http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v7i9.110

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

A Comparative Evaluation of Fosfomycin activity with other Antimicrobial agents against *Enterobacteriaceae* Uropathogen

Authors
Dr Ved Prakash Mamoria[1], Kajal Meena[2], Dr Richa Sharma[3*]
[1]Professor & Head, Department of Microbiology, Mahatma Gandhi University of Medical Sciences & Technology, Jaipur
[2]PGStudent, Department of Microbiology, Mahatma Gandhi University of Medical Sciences & Technology, Jaipur
[3]*Assistant Professor, Department of Microbiology, Mahatma Gandhi University of Medical Sciences & Technology, Jaipur
*Corresponding Author
Dr Richa Sharma
Assistant Professor, Department of Microbiology, Mahatma Gandhi University of Medical Sciences & Technology, Jaipur, India

Abstract
Urinary Tract Infection is the common bacterial diseases that affect a large part of the world's population, in both hospitals and in the community. Escherichia coli, Klebsiella spp., and Proteus spp. are the uropathogens with the highest prevalence among patients with UTIs. The gender and sexual anatomy are among the major determinants of UTI. They are more common in women in comparison with men. In the present study, out of 591 Enterobacteriaceae isolates, majority of the urine specimens were from inpatients 377 (64%) than from outpatients 214 (36%). Out of 4176 urine specimens 591 (100%) Enterobacteriaceae isolates were obtained, of which 432 (73.09%) were Escherichia coli, followed by Klebsiella pneumoniae 97 (16.41%), Providenciarettgeri21 (3.55%), Proteus mirabilis 14 (2.37%), Enterobacter cloacae 12 (2.03%),Morganella morganii and Citrobacter koseri 6 (1.02%), Serratia marcescens 3 (0.51%). So total positive Enterobacteriaceae isolates were 591.Out of the 591 isolated patient's majority of isolates were from female patients in the age group of 21-30 i.e. 102 (17.26%), followed by age group 31-40 i.e. 51(8.63%). In the present findings, also reported the sensitivity to fosfomycin was significantly higher in E. coli i.e. 419(97%), followed by Klebsiella pneumoniae 61 (62.9%). It concludes that Fosfomycin may be given empirically in patients suffering from UTI due to Enterobacteriaceae.
Keywords: *UTI, E.coli, Sensitivity, Uropathogens.*

Introduction

Among the most common infectious diseases, urinary tract infections (UTIs), considered the most common bacterial diseases that affect a large part of the world's population, in both hospitals and in the community. It can be spread and caused by gram-negative bacteria such as *Enterobacteriaceae* particularly *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species and *Proteus* species(1). E coli is the most common organism causing both community as well as hospital acquired UTI. The

gender and sexual anatomy are among the major determinants of UTI. They are more common in women in comparison with men. UTI is rare in males unless microorganisms are introduced artificially with catheters. In women, the urethra is much shorter and very close to the anus, which is a constant source of faecal bacteria [2].*Escherichia coli*, *Klebsiella spp.*, and *Proteus spp.* are the uropathogens with the highest prevalence among patients with UTIs. However, the antibiotic susceptibility patterns of *Enterobacteriaceae* have been constantly changing due to the continuous development of new resistance mechanisms, like the production of extended-spectrum β-lactamases (ESBLs) or carbapenemases by bacteria and the spread of genes on mobile elements [3]. Fosfomycin is an old bactericidal antibiotic, discovered in Spain in 1969 from cultures of *Streptomyces*[4]. That antibiotic have unique properties of not sharing any structural similarity and lack of cross-resistance with other antimicrobial agents. It inhibits cell wall formation by inhibiting the initial step involving phosphoenolpyruvate synthetase. Fosfomycin was previously used mainly as oral treatment for uncomplicated urinary tract infections (UTIs), currently attracts clinicians' interest worldwide. IDSA (Infectious Disease Society of America) and ESCMID (European Society of Clinical Microbiology and Infectious Diseases) recommends Fosfomycin as one of the first line agents for uncomplicated cystitis and pyelonephritis[5].

The emergence and spread of multidrug-resistant (MDR) Gram-negative bacteria related to urinary tract infections (UTIs) is increasing worldwide, both in hospitals and in the community. Fosfomycin tromethamine (FOF), a stable salt of Fosfomycin, has been found to be effective for the treatment of UTIs related to *Escherichia coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., and Enterococcus faecalis*[6]. The current study was undertaken to evaluate in-vitro activity of Fosfomycin against *Enterobacteriaceae* uropathogens and also assessed to compare Fosfomycin activity with the other antimicrobial agents against isolated *Enterobacteriaceae* uropathogens.

Material and Methods

The study was conducted prospectively in the Department of Microbiology at Mahatma Gandhi Medical College and Hospital Jaipur, Rajasthan, during one year period from June 2018 to May 2019 after receiving clearance from the Institutional Ethics Committee (IEC). The test group selected was the population of patients admitted in various OPD and IPD wards in the hospital regardless of their age, sex, occupation, religion and ethnicity.

Source of Data

Urine samples (4176) were received in lab between June 2018 to May 2019 for bacterial culture and sensitivity from various outdoor patient departments (OPDs) and indoor patient departments (IPDs) wards of Mahatma Gandhi Hospital (MGH) Sitapura Jaipur, Rajasthan.

Collection of urine specimen

Sample were collected with universal precautions by prescribed sterile techniques and transported to the laboratory as soon as possible maintaining optimum transportation condition. Detailed relevant history was taken as age, sex, the history of any in-dwelling medical devices used and the duration of wards and ICU stay. Urine samples were collected from various IPD and OPD wards.

(i) Mid-stream urine/ Urine in non-catheterise Patient: The clean catch mid-stream technique was employed to collect urine samples. Following the verbal consent of the patient /attendants, a mid-stream urine sample was collected in a wide mouthed sterile container with lid, labelled with the details of the patient. For clean catch mid-stream urine, patient will be instructed to cleanse the area with soaped swabs, then pass a small amount of urine

into toilet, and finally urinate into the wide mouthed container.

(ii) **Urine from Catheter:** For catheterized patient-Urine was collected through the draining portal of the urinary catheter using aseptic precaution.

Transport and storage of urine specimen

After collection the urine sample, the container was properly labelled with patient's name, ID number etc. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 hour after collection and processed as soon as possible. When the processing was delayed, they were stored at 4^0C.

Processing of urine specimen

Primary inoculation was done on Blood agar and Mac-conkey agar culture media using calibrated wire loop containing 0.001 ml of urine sample. The inoculums were spread with the wire loop on the media plate. They were incubated aerobically at 37°C for 18-24 hours. $>10^5$ CFU/ml for mid-stream urine &$>10^3$ CFU/ml in catheterized urine sample was taken as significant Bacteriuria. Colony characteristics were noted of the bacterial growth. Then Gram's staining was done of the growth. Only gram-negative bacilli were further processed by battery of tests for identification of bacterial isolates. Only *Enterobacteriaceae spp*. of bacterial isolates were taken in this study. Then antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method and the interpretation of antibiotic susceptibility was interpret as per CLSI guidelines [7]. All culture media were obtained from Hi-media laboratories, Mumbai, India.

Results

The study was carried out in the Department of Microbiology during 12 months period from June 2018 to May 2019. A total of 4176 urine samples were studied from patients with clinically suspected cases of UTI. The result was analysed as follows; out of 4176 urine specimens 591 (100%) *Enterobacteriaceae* isolates were obtained, of which 432 (73.09%) were *Escherichia coli*, followed by *Klebsiella pneumoniae* 97 (16.41%), *Providencia rettgeri* 21 (3.55%), *Proteus mirabilis* 14 (2.37%), *Enterobacter cloacae* 12 (2.03%), *Morganella morganii* and *Citrobacter koseri* 6 (1.02%), *Serratia marcescens* 3 (0.51%). So total positive *Enterobacteriaceae* isolates were 591. Out of 591 (100%) isolates of *Enterobacteriaceae*, majority were *E. coli* that is 432 (73.09%), followed by *Klebsiella pneumoniae* 97 (16.41%) **(Figure 1).** Of these 591 *Enterobacteriaceae* isolates,377 (63.79%) were from IPD and 214 (36.21%) were from OPD hospital wards as shown in **Table 1**.Of the 591 isolated patient's majority of isolates were from female patients in the age group of 21-30 i.e. 102 (17.26%), followed by age group 31-40 i.e. 51(8.63%) **(Table 2, Figure 2)**. Sensitivity to fosfomycin was significantly higher in *E. coli* i.e. 419(97%), followed by *Klebsiella pneumoniae* 61 (62.9%) **(Table 3, Figure 3).**

Of the 591 urine *Enterobacteriaceae* isolates studied, out of 432 *E. coli* isolates highly sensitive to Tigecycline i.e. 427(98.9%), followed by Colistin 424(98.1%) and Fosfomycin 419(97%).In total 97 *Klebsiella pneumoniae* isolates were highly sensitive to Colistin i.e. 94 (96.9%), followed by Fosfomycin i.e. 61(62.9%). In total 21 *Providencia rettgeri* isolates were highly sensitive to Fosfomycin i.e.15 (71.4%), followed by Trimethoprim/ Sulfamethoxazole i.e. 4 (19.05%). Out of 14 *Proteus mirabilis* isolates, 12 (85.7%) isolates were sensitive to Fosfomycin and Piperacillin/Tazobactum, followed by 11 (78.6%) Cefoperazone/Sulbactam, 10 (71.4%) Ertapenem and Amikacin. In total 12 *Enterobacter cloacae* isolates were highly sensitive to Colistin i.e. 11 (91.7%), followed by Tigecycline i.e. 8 (66.7%) and 7 (58.3%) Fosfomycin, Amikacin, Imipenem and Ertapenem. Out of 591 (100%) *Enterobacteriaceae* isolates were highly sensitive to Colistin (90.5%), Fosfomycin (89%) and Tigecycline (82.7%) **(Table 4).**

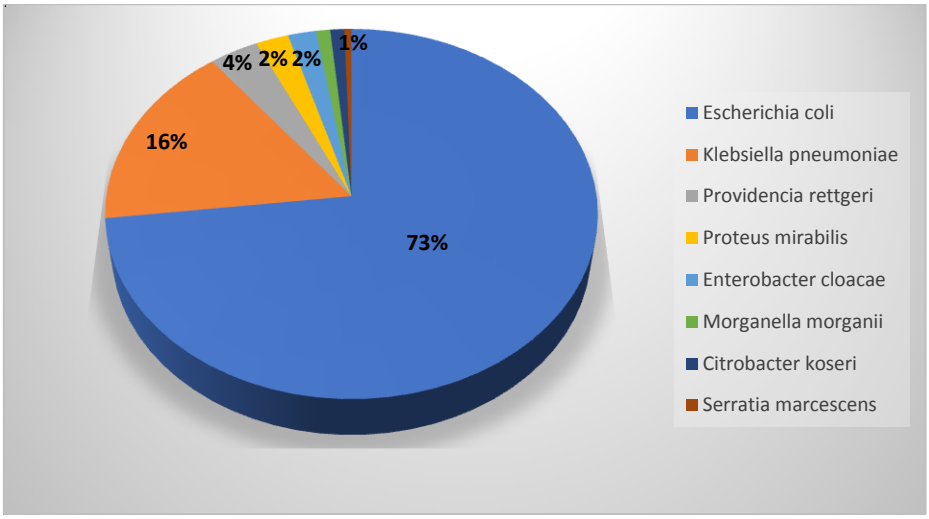

Fig. 1: Total No. of isolates obtained from *Enterobacteriaceae* family

Table 1: Distribution of positive *Enterobacteriaceae* isolated with respect to OPD/IPD

Distribution of OPD/IPD	No. of isolates	Percentage
OPD	214	36.21%
IPD	377	63.79%
Total	591	100%

Table 2: Gender wise distribution of patients

Age group	Male	Female	Total
0-10 yrs.	15	11	26
11-20 yrs.	21	25	46
21-30 yrs.	39	102	141
31-40 yrs.	27	51	78
41-50 yrs.	38	38	76
51-60 yrs.	58	35	93
61-70 yrs.	52	25	77
71-90 yrs.	31	23	54

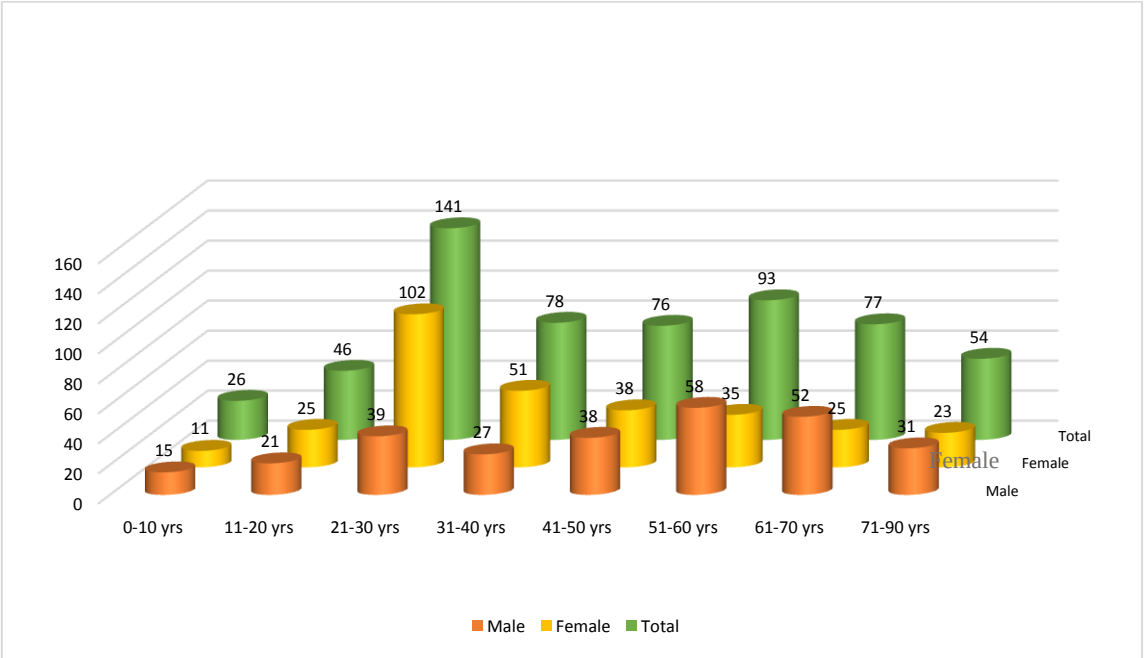

Fig. 2: Gender wise distribution of patients

Table 3: Antibiotic susceptibility pattern of *Enterobacteriaceae* with respect to Fosfomycin

Enterobacteriaceae spp.	**Fosfomycin susceptibility**	
	S	**R**
Escherichia coli	419 (97%)	13 (3%)
Klebsiella pneumoniae	61 (62.9%)	36 (37.1%)
Providenciarettgeri	15 (71.4%)	6 (28.6%)
Proteus mirabilis	12 (85.7%)	2 (14.3%)
Enterobacter cloacae	7 (58.3%)	5 (41.7%)
Morganellamorganii	3 (50%)	3 (50%)
Citrobacterkoseri	6 (100%)	-
Serratia marcescens	3 (100%)	-
Total	526	65

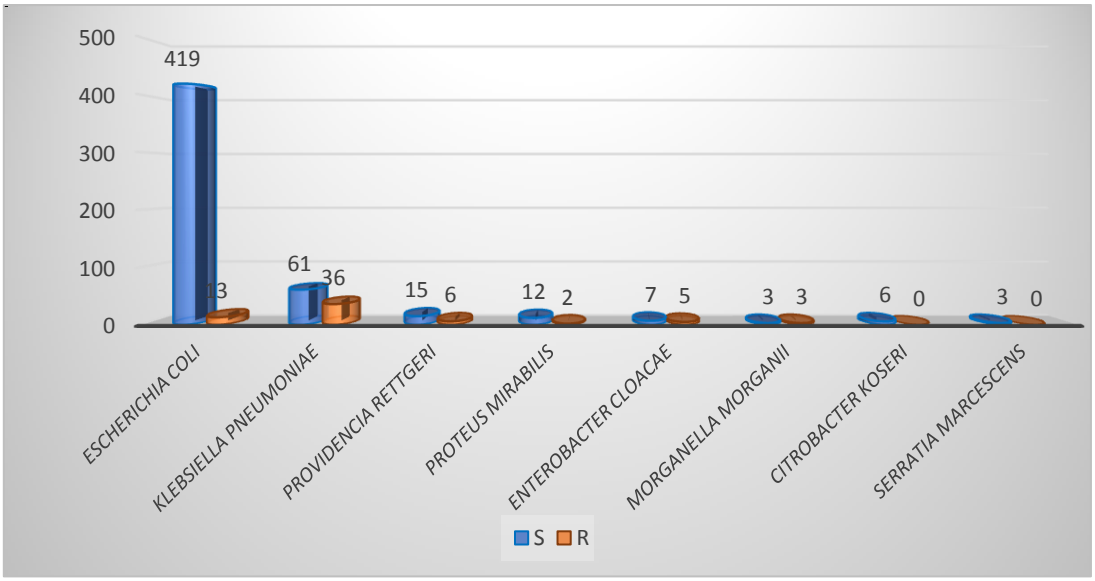

Fig. 3: Antibiotic susceptibility pattern of *Enterobacteriaceae* with respect to Fosfomycin

Table 4: Antibiotic susceptibility pattern of isolated *Enterobacteriaceae spp.*

Entero. Spp.	TOTAL NO.	AMC	PIT	CXM	CTR	CPZ/S	CPM	ETP	IPM	AK	GEN	LE	MINO	TGC	FO	NIT	CL	COT
Escherichia coli	432	128	234	66	78	249	121	285	303	324	227	82	239	427	419	313	424	145
Klebsiella pneumoniae	97	28	36	19	24	38	28	40	42	52	43	28	30	46	61	26	94	39
Providenciarettgeri	21	-	1	-	1	1	2	1	1	1	2	1	2	-	15	-	-	4
Proteus mirabilis	14	9	12	6	6	11	8	10	1	10	6	5	1	-	12	-	-	3
Enterobacter cloacae	12	-	5	-	4	4	4	7	7	7	3	5	3	8	7	3	11	5
Morganellamorganii	6	-	5	-	3	5	4	4	2	5	3	1	-	-	3	-	-	1
Citrobacterkoseri	6	6	6	5	5	6	6	6	6	6	6	6	6	6	6	5	6	6
Serratia marcescens	3	-	-	-	-	2	3	-	-	3	3	2	1	2	3	-	-	3
Total	591	171	299	96	121	316	176	353	362	408	293	130	282	489	526	347	535	206
%	100%	29.1	50.6	16.2	20.5	53.5	29.8	59.7	61.3	69	49.6	21.9	47.7	82.7	89	58.7	90.5	34.9

Discussion

This study shows the comparative evaluation of Fosfomycin activity with other Antimicrobial agents against *Enterobacteriaceae* Uropathogen in the Microbiology Department of Mahatma Gandhi Medical College & Hospital, Jaipur. Despite the widespread availability of antibiotics, Urinary Tract Infection (UTI) remains the most common bacterial infection in the human population. Antibiotic resistance is a common phenomenon in

developing countries where drugs are available freely without prescription. The resistance pattern varies from one country to another. In the present study samples were obtained from various outdoor patient departments (OPDs) and indoor patient departments (IPDs) wards of Mahatma Gandhi Hospital (MGH) Sitapura Jaipur, Rajasthan.

In our study Out of 591 *Enterobacteriaceae* isolates, majority of the urine specimens were from inpatients 377 (64%) than from outpatients 214 (36%), which are in correlation with the findings of Ekadashi Rajni Sabharwal et al.[8], reported 199 (75.1%) urine specimens were from inpatients and 66 (24.9%) were from outpatients. Similarly, to the study Sujatha R et al. [9], reported 314 (68.86%) urine specimens were from inpatients and 142 (31.14%) were from outpatients.In our study highest prevalence was observed in female patients i.e. 310 (52.45%) than from male patients 281 (47.55%), and in the study of Gamal A. et al. [10], reported highest prevalence in female patients 44 (66.7%) than from male patients 22 (33.3%) and study conducted by Thana Khawcharoenporn et al. [11], reported 81% female and 19% male patient. The highest prevalence of UTI were observe in female patients rather than male patients because the female urethra is of particular importance to the pathogenesis of UTIs. The female urethra is relatively short compared with the male urethra and also lies in close proximity to warm, moist, perirectal region which is abundant with microorganisms. Because of the shorter urethra, bacteria can reach the bladder more easily in the female host.In present study, 591 (100%) isolates from urine samples shows the *Enterobacteriaceae* growth, of which 432 (73.09%) were *Escherichia coli* followed by 97 (16.41%) *Klebsiella pneumoniae,* 21 (3.55%) *Providencia rettgeri,* 14 (2.37%) *Proteus mirabilis,* 12 (2.03%) *Enterobacter cloacae,* 6 (1.02%) *Morganella morganii* and *Citrobacter koseri* and 3 (0.51%) *Serratia marcescens,* that is similar to the study conducted by Ekadashi Rajni Sabharwal et al. [8], reported 68.8% *Escherichia coli*, followed by 24.9% *Klebsiella spp.* and 5.28% *Proteus spp.*. Similarly, to the study of Sujatha R et al.[9], showed 260 (57.02%) *Escherichia coli*, followed by *Klebsiella* 122 (26.75%), *Proteus* 41 (8.99%), *Enterobacter* 18 (3.95%), *Citrobacter* 15 (3.29%).

On studying the antibiotic susceptibility pattern for Fosfomycin, we found that 526 (89%) is susceptible for *Enterobacteriaceae* isolates. In other similar studies given by Dr. Nandita pal et al. [12], showed Fosfomycin was sensitive to 362 (93.29%) *Enterobacteriaceae* isolates and Ekadashi Rajni Sabharwal et al. [8], reported 249 (93.96%) Fosfomycin susceptible to *Enterobacteriaceae* isolates. In another study of Sayantan Banerjee et al. [13], overall 279 (97.21%) *Enterobacteriaceae* isolates were susceptible to Fosfomycin, Similarly, to the study conducted by Asfia sultan et al.[14], showed 368 (98.92%) Fosfomycin sensitive *Enterobacteriaceae* isolates. It concludes that Fosfomycin may be given empirically in patients suffering from UTI due to *Enterobacteriaceae*. In our study, we observe that Colistin was sensitive to 535 (90.5%) *Enterobacteriaceae* isolates. In other similar studies conducted by Dr. Nandita pal et al. (2017) [12] and Sayantan Banerjee et al. (2017) [13], reported 373 (96.13%) and 202 (70.38%) *Enterobacteriaceae* isolates susceptible to Colistin respectively. In the present study, 408 (69.04%) *Enterobacteriaceae* isolates were sensitive to Amikacin. Similarly, to the study conducted by Asfia sultan et al (2015)[14] shows 362 (97.31%) *Enterobacteriaceae* isolates were susceptible to Amikacin, Sayantan Banerjee et al. (2017) [13] reported 232 (80.84%) *Enterobacteriaceae* isolates were susceptible to Amikacin, Dr. Nandita pal et al. (2017) [12] observe 332 (85.57%) *Enterobacteriaceae* isolates were susceptible to Amikacin and Ekadashi Rajni Sabharwal et al. [8], shows 162 (61.13%) *Enterobacteriaceae* isolates were sensitive to Amikacin.

Conclusion

The present findings concluded that Colistin was most sensitive drugs and Fosfomycin is also

comparably sensitive against *Enterobacteriaceae* isolates. But as Fosfomycin is cheaper in comparison to Colistin and it can be taken orally so for patients suffering from UTI caused by *Enterobacteriaceae*, Fosfomycin is a better option.

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