Indian Journal of Microbiology Research 2021;8(2):142–145

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: https://www.ijmronline.org/

Original Research Article

Fosfomycin susceptibility among ESBL producing gram negative bacilli causing urinary tract infections

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ARTICLE INFO

Article history:
Received 28-06-2021
Accepted 28-06-2021
Available online 30-07-2021

Keywords:
Ecoli
ESBL
Fosfomysin
Kpneumoniae

ABSTRACT

Urinary tract infection is one of the common infection encountered in day to day practice. Due to emergence of drug resitance among uropathogens treatment options have become limited. Fosfomycin being a safe oral antibiotic is being used widely to treat multidrug resistant uropathogens. In the present study 831 (48.45%) samples that yielded significant growth were processed out of 1715 sample for ESBL detection by double disc synergy and phenotypic confirmatory method. E.coli constituted the predominant isolate (60.4%) followed by K.pneumoniae. 256 (30.80%) samples yielding growth were from out patients and 575 from inpatients. Over all 44% of isolates in the present study were ESBL producers. 50% of Ecoli were ESBL producers. 70.64% of ESBL isolates were susceptible to fosfomycin in vitro. Present study finding suggest that resistance to fosfomysin is on rise even though majority of ESBLs were sensitive to it. The current study recommends to use fosfomycin only after testing susceptibility among uropathogens.

1. Introduction

Infection of any part of Urinary system – kidneys, ureters, bladder and urethra is known as Urinary tract infection. The term urinary tract infection encompasses various entities like asymptomatic bacteriuria (ASB), Cystitis, Prostatitis and pyelonephritis.[1] Lower urinary tract infection is frequently encountered in day to day clinical practice. Members of the family Enterobacteriaceae are the most common agents causing UTI with E.coli (Escherichia Coli) being the predominant pathogen.

Erroneous antibiotic prescription practices, readily available Over the counter (OTC) antibiotics, increased use of antibiotics in livestock rearing has dwindled the emergence of Multi drug resistant organisms, thus making outpatient therapy difficult. The organisms causing UTI frequently carry multiple drug resistance (MDR) mechanisms against the commonly used oral antimicrobias like fluoroquinolones, trimethoprim-sulfamethoxazole, nitrofurantoin, and second and third-generation cephalosporins.[2]

Extended-spectrum Beta lactamases (ESBLs) are a group of enzymes that are resistance to Beta lactam antibiotics. Carbapenems are the drugs of choice for treatment of ESBL producers. Increased use of Carbapenems for the treatment of UTI's caused by ESBL producers has led to the emergence of Multi drug resistant bacteria (especially Carbapenem resistant Enterobacteriaceae- CRE). MDR is defined as resistance to at least one agent in three or more antimicrobial categories.[3]

Usage of Fosfomycin is one of the treatment options for MDR bacteria. Fosfomycin is a bactericidal agent which acts by inhibiting the cell wall synthesis.[4]

Bioavailability of oral Fosfomycin ranges between 34 to 58%, with absorption occurring predominantly in the small intestine thus, consumption of food reduce the absorption of Fosfomycin (37%- fasting v/s 30% with food).[5]

The peak serum concentration occurs within 4 hours of a 3g dose, with detectable levels (100 mg/L) at the end of 48 hours after the first dose. Hence, dosing of Fosfomycin

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https://doi.org/10.18231/j.ijmr.2021.029

is once in 48 hours.[6]

This study was conducted to determine the susceptibility of Fosfomycin among the uropathogens and also to study the susceptibility profiles of various bacteria isolated from Urine.

2. Materials and Methods

This is a retrospective study conducted between January 2020 to December 2020 in the Department of Microbiology in a 250 bedded Tertiary care hospital in Bengaluru. The urine culture samples received in the department both from Inpatient and Outpatient requiring urine culture as prescribed by the clinician was included in the study. Midstream urine sample from conscious, alert & oriented patients and from catheterised patients the sample was collected by following standard technique guidelines.[7]

The samples were processed as per the Standard operating procedure laid down which includes – sample processed within 30 minutes of collection, Direct microscopy of uncentrifuged sample to look for presence of pus cells, RBC's and bacteria. The samples were inoculated on Urichrom agar by semiquantitative method and were incubated overnight at 37° C. The reading of plates was done and samples with significant bacterial growth and with presence of significant pus cells on Microscopy were included in the study.

VITEK 2 Compact System (BioMérieux Inc., France) was used for the identification and susceptibility testing of the bacteria.

3. Detection of ESBL

3.1. Double disc diffusion synergy test[8]

All Enterobacteriaceae were screened for ESBL by Double disc diffusion synergy test (DDST). Ceftazidime (30μg), Cefotaxime (30μg), Ceftriaxone (30μg) and Amoxycillin / Clavulanic acid (20μg) discs were used. Bacterial isolates in BHI broth matched to 0.5 Mac Farland turbidity was uniformly swabbed on the sterility checked Mueller Hinton Agar using a sterile cotton swab. Ceftazidime(30μg), Cefotaxime(30μg), Ceftriaxone(30μg) were placed at distance of 20 mm from center to center around Amoxycillin / Clavulanic acid(20/10μg) disk. Plates were incubated at 37°C overnight. Extention of zone of inhibition of the any one of the cephalosporin towards the Amoxycillin / Clavulanic acid disc was considered positive result.

3.2. Phenotypic confirmatory method[9]

ESBL phenotypic method was done for all the 100 klebsiella pnuemoniea isolates screening for ESBL. Based on the CLSI guidelines the klebsiella pnuemoniae isolates were subjected for ESBL detection method using combined disc method. Combined disc method was done to detect the ESBL producers organisms in which cefotaxime 30μg and a cefotaxime/ clavulanic acid (30μg+10μg) discs were applied on the surface of Muller-Hinton Agar plate after a lawn culture of the isolated is done matching to 0.5 MacFerland then the plates were incubated overnight under 37 C. The result noted after taking the size of inhibition zone in which the zone of CEC should by ≥ 5mm in diameter comparing to the zone of CTX. This confirms the ESBL production.

Fosfomycin MIC was also tested on VITEK 2 system using M364 card. The MIC interpretation is based on CLSI guidelines with MIC <=64 is considered as significant and >=128 as resistant.

4. Results

A total of 1715 urine samples were received during the study period. Of which 831 (48.45%) samples that yielded significant growth were processed. Gram negative bacilli were the predominant isolate from the samples followed by Gram positive cocci and Candida species.

E.coli constituted the predominant isolate (60.4%) followed by K.pneumoniae (17.32%), Enterobacter spp (4.21%) and Pseudomonas spp (4.45%) as described in Table 1.

Out of the 831 samples 256 (30.80%) samples yielding growth were from Out patients. Out of the 575 IP samples yielding growth, 508 (88.3%) samples sent from wards and67 (11.6%) were from ICU. Ward wise distribution of gram negative bacteria is shown in Table 2.

All the Enterobacterales which were ESBL producers by Double disc diffusion synergy test were confirmed by phenotypic confirmatory method. All most 50% of E.coli were ESBL producers, 70.5% of Citrobacter spp, 62.8% of Enterobacter spp and 22.2% of Klebsiella pneumonia were also ESBL producers as shown in Table 3.

Fosfomycin sensitivity was performed only on 218 ESBL isolates as depicted in the Table 4.

77% of E.coli isolates were sensitive to Fosfomycin, 69.8% of Klebsiella isolates were sensitive. 17 Enterobacter isolates among 30 isolates tested for Fosfomycin were sensitive.

Table 1: Showing distribution of organisms isolated from urine sample

Organism	Growth
E. coli	502 (60.4%)
Klebsiella pneumoniae	144 (17.32%)
Enterobacter spp	35 (4.21%)
Citrobacter spp	17 (2.04%)
Proteus spp	28 (3.00%)
Pseudomonas spp	37 (4.45%)
A.baumanii	06 (0.72%)
Others (Gram positive bacteria and yeast)	62 (7.46%)
Total	831

Table 2: Distribution of organisms in the ward ICU out patient

Bacteria	OPD	Ward	ICU	Total
E.coli	153 (30.04%)	305 (60.7%)	44 (8.76%)	502 (60.4%)
Klebsiella pneumoniae	42 (29.16%)	88 (61.1%)	14 (9.72%)	144 (17.32%)
Proteus spp	06 (21.4%)	20 (71.42%)	02 (7.14%)	28 (3.00%)
Pseudomonas spp	05 (13.51%)	24 (64.86%)	08 (21.62%)	37 (4.45%)
Enterobacter spp	13 (34.21%)	19 (50%)	03 (08.57%)	35 (4.21%)

Table 3: ESBL producing enterobacterales

Organism	ESBL Producers	Non ESBL Producers	Total
E.coli	248 (49.4%)	254 (50.6%)	502
Klebsiella pneumoniae	32 (22.2%)	112 (78.8%)	144
Proteus spp	6 (21.42%)	22 (78.8%)	28
Enterobacter spp	22 (62.85%)	13 (37.14%)	37
Citrobacter spp	12 (70.5%)	05 (29.4%)	17
Total	320 (43.9%)	406 (55.76%)	728

Table 4: Sensitivity to fosfomycin of various ESBL isolates

Organism	Isolates tested for Fosfomycin sensitivity	Sensitive to Fosfomycin	Resistant to Fosfomycin
E.coli	102	79 (77.45%)	23 (22.54%)
K.pneumonaie	63	44 (69.8%)	19 (30.1%)
Enterobacter spp	30	17 (56.6%)	13 (43.3%)
Citrobacter spp	08	5 (62.5%)	3 (37.5%)
Proteus spp	15	9 (60%)	6 (40%)
Total	218	154 (70.64%)	64(29.9%)

5. Discussion

Due to the development of multi drug resistance and extreme drug resistance physicans are moving towards older antibiotics such as aminoglycosides, polymixins, etc.[10]

Fosfomycin is a novel antibiotic with broad spectrum activity against drug resistance bacteria.[11] It acts by mimicking the phosphoenol pyruvate and binds to binds MurA (UDP-GlcNAc enopyruvyl transferase that is found in the cytoplasm of the bacteria and thus inhibits enolpyruvyl transferase, which is required for peptidoglycan synthesis.[12] Hence effective in covering gram positive, gram negative and multi drug resistance organisms. Studies have demonstrated a synergistic activity of Fosfomycin with antibiotics such as beta lactams in mechanism of action as well as reducing the dosage and adverse effects as well.[13] On the contrary reports on resistance have also been seen due to the involvement of 6 genes of which MurA is also one and was seen in E coli.[14]

In the present study Out of the 831 samples 256 samples yielding growth were from out patients and 575 samples were from IP samples.67 samples showing growth were from ICU. As the COVID pandemic was at its peak and lockdown explains the smaller number of OP samples and more number of IP admissions. With COVID first wave affecting predominantly people with comorbidities like Diabetes, these patients also had secondary bacterial infections both due to the comorbid condition & due to increased use of steroid.

Out of 831 samples 769(92.5%) grew Gram negative bacilli followed by Gram positive cocci these findings are in parallel with Ekadashi Rajni Sabharwal et al.[15] study in which 82.9% were the gram negative bacilli. E.coli(60.4%) was the predominant isolate followed by K.pneumoniae (17.3%) in this study which is in consistent with study by Banerjee S et al.[16] and Ekadashi Rajni Sabharwal et al.[15]

Over all 44% of isolates in the present study were ESBL producers where as Banerjee S et al[16] reported slightly higher percentage of (64.78%) of ESBLs. In the current study 50% of E.coli isolates were ESBL producers similarly in the study by Gupta V et al[17] also reported 52.6% of Ecoli isolates as ESBL producers.

No significant difference was found between the use of carbapenem and fosfomycin in the treatment of lower urinary tract infections in ESBL producing bacteria.[18] In comparison to another antibiotics in a randomised control trial involving 27 trials in a mixed population there was no significant difference demonstrated between the fosfomycin and other drugs in terms of clinical and microbiological efficacy.[19] However in the present study only 70.64% of isolates were susceptible to fosfomycin in vitro. In contrast to the present study findings Banerjee S et al[16] reported 95.18% of the total urinary isolates and 95.93% of MDRs were susceptible to fosfomycin in 2017. These findings

indicate that urinary gram negative bacteria are gradually developing resistance to fasfomycin. One of the reasons for increased resistance could be due to its increased usage because of its ease of oral administration especially in treating UTI in out patients.

6. Conclusion

Many previously reported studies have shown that fosfomycin has high in vitro activity against common uropathogens, including ESBL producers, and carbapenem-resistant Enterobacteriaceae. it is being widely used as one of the safe oral antibiotic for the treatment of UTI. However present study finding suggest that resistance to fosfomysin is on rise even though majority of ESBLs were sensitive to it. The current study recommends to use fasfomycin only after testing susceptibility among uropathogens.

7. Source of Funding

None.

8. Conflict of Interest

The author declares no conflict of interest.

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Cite this article: Rajeshwari K G. Fosfomycin susceptibility among ESBL producing gram negative bacilli causing urinary tract infections. *Indian J Microbiol Res* 2021;8(2):142-145.