Title- Fosfomycin Susceptibility and fosA-Mediated Resistance Among Gram-Negative Uropathogens in a Tertiary Care Centre

Authors - Sandeepika Dubey, Areena Hoda Siddiqui, Meenakshi Sharma

Affiliation- Integral Institute Of Medical Sciences Research, Integral University, Lucknow

Introduction-

Urinary tract infections (UTIs) remain a major public health concern, increasingly complicated by the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative uropathogens. Fosfomycin has re-emerged as a valuable oral therapeutic option against resistant pathogens; however, the emergence of fosfomycin resistance, particularly mediated by the fosA gene, threatens its long-term clinical effectiveness. Understanding susceptibility patterns and resistance mechanisms is essential within a One Health framework to combat antimicrobial resistance (AMR).

Objectives: To evaluate fosfomycin susceptibility among Gram-negative uropathogens using disc diffusion and agar dilution MIC methods, assess concordance between phenotypic techniques, and detect fosA-mediated resistance in fosfomycin-resistant isolates.

Material and methods- This prospective study (April 2023–March 2025) at the Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, analyzed 2,550 urine samples. Significant gram-negative isolates were subjected to antimicrobial susceptibility testing, ESBL and CRE screening, and fosfomycin susceptibility using Kirby–Bauer disc diffusion and agar dilution MIC (CLSI/EUCAST). Fosfomycin-resistant isolates underwent molecular confirmation of fosA using conventional PCR. Concordance between phenotypic and MIC methods was assessed to validate diagnostic reliability. Data were analyzed using SPSS v20.0.

Results –

Of 2,550 samples, 651 showed significant growth, with 371 gram-negative isolates. *E. coli* (79.5%) was the predominant pathogen. Fosfomycin demonstrated high overall susceptibility (91.1% by disc diffusion; 94.8% by agar dilution). Strong concordance (96%) between both methods supports routine disc diffusion testing. High susceptibility was maintained even among ESBL (93%), CRE (89.6%), and MDR isolates (94%). MIC values (2–64 µg/mL) indicated overall low-level resistance. Molecular testing identified the fosA gene in 78.9% of phenotypically resistant isolates, mainly in *E. coli* (85.7%) and *K. pneumoniae* (100%).

Table 1. AST pattern of Gram-negative uropathogens

Antibiotic	*E.coli* (N=295)		*Klebsiella* spp. (N=34)		*Citrobacter* spp. (N= 11)		*Enterobacter* spp. (N=3)		*Proteus* spp. (N=6)		*Pseudomonas* spp. (N=17)		*Acinetobacter* spp. (N=5)		Total (N=371)	
	S(%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
AS (10/10 µg)	38.4	61.6	44.1	55.9	54	46	33	67	66	34	NA	NA	60	40	40	60
PIT (100/10 µg)	69.6	30.4	79.4	20.6	80	20	100	0	100	0	76	24	80	20	79	21
CZ (30 µg)	17.3	82.7	43.3	56.7	22	78	66	34	33	67	NA	NA	0	100	20.5	79.5
CTR (30 µg)	20.2	79.8	55.8	44.2	36	64	66	34	83	17	NA	NA	40	60	26	74
CAZ (30 µg)	29.6	70.4	55.8	44.2	36	64	66	34	83	17	31	69	20	80	33.4	66.6
CPM (30 µg)	33.3	66.7	61.7	38.3	54	46	100	0	83	17	53	47	20	80	38	62
AT (30 µg)	32.7	67.3	64.7	35.3	63	37	100	0	100	0	64.7	35.3	40	60	38.6	61.4
TE (30 µg)	28	72	47	53	45	55	66	34	20	80	NA	NA	60	40	32	68
GEN (10 µg)	67	33	73.5	26.5	72	28	100	0	66	34	62	38	80	20	68	32
AK (30 µg)	69	31	68.7	31.3	72	28	100	0	66	34	64	36	60	40	69	31
TOB (10 µg)	61.3	38.7	64.7	35.3	63	37	100	0	66	34	43	57	100	0	63.4	36.6
CIP (5 µg)	16.2	83.8	45.4	54.6	45	55	50	50	33	67	37.5	62.5	40	60	20	80
NX (10 µg)	24.4	76	61.7	38.3	63	37	66	34	66	34	29	71	0	100	31	69
IMP (10 µg)	79.2	20.8	79.4	20.6	63	37	100	0	100	0	53	47	60	40	79	21
DOR (10 µg)	79.8	20.2	79.4	20.6	81	19	100	0	100	0	65	35	80	20	80	20
MRP (10 µg)	77.8	22.2	76.4	23.6	72	28	100	0	100	0	65	35	80	20	79	21
COT (1.25/23.75 µg)	32.7	67.3	55.8	44.2	57	43	50	50	66	34	NA	NA	0	100	37.5	62.5
NIT (300 µg)	87.5	12.5	62.5	37.5	63	37	77	23	76	24	100	0	60	40	83	17
FO (200 µg)	92.5	7.5	91.1	8.9	81	19	77	23	100	0	100	0	80	20	91.6	8.4

Table 2: Fosfomycin susceptibility in Enterobacteriaceae by resistance phenotype

Resistance Category	Organism	Total Isolates (n)	Fosfomycin Sensitive n (%)	Fosfomycin Resistant n (%)
ESBL Producers	*E. coli*	118	109 (92.3)	9 (7.7)
	Klebsiella spp.	8	6 (75.0)	2 (25.0)
	Citrobacter spp.	1	1 (100)	0 (0)
	Enterobacter spp.	1	0 (0)	1 (100)
	Proteus spp.	1	1 (100)	0 (0)
	Total ESBL	129	120 (93.0)	9 (7.0)
Non-ESBL	All species	220	209 (94.5)	11 (5.5)
MDRE	*E. coli*	58	56 (91.0)	2 (9.0)
	Klebsiella spp.	6	5 (82.0)	1 (18.0)
	Proteus spp.	1	1 (100)	0 (0)
	Citrobacter spp.	2	1 (50.0)	1 (50.0)
	Enterobacter spp.	0	0	0
	Total MDRE	67	63 (94.0)	4 (6.0)
Non-MDRE	All species	122	116 (95.0)	6 (5.0)
CRE	*E. coli*	50	45 (90.0)	5 (10.0)
	Klebsiella spp.	6	6 (100)	0 (0)
	Citrobacter spp.	2	1 (50.0)	1 (50.0)
	Total CRE	58	52 (89.6)	6 (10.4)
Non-CRE	All species	291	271 (93.0)	20 (7.0)
XDR	*E.coli*	8	7 (87.5)	1 (12.5)
	Klebsiella spp.	2	2(100)	0
	Total XDR	10	9 (90)	1 (10)
PDR	*E. coli*	2	2 (100)	0
	Total	2	2 (100)	0

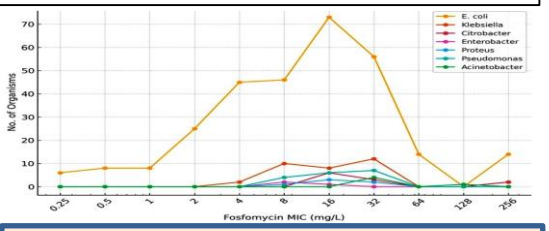

Fig. 2: Distribution of fosfomycin MIC values among Gram-negative uropathogens by agar dilution method

S.No	Sample ID	Organism	Fos A gene
1	EC1	*E.coli* (ESBL)	Detected
2	EC2	*E coli*	Detected
3	EC3	*E. coli* (Carbapanamase producer)	Detected
4	EC4	*E coli* (ESBL)	Detected
5	EC5	*E coli* (ESBL, carbapanamase producer)	Detected
6	EC6	*E.coli* , (carbapanamase producer)	Detected
7	EC7	*E.coli* , (Carbapanamase producer)	Detected
8	CF8	*Citrobacter freundii* (Carbapanamase producer)	Detected
9	EC9	*E. coli* (carbapanamase producer)	Detected
10	EC10	*Escherichia coli* (Carbapenamase producers)	Not Detected
11	EC11	*Escherichia coli*	Not Detected
12	KP12	*Klebsiella pneumoniae* (ESBL)	Detected
13	AB13	*Acinetobacter spp.*, (ESBL, carbapanamase)	Not Detected
14	CF14	*Citrobacter spp.*	Not Detected
15	KP15	*Klebsiella pneumoniae* ESBL	Detected
16	EC16	*Escherichia coli* ESBL	Detected
17	EC17	*E. coli* (ESBL)	Detected
18	EC18	*E. coli* (ESBL)	Detected
19	EC19	*E.coli* (ESBL)	Detected

Table 3. Distribution of *fosA* Gene among Fosfomycin-Resistant Isolates

Conclusion- Fosfomycin demonstrates strong in-vitro activity against Gram-negative uropathogens, including MDR and CRE isolates, supporting its use as an effective oral therapy for UTIs. High concordance between disc diffusion and MIC confirms disc diffusion as a reliable routine test in resource-limited settings. The detection of fosA highlights the need for ongoing molecular surveillance and integrated antimicrobial stewardship within a One Health framework to preserve fosfomycin efficacy and combat AMR.

Key words: Fosfomycin; fosA gene; Gram-negative uropathogens; Antimicrobial resistance; Urinary tract infections