes in Sohag University Hospital.

METHODOLOGY

Study design and patients

A Cross-sectional study was conducted at Department of Medical Microbiology and Immunology, Sohag Faculty of Medicine from December 2019 to December 2022. The study included patients with catheter associated UTI (CAUTI) admitted in the different Departments of Sohag University Hospitals and the ICU.

The study included patients who met the criteria of CAUTI irrespective to age and gender. CAUTI was defined according to Centers for Disease Control and Prevention (CDC)[2] which included the following: Patient with an indwelling urinary catheter in place for >48 hours with at least one of the following signs or symptoms of acute UTI with no other identified source of infection, fever (>38°C), suprapubic tenderness, Costovertebral angle pain or tenderness and positive urine culture of $\geq 10^5$ CFU/mL and with no more than 2 species of microorganisms.

The following patients were excluded: patients with indwelling urinary catheter less than 2 days, asymptomatic bacteriuria (ASB), UTI before 48 h from time of catheter insertion or after 48h from catheter removal. More than two types of organisms grown on culture media from the clinical sample, were recognized as contaminated and consequently, excluded from the study.

Ethical considerations:

The study was approved by Sohag faculty of medicine ethical committee on December, 2019. An informed written consent was taken from all patients in the study.

Data collection:

The following data were collected from hospitalized catheterized patients in a preformed study sheet. Patient's demographic data as age and gender. Associated comorbid conditions: Renal failure, liver cirrhosis, and diabetes. Possible risk factors: Duration of catheterization, place of catheter insertion, duration of hospital stay and use of broad-spectrum antibiotics.

Isolation and identification of Gram-negative organisms causing CAUTI:

Urine samples from catheterized patients were obtained under complete aseptic conditions. The catheter port or wall of the tubing was then cleaned with 70% ethanol, and urine aspirated via a sterile syringe [16]. The samples were collected in a sterile cup then labeled and transported to the laboratory immediately and subjected to methods of detection or kept refrigerated within 30 minutes at 4^{o} C for 24 hours maximally. Samples were subjected to direct smear and Gram staining. Urine culture was done on Cysteine Lactose Electrolyte Deficient Agar (CLED) agar using semi-quantitative method called the calibrated loop/surface streak method[17]. Isolates on CLED agar were identified by colony morphology, Gram staining, subculture on MacConkey culture medium to get pure growth of the microorganisms. Complete identification and antibiotic sensitivity of the isolated GN organisms were done by BD Phoenix automated identification system and susceptibility testing system using NMIC-50 panel (Becton Dickinson Diagnostic Systems, Sparks, MD, USA)[18].

Detection of ESBL production by double-disk synergy test (DDST):

On Muller Hinton agar inoculated with 0.5 MacFarland bacterial suspension and using a disk of one of the extended spectra cephalosporins (cefotaxime, ceftriaxone, ceftazidime, aztreonam and cefpodoxime) 30-µg and a disk of amoxicillin–clavulanate[19].

Detection of Carbapenemase production by the modified Hodge test (MHT):

Using the indicator organism, *E. coli* ATCC 25922 and the carbapenem disk either ertapenem disks (10 µg) or meropenem disk (10 µg)[20].

Detection of MBL production by EDTA (Ethylene diamine tetra acetic acid) combined disk synergy test (CDST):

Based on the demonstration of synergy between imipenem and EDTA[20].

Antimicrobial susceptibility testing of Fosfomycin

Sensitivity to fosfomycin was done by modified Kirby Bauer's disc diffusion method using discs containing 200µg fosfomycin and 50µg Glucose -6-Phosphate (Oxoid, UK). Interpretations of zone diameters were ≥16 mm as sensitive, 13-15 mm as intermediate and ≤12 mm as resistant[18]. Fosfomycin MIC (Minimum Inhibitory Concentration) was determined by E-test by using Fosfomycin E-test strip papers (Liofilchem® s.r.l.) adjusted on inoculated Mueller Hinton medium supplemented with 25 mg/L of G-6-P (glucose-6-phosphate). MIC ≤64 µg/mL as sensitive, 128 µg/mL as intermediate and ≥256 µg/mL as resistant[18].

Detection of Fos genes by simple qualitative PCR:

Simple qualitative polymerase chain reaction was performed for all strains resistant to fosfomycin as detected by E-test for identification of genes responsible for fosfomycin resistance. DNA extraction was done by boiling method; few colonies were dissolved in 50µl of sterile distilled water and heated for 10 min at 100°C by heat blocks. The bacterial cells were centrifuged for 2 min at 3000 rpm. The supernatant was transferred to Eppendorf tubes, taken as template DNA and stored at -20°C[21].

The PCR reaction was done in a sterile Eppendorf tube. The reaction mix included 12.5µl mastermix (COSMO PCR RED M.MIX, Willofort, UK, catalog number W10203001), PCR grade water 8µl (Invitrogen, USA), forward primer 1.25µl, reverse primer 1.25µl (Invitrogen, USA), 2µl genomic extracted DNA. Detailed sequence of the used primers for each gene are shown in table1.

DNA amplification was done by using a Biometra thermal cycler -T Gradient software version 5.0 PCR system (Biometra, USA). Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of (denaturation at 95 for 5-minute, primer annealing for 1 minute (detailed annealing temperature for each primer are listed in table 1), extension for 45s at 71°C) followed by final elongation step at 72°C for 7 minutes. The reaction was stopped by cooling at 4°C.

Table 1: list of primers used for detection of Fosfomycin resistance genes

Gene	Primer	Nucleotide Sequence	Annealing temperature	Amplicon size	Reference
FosA	Forward Reverse	5′ ATCTGTGGGTCTGCCTGTCGT 3′ 5′ ATGCCCGCATAGGGCTTC T 3′	50.6 °C	271 bp	[22]
FosA2	Forward Reverse	5 ′ GCTGCAATCACTCAACCATC 3 ′ 5 ′ CACGTGCAGCTCCAGCTT 3 ′	57.4 °C	346 bp	[23]
FosA3	Forward Reverse	5′ GCGTCAAGCCTGGCATTT 3′ 5′ GCCGTCAGGGTCGAGAAA 3′	53 °C	282 bp	[23]
FosC2	Forward Reverse	5 ′ TGGAGGCTACTTGGATTTG 3 ′ 5′AGGCTACCGCTATGGATTT3′	50.5 °C	217 bp	[22]

The amplified PCR products were detected by gel electrophoresis on a 2 % agarose gel and stained with 0.5 µg/mL ethidium bromide and DNA molecular weight marker, 100bp ladder (DL004, Biomatik). Following electrophoresis, the agarose gels were visualized and photographed by transillumination with UV light using InGenius3 gel documentation system (Syngene™ IG3, England).

Statistical analysis

Data were analyzed using STATA version 14.2 (Stata Statistical Software: Release 14.2 College Station, TX: Stata Corp LP.). Quantitative data were represented as mean, standard deviation, median and range. Qualitative data were presented as numbers and percentage and compared using the Chi square test. Graphs were produced by using Excel program. P value was considered significant if it was less than 0.05.

RESULTS

The study was conducted at Sohag University Hospital in the period between December 2019 and December 2022. Our study included 180 Gram negative isolates which were collected from catheterized patients with CAUTI recruited from different departments. Gram negative bacilli were detected in (180) samples representing 58% from the total collected samples (310) during the period of the study.

Our study group included all patients from whom Gram negative bacilli were isolated. The mean age of the studied patients was 52.8±11.7 years. The median age was 55 years. The range for age was (15:65) years. Males were 99 representing (55%) of the studied group (table 2). The most common isolated organism was *E. coli* 75 (41.7%) of the total isolated Gram-negative bacilli the frequency of isolated organism and their antibiotic sensitivity pattern shown in table 3.

Among the 180 isolated Gram-negative strains, 156 were MDR (defined as bacteria that are resistant to at least one drug in three or more classes of antimicrobial drugs). Most MDR isolates 95 (60.9%) were isolated from ICU. Risk factors related to having MDR in our study were age, diabetes, hospital stay, duration of catheterization. details in table 2.

Table 2: Demography and characteristic of the study group as regard isolation of MDR Gram negative isolates

Socio-demographic characters	Total	Patients with MDR				*P value*
		yes	%	*No*	*%*	
Age /year						
mean	52.8±11.7					
Median (Range)	55.0(15:65)					
Age groups						<0.001***
<20	2(1.1%)	0	0%	2	8.3%	
20-50	60(33.3%)	45	28.8%	15	62.5%	
>50	118(65.6%)	111	71.2%	7	29.2%	
Gender						0.09 NS
Male	99(55.0%)	82	47.4%	17	70.8%	
Female	81(45.0%)	74	52.6%	7	29.2%	
Place of urinary catheter insertion						0.001**
ICU	39(21.7%)	38	24.4%	1	4.2%	
Operation room	39(21.7%)	27	17.3%	12	50%	
Ward	102(56.7%)	91	58.3%	11	45.8%	
Duration of urinary catheterization days						0.001**
<3 days	0 (0.0%)	0	0.0%	0	0%	
3-7 days	59 (32.8%)	44	28.2%	15	62.5%	
>7days	121(67.2%)	112	71.8%	9	37.5%	
Diabetes Mellitus						<0.001***
Yes	128(71.1%)	121	77.6%	7	29.2%	
No	52 (28.9%)	35	22.4%	17	70.8%	
Use of broad-spectrum antibiotics						0.44 NS
Yes	152(84.4%)	133	85.3%	19	79.2%	
No	28(15.6%)	23	14.7%	5	20.8%	
Old age (≥65 years)						0.22 NS
Yes	73(40.6%)	66	42.3%	7	29.2%	
No	107(59.4%)	90	57.7%	17	70.8%	
Duration of hospital stay in days						
< 7 days						
7-14 days	25(13.9%)	13	8.3%	12	50%	<0.001***
>14 days	122(67.8%)	110	70.5%	12	50%	
	33(18.3)	33	21.2%	0	0.0%	
Department of the cases						
ICU	102 (56.7%)	95	60.9%	7	29.2%	
General surgery	21(11.7%)	13	8.3%	8	33.3%	
Vascular surgery	11(6.1%)	11	7.1%	0	0%	<0.001***
Chest	13(7.2%)	11	7.1%	2	8.3%	
Orthopedics	11(6.1%)	9	5.8%	2	8.3%	
Gynecology& obstetric	6(3.3%)	6	3.8%	0	0%	
Internal medicine	6(3.3%)	6	3.8%	0	0%	
Plastic	6(3.3%)	3	1.9%	3	12.5%	
Urology	4(8.3%)	2	1.3%	2	8.3%	

Table 3: Species frequency and antibiotic sensitivity testing of isolated Gram-negative bacilli (by phoenix BD)

GN bacilli N=180 100%	***E. coli N= 75 41.7%***		***klebsiella Pneumoniae N=39 21.7%***		***Pseudomonas aeruginosa N=12 6.7%***		**Enterobacter *cloacae* N=24 13.3%**		***Actinobacter baumanii N=15 8.3%***		***Citrobacter freundi N=8 4.4%***		***Proteus Mirabilis N=7 3.9%***	
Antibiotic	**N**	**%**	N	%	N	%	N	%	N	%	N	%	N	%
Piperacillin	2	2.7%	0	0	0	0	0	0	0	0	0	0	0	0
Ampicillin	4	5.3%	0	0	0	0	0	0	0	0	0	0	0	0
Piperacillin-Tazobactam	29	38.7%	8	20.5%	5	41.7%	17	70.8%	5	33.3%	5	62.5%	1	14.3%
Amoxicillin-clavulanate	14	18.7%	7	17.9%	1	8.3%	17	70.8%	0	0	2	25%	0	0
Cefotolozone-Tazobactam	31	41.3%	12	30.8%	6	50%	18	75%	1	6.7%	5	62.5%	2	28.6%
Ceftazidime	16	21.3%	2	5.1%	2	16.7%	11	45.9%	5	33.3%	5	62.5%	0	0
Cefotaxime	5	6.7%	2	5.1%	0	0%	0	0	0	0	2	25%	0	0
Cefazolin	5	6.7%	2	5.1%	0	0%	2	8.3%	0	0	2	25%	0	0
Cefuroxime	6	8%	2	5.1%	0	0%	4	16.7%	0	0	2	25%	0	0
Ceftriaxone	17	22.7%	2	5.1%	1	8.3%	12	50%	0	0	5	62.5%	0	0
Cefepime	8	10.7%	2	5.1%	0	0%	7	29.2%	0	0	5	62.5%	1	14.3%
Aztreonam	38	50.6%	16	41%	4	33.3%	21	87.5%	4	26.7%	5	62.5%	5	71.4%
Ertapenem	40	53.3%	9	23.1%	6	50%	21	87.5%	1	6.7%	5	62.5%	7	100%
Imipenem	31	41.3%	8	20.5%	7	58.3%	13	54.2%	6	40%	0	0	6	85.6%
Meropenem	41	54.7%	12	30.8%	7	58.3%	23	95.8%	1	6.7%	5	62.5%	7	100%
Gentamicin	41	54.7%	20	51.3	7	58.3%	24	100%	0	0	6	75%	6	85.6%
Amikacin	42	56.0%	16	41%	7	58.3%	24	100%	0	0	6	75%	7	100%
Ciprofloxacin	26	34.7%	3	7.7%	1	8.3%	16	66.7%	0	0	6	75%	1	14.3%
Levofloxacin	32	42.7%	8	20.5%	5	41.7%	19	79.2%	1	6.7%	6	75%	6	85.6%
Trimethoprim-Sulfamethoxazole	29	38.6%	15	38.5%	4	33.3%	14	58.3%	6	40%	6	75%	5	71.4%
Nitrofurantoin	59	78.7%	17	43.6%	5	41.7%	14	58.3%	6	40%	7	87.5%	5	71.4%
Chloramphenicol	54	72%	21	53.9%	1	8.3%	13	54.2%	1	6.7%	5	62.5%	6	85.6%
Tetracycline	17	22.7%	11	28.2%	0	0	10	41.7%	1	6.7%	1	12.5%	5	71.4%
Tigecycline	54	72.0%	7	17.9%	4	33.3%	9	37.5%	6	40%	6	75%	5	71.4%
Fosfomycin *	50	66.7%	21	53.8%	7	58.3%	18	75	6	40%	7	87.5%	6	85.7%

*Fosfomycin was done by E-test

ESBL producers by double disc synergy test

Among the Total GN isolates. There was 67 (37%) ESBL producers. The highest were *Kebsiella pneumoniae* 92 (43.3%) of all ESBL producers, followed by *E. coli* 23 (34.3%), and the least were *Citrobacter freundii* 2 (3%). Details in table 4.

Table 4 : Distribution of MDR, ESBL, Carbapenemase and MBL producers among isolated Gram-negative bacilli species

Species	***N (%)***	***MDR****	***ESBL Producers*** **	***Carbapenemase producers**** **	***MBL producers*****
Total	180 (100%)	156(86%)	67(100%)	49 (27.2%)	40(22.2%)
Acinetobacter baumanni	15 (8.3%)	15(100%)	5(7.5%)	5 (33.3%)	0(0.0%)
Citrobacter freundi	8 (4.4%)	7(87.5%)	2(3.0%)	1 (12.5%)	0 (0.0%)
E. Coli	75 (41.7%)	62(82,7)	23(34.3%)	8 (10.7%)	10(13.3%)
Enterobacter cloacae	24 (13.3%)	18(75)	5(7.5%)	2 (8.3%)	1 (4.2%)
Klebsiella pneumoniae	39 (21.7%)	36(92.3)	29(43.3%)	28 (71.8%)	28(71.8)
Proteus mirabilis	7 (3.9%)	7(100%)	0 (0.0%)	0 (0.0%)	0(0.0%)
Pseudomonas aeruginosa	12 (6.7%)	11(91.7)	3(4.5%)	5 (41.7%)	1(8.3)

*Defined as resistant to at least one agent from three or more groups of antibiotics, **done by double disc synergy test, *** done by modified Hodge test, ****done by EDTA combined disc synergy test

Carbapenemase production modified Hodge test:

Carbapenemase producers by modified Hodge test were **49** strains representing **27%** of total isolated strains. Risk factors for Isolation of Carbapenemase producing Gram negative bacilli were prolonged duration of catheterization, prolonged hospital stay, diabetes mellitus, use of broad-spectrum antibiotics and age ≥ 50 years with P-value **(P-value <0.05).**

Metallo-ß-lactamase production by EDTA combined disc synergy test:

MBL producers by EDTA combined disc synergy test were **40** strains representing **22%** of total isolated strains. Twenty-eight **(71.8%)** strains of *Klebsiella pneumoniae*, **10(13.3%)** strains of *E. coli*. For more details are shown table 2.

Antimicrobial susceptibility profile of Fosfomycin

Using the disc diffusion method; **(61%, n=103)** were sensitive, **(5%, n=17)** were intermediate, **(34, n=60)** were resistant. While by using E- test; (**66%, n=115)** were sensitive, **(34%, n=65)** were resistant. Details of distribution of fosfomycin sensitivity among isolated species are shown in table 3.

Fosfomycin sensitivity among MDR Gram- negative isolates:

Among 156 (100%) MDR Gram negative isolates 92 (59%) were sensitive to fosfomycin with highest sensitivity among MDR *Citrobacter freundii* 6 (85.7%) and MDR *Proteus Mirabilis* 6 (85.7%) and least sensitivity at MDR *Acinetobacter baumanii* 6 (40%) while sensitivity in MDR *E. coli* was 37 (59.7%), MDR *Klebsiella pneumonia* was 19 (52.8%), MDR *Pseudomonas aeruginosa* 6 (54.5%) was and MDR *Enterobacter cloacae* was 12 (66.7%).

Detection of Fosfomycin resistance genes (Fos genes) by PCR:

Among the **65** strains resistant to Fosfomycin by E-test, FosA, fosA2, fosA3, fosc2 genes were detected in **47** strains representing **72.3%** of the total isolates. Agarose gel (1.5%) electrophoresis of the tested Fos genes are shown in figure 1-4.

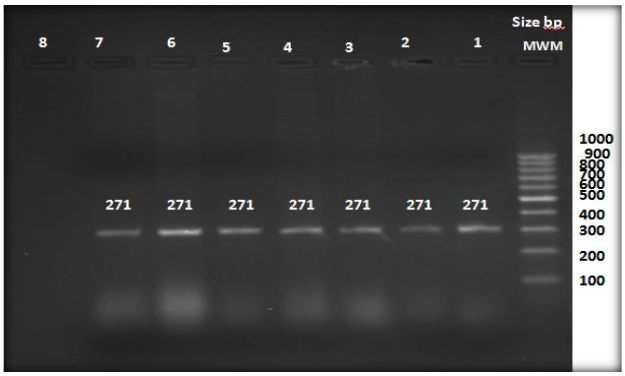

Fig. 1: Agarose gel electrophoresis of PCR products after amplification of FosA gene. MWM-molecular weight marker (100 bp DNA ladder). Lanes 1, 2, 3, 4, 5, 6 and 7 are positive for FosA gene (FosA gene products at 271 bp). Lane 8 is negative for FosA gene.

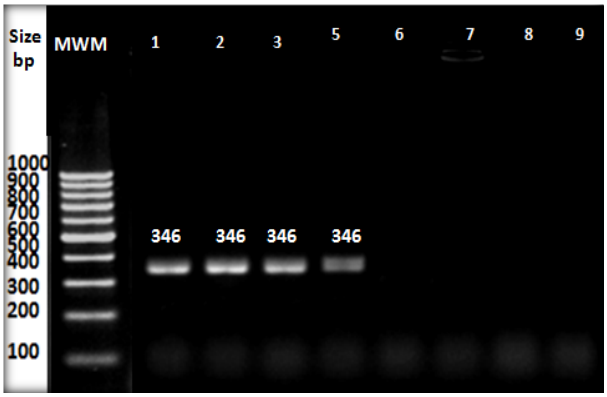

Fig. 2: Agarose gel electrophoresis of PCR products after amplification of FosA2 gene. MWM-molecular weight marker (100 bp DNA ladder). Lanes 1, 2, 3 and 5 are positive for FosA2 gene (FosA2 gene products at 346 bp). Lanes 6, 7, 8 and 9 are negative for FosA2 gene.

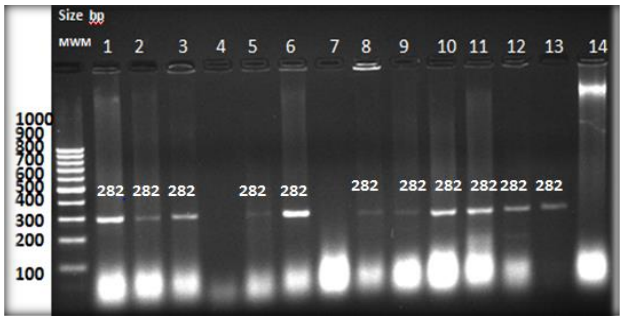

Fig. 3: Agarose gel electrophoresis of PCR products after amplification of FosA3 gene. MWM-molecular weight marker (100 bp DNA ladder). Lanes 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13 are positive for FosA3 gene (Fos A3 gene products at 282 bp). Lanes 4, 7 and 14 are negative for FosA3gene.

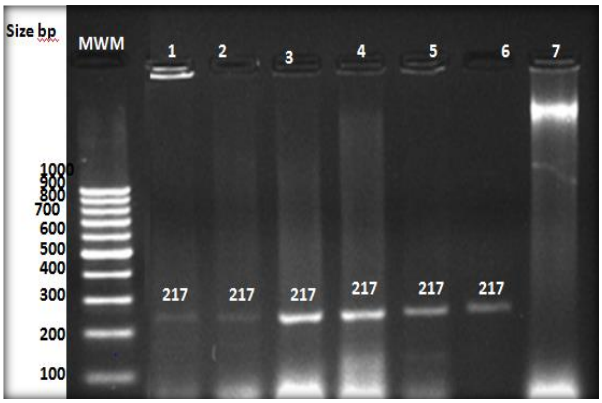

Fig. 4: Agarose gel electrophoresis of PCR products after amplification of FosC2 gene. MWM-molecular weight marker (100 bp DNA ladder). Lanes 1, 2, 3,4, 5 and 6 are positive for FosC2 gene (FosC2 gene products at 217 bp). Lane 7 is negative for Fos C2 gene.

DISCUSSION

CAUTI is the most common nosocomial infection in health care settings. This study evaluated the Gram-negative bacilli as an important causative organism, their resistance pattern in our setting and detect fosfomycin sensitivity in isolated organisms.

In this study 180 Gram negative bacilli were isolated from 310 collected urine samples representing 58%. Similar was reported by Gharieb et al.,[23] in Egypt found that out of 155 studied patients, 99 (64%, n= 99) isolates were Gram negative bacilli. Several studies reported more than 50 % of their CAUTI were caused by Gram negative organisms. The median age of our study group was 55 years with 118 cases more than 50 years (65.6%). The age of our study group agreed with that reported by many previous studies[6,24,25].

In this study males were slightly more than females 55% versus 45%. Other researchers reported similar results[6,26]. However, *Hussain et al.,*[27] *& Khan et al.,*[28] found that females have a stronger predilection for CAUTI compared to males due to the shorter female urethra that allows easier access of the perennial flora to the bladder along the catheter.

The most frequent isolates were *E. coli* (41.7%, n=75), followed by *K. pneumonia* (21.7%, n= 39). This result was in agreement with recent studies by Hussain et al.,[29] and Bashir et al.,[24]. This highlighted the principal role of *E. coli* in causing UTI. due to the significant abundance of *E. coli* in the rectal area, which in turn via contamination ascends through the catheter to the urinary tract [27,30].

In our study, (86.7%, n= 156) strains were MDR, and most of them (60.9%, n=95) were isolated from ICU, and (39.7%, n=62) of them were *E. coli* which represents (82,7%, n=62) of all *E. coli* isolates. *Gharieb et al.,*[23] reported (82,7%, n=62) of isolated *E. coli* were MDR and *Shrief et al.,*[15] *reported (*100% n=54) of carbapenem resistant *E. coli* were MDR in Egypt. Also, *Arina etal.,*[31] reported (60.8%) of isolated *E. coli* were MDR in Bangladesh. Lalezadeh et al.[11] reported 80% MDR in *Enterobacterales* isolated from UTI in Iran. The high prevalence of MDR in health care setting reflects the lack of infection control strategies and antibiotic stewardship programs.

Isolation of MDR Gram negative bacilli causing CAUTI was significantly higher in patients with risk factors such as prolonged duration of catheterization, insertion of urinary catheter in the ward, prolonged hospital stays, diabetes mellitus and age ≥ 50 years with (P-value <0.05). Several studies had reported these risk factors in their CAUTI patients[4,24,30].

In this study ESBL producers by double disc synergy test were 67 (37%) of the total isolated strains. 29 (43.3%) of all ESBL producers were *Klebsiella pneumoniae* and 23 (34.3%) were *E. coli*. A recent survey and review of literature of MDR in Gram negative bacilli isolated from Egypt reported ESBL (19–85.24% of E. coli, and 10–87% of K. pneumoniae) reflecting the variability in health care setting in Egypt[32]. Much lower percentage was reported by Obaid et al.,[33] in Saudia Arabia where ESBL-producing E. coli 4.9% in and *Klebsiella pneumoniae 2.8% in CAUTI infections*.

This study detected Carbapenemase production in the isolated Gram-negative bacilli causing CAUTI by modified Hodge test were (27%, n= 49) *with K. pneumoniae* was the most frequently resistant isolate (71.8%, n=28) followed by *E. coli* (10.7%, n=8). Raheel et al.,[34] described that 34.1% of the isolated *Enterobacterales* from Suez Canal University Hospitals, Egypt were Carbapenemase producers. Also, *Abdelaziz,*[35] found that (80.9%) of the isolated *Enterobacterales* from four Egyptian hospitals were carbapenem-resistant and the most detected strains was *K. pneumoniae* (93.4%, n= 71) and E. coli (57.9%, n=11). A much lower rate (4.88% n=6) was reported by *Hussaini et al.,*[29] in Nigeria.

In this study, the EDTA combined disc synergy test used for detection of MBLs revealed (22%, n=40) of isolated GN strains were MBL producers. Most of them were *Klebsiella pneumoniae (71.8%, n=28)* and of *E. coli (13.3%, n=10). A recent study by Fawzy et al.,* [36] reported MBL production in 50% of Gram-negative organisms isolated from four ICU in El Minia, Egypt**.**

In this study by using the E test (36.1%, n=65) of isolated GN bacilli were resistant to fosfomycin. Gharieb et al.,[23] reported (43,7% n=28) fosfomycin resistance among ESBL Enterobacterales from Menoufia in Egypt. However, Shrief et al,[15] reported (17.4%, n= 16) resistance to fosfomycin in uropathogenic CRE strains from Mansoura, Egypt. A lower frequency of resistance to fosfomycin (7.1%, n=15) among Enterobacterales causing UTI was reported by *Lalezadeh etal.,*[11] in Iran. The difference in the rate of resistance to fosfomycin may be due to the amount of antibiotic usage in the different area.

In this study Fos genes were detected in (72.3%, n=47). The prevalence of fos genes in our fosfomycin resistant isolates was high similar to recent studies performed in Egypt where *Shrief et al.,*[15] detected fosA gene in 68.75% of Gram-negative isolates. *Bahy et al.,*[8] found resistance to fosfomycin in 71.4% of isolates was mostly because of FosA3. Galindo et al.,[1] found that among the 38 fosfomycin resistant strains, 23 (60.5%) were identified as fos producing organisms.

In our study FosA3 was the most prevalent (85%, n=40) followed by FosA (54%, n=25), FosA2 (17%, n=8) and FosC2 (13%, n= 6) of all isolates carrying Fos genes

A recent study at Fayoum, Egypt detect fos3A genes only in their fosfomycin resistant *E. coli* strains [8]. Also, Hameed et al.,[37] in China reported that the prevalence of fosA3 gene was 38.58% and 13.3% of

isolated fos genes in the study conducted by *lalezadeh etal.*,[11] in Iran. However, the prevalence of *fosA3*gene was (60.5%) in a study by Galindo et al.,[1] in Mexico.

In our study there were 18 strains strains negative for tested fosfomycin resistance genes, resistance due to other mechanisms as mutation of the target *murA*, *glpT* which was detected in other studies [1,8,11].

CONCLUSIONS

There is high level of MDR in our setting necessitate combined measures as surveillance of Gram negative MDR, infection control measures and policy to prevent their spread as hand hygiene, isolation. Antimicrobial stewardship program is essential. Measures to reduce CAUTI as reducing duration of hospital stay, duration of catheterization. Much care should be targeted to old age, diabetic patients, ICU patients. Fosfomycin can be used as a potential line in managing CAUTI caused by MDR Gram negative bacilli.

Declarations:

Consent for publication

Not applicable

Availability of data and material

Data are available upon request

Competing interests

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REFERENCE

1. Galindo-Méndez M, Navarrete-Salazar H, Baltazar-Jiménez F, et al. Emergence of Fosfomycin Resistance by Plasmid-Mediated fos Genes in Uropathogenic ESBL-Producing E. coli Isolates in Mexico. Antibiotics. 2022;11(10). doi:10.3390/antibiotics11101383.
2. CDC. *Catheter-Associated Urinary Tract Infection (CAUTI) and Non-Catheter-Associated Urinary Tract Infection (UTI) (and Other Urinary System Infection (USI))*. Accessed June 9, 2023. https://www.cdc.gov/nhsn/psc/uti/index.html
3. Venkataraman R, Yadav U. Catheter-associated urinary tract infection: an overview. *J Basic Clin Physiol Pharmacol*. 2022;34(1):5-10.
4. Verma S, Naik SA, Ts D, Pradesh U. Etiology and risk factors of catheter associated urinary tract infections in ICU patients. International Journal of Medical Microbiology and Tropical Diseases.2017; 3(2):65-70. doi:10.18231/2455-6807.2017.0015
5. Hassan R, El-Gilany AH, Abd elaal AM, El-Mashad N, Azim DA. An overview of healthcare-associated infections in a tertiary care hospital in Egypt. Infection Prevention in Practice. 2020;2(3). doi:10.1016/j.infpip.2020.100059
6. Saleem M, Syed Khaja AS, Hossain A, et al. Catheter-Associated Urinary Tract Infection in Intensive Care Unit Patients at a Tertiary Care Hospital, Hail, Kingdom of Saudi Arabia. Diagnostics. 2022;12(7). doi:10.3390/diagnostics12071695
7. Anggi A, Wijaya DW, Ramayani OR. Risk factors for catheter-associated urinary tract infection and uropathogen bacterial profile in the intensive care unit in hospitals in Medan, Indonesia. Open Access Maced J Med Sci. 2019;7(20):3488-3492. doi:10.3889/oamjms.2019.684
8. Bahy R, Abu El-Wafa W, Abouwarda A. Molecular Mechanisms of Fosfomycin Resistance in MDR Escherichia coli Isolates from Urinary Tract Infections. Egyptian Journal of Medical Microbiology, 2023; *32*(2), 25-29. doi: 10.21608/ejmm.2023.279741
9. Zurfluh K, Treier A, Schmitt K, Stephan R. Mobile fosfomycin resistance genes in Enterobacteriaceae—An increasing threat. *Microbiologyopen*. 2020;9(12). doi:10.1002/mbo3.1135
10. Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. Int J Infect Dis. 2011 Nov; 15(11): e732-9. doi: 10.1016/j.ijid.2011.07.007. Epub 2011 Sep 25. PMID: 21945848.
11. Lalezadeh A, Ghotaslou P, Ghotaslou R. The Detection of Fosfomycin-Modifying Enzymes (fos) in Uropathogenic Enterobacterale, Azerbaijan, Iran. Canadian Journal of Infectious Diseases and Medical Microbiology. 2023; vol. 2023, Article ID 3766269, 7 pages, doi:10.1155/2023/3766269
12. López-Montesinos I, Horcajada JP. Oral and intravenous fosfomycin in complicated urinary tract infections. Rev Esp Quimioter. 2019 May;32 Suppl 1(Suppl 1):37-44. PMID: 31131591; PMCID: PMC6555162.
13. Ruiz Ramos J, Salavert Lletí M. Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. Rev Esp Quimioter. 2019 May;32 Suppl 1(Suppl 1):45-54. PMID: 31131592; PMCID: PMC6555168.
14. Munny NN, Shamsuzzaman SM, Hossain T. Emergence of fosfomycin resistance among isolates of multidrug-resistant *Enterobacter species* at a tertiary care hospital in Dhaka, Bangladesh. Bangladesh Med Res Counc Bull [Internet]. 2023 May 30;48(3):203–10. doi:10.3329/bmrcb.v48i3.63812. Available from:

https://www.banglajol.info/index.php/BMRCB/article/view/63812

15. Shrief R, El-Ashry AH, Mahmoud R, El-Mahdy R. Effect of Colistin, Fosfomycin and Meropenem/Vaborbactam on Carbapenem-Resistant *Enterobacterales* in Egypt: A Cross-Sectional Study. Infect Drug Resist. 2022 Oct 27;15:6203-6214. doi: 10.2147/IDR.S385411. PMID: 36324668; PMCID: PMC9621046.
16. Chuang L, Tambyah PA. Catheter-associated urinary tract infection. J Infect Chemother. 2021 Oct;27(10):1400-1406. doi: 10.1016/j.jiac.2021.07.022. Epub 2021 Aug 4. PMID: 34362659.
17. Tille Patricia M. Bailey and Scott's Diagnostic Microbiology. St. Louis, Missouri: 15th ed. Elsevier; 2021.
18. CLSI (2022), Performance Standards for Antimicrobial Susceptibility Testing, 32 nd Ed, CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA
19. Mohammed AB, Anwar KA. Phenotypic and genotypic detection of extended spectrum beta lactamase enzyme in *Klebsiella pneumoniae*. *PLoS One*. 2022;17(9 September). doi:10.1371/journal.pone.0267221
20. Vadlamannati RV, Ayesha Vaseem Y. Detection of Carbapenem Resistance in Gram Negative Bacteria. European Journal of Molecular & Clinical Medicine. 2022 Aug 7;9(4):2101-7.
21. Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait Med J. 2009 Jun 1;41(2):117-22.
22. Liu P, Chen S, Wu ZY, Qi M, Li XY, Liu CX. Mechanisms of fosfomycin resistance in clinical isolates of carbapenem-resistant Klebsiella pneumoniae. Journal of global antimicrobial resistance. 2020 Sep 1; 22:238-43. doi:10.1016/j.jgar.2019.12.019
23. Gharieb MM, Ghonaim MM, Makled AF, Sharweed M. Activity of Fosfomycin against Extended-Spectrum-Beta-Lactamase producing Enterobacteriaceae isolates from Menoufia University Hospitals. Egyptian Journal of Medical Microbiology. 2020 Jan 1;29(1):111-9. www.ejmm-eg.com
24. Bashir M, Isa H, Chimbekujwo K, Kachalla N, Umar A, Bilyaminu M. Antibiotic Sensitivity Profile of Bacteria Isolated from Urinary Catheters in Urinary Tract Infections (UTIs) Patients. Microbes and Infectious Diseases. 2021 Nov 1;2(4):706-14. doi:10.21608/mid.2020.44217.1067
25. Negm EM, Elgharabawy ES, Badran SG, et al. Analysis of cumulative antibiogram reports in intensive care units at an Egyptian University Hospital. *J Infect Public Health*. 2023;16(8):1220-1229. doi:10.1016/j.jiph.2023.05.032
26. Juanjuan D, TianTian Z, Yue D, Lili W, Ping X, Xu H. Analysis of etiology and risk factors of catheter-associated urinary tract infection in critically ill patients and research on corresponding prevention and nursing measures. Applied Bionics and Biomechanics. 2021 Dec 20;2021. doi:10.1155/2021/8436344.
27. Hussain MS, Cheema UK, Irim S, Sajjad M, Anwar M, Raees S. Prevalence of uropathogens in catheter associated urinary tract infections and their antimicrobial susceptibility. The Professional Medical Journal. 2023 Jan 31;30(02):245-51. doi:10.29309/tpmj/2023.30.02.7069
28. Khan AM, Ali Z, Salahuddin GS, Kamal M, Asghar M, Afridi MA. Uropathogen isolates and their antibiotic sensitivity in patients with indwelling catheter associated urinary tract infections. Journal of Postgraduate Medical Institute. 2020 Aug 27;34(1).
29. Hussaini IM, Suleiman AB, Olonitola OS, Oyi RA. Phenotypic and molecular detection of carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae*. Microbes and Infectious Diseases. 2023 Feb 1;4(1):151-9. doi:10.21608/mid.2022.124181.1251
30. Patil HV, Patil VC. Clinical, bacteriology profile, and antibiotic sensitivity pattern of Catheter associated Urinary tract infection at tertiary care hospital. Int J Health SciRes. 2018;8(1):25-35. www.ijhsr.org
31. Arina S, Shamsuzzaman S. Antibiotic sensitivity patterns of uropathogens isolated from catheterized patients in a tertiary care hospital in Dhaka, Bangladesh. Urology & Nephrology Open Access Journal. 2021;9(3):61-66. doi:10.15406/unoaj.2021.09.00309
32. El-Kholy A, El-Mahallawy HA, Elsharnouby N, Aziz MA, Helmy AM, Kotb R. Landscape of multidrug-resistant Gram-negative infections in Egypt: Survey and literature review. Infect Drug Resist. 2021; 14:1905-1920. doi:10.2147/IDR.S298920
33. Obaid NA, Abuhussain SA, Mulibari KK, Alshanqiti F, Malibari SA, Althobaiti SS, Alansari M, Muneef E, Almatrafi L, Alqarzi A, Alotaibi N. Antimicrobial-resistant pathogens related to catheter-associated urinary tract infections in intensive care units: A multi-center retrospective study in the Western region of Saudi Arabia.

Clinical Epidemiology and Global Health. 2023 May 1;21: 101291. doi:10.1016/j.cegh.2023.101291.

34. Raheel A, Azab H, Hessam W, Abbadi S, Ezzat A. Detection of carbapenemase enzymes and genes among carbapenem-resistant Enterobacteriaceae isolates in Suez Canal University Hospitals in Ismailia, Egypt. *Microbes and Infectious Diseases*. 2020;1(1):24-33. doi:10.21608/mid.2020.25702.1007.
35. Abdelaziz NA. Phenotype-genotype correlations among carbapenem-resistant Enterobacterales recovered from four Egyptian hospitals with the report of SPM carbapenemase. Antimicrob Resist Infect Control. 2022;11(1). doi:10.1186/s13756-022-01061-7.
36. Fawzy RH, Gad GF, Mohamed HA. Phenotypic and genotypic detection of resistance mechanisms in carbapenem-resistant Gram-negative bacteria isolated from Egyptian ICU patients with first emergence of NDM-1 producing *Klebsiella oxytoca*. Iranian Journal of Microbiology. 2022 Dec; 14(6):832. http://ijm.tums.ac.ir.
37. Hameed MF, Chen Y, Bilal H, Khan S, Ge H, Xiaofang C, Gu P. The Co-occurrence of mcr-3 and fosA3 in IncP plasmid in ST131 *Escherichia coli*: A novel case. The Journal of Infection in Developing Countries. 2022 Apr 30;16(04):622-9. doi: 10.3855/jidc. 15943.