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RESEARCH ARTICLE

FOSFOMYCIN SUSCEPTIBILITY IN BACTERIAL ISOLATES FROM UROPATHOGENS WITH SPECIAL REFERENCE TO EXTENDED SPECTRUM B-LACTAMASE(ESBL) PRODUCERS AND MULTIDRUG RESISTANT(MDR) ORGANISMS

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Urinary Tract Infection(UTI), Escherichia Coli, Klebsiella Species, Fosfomycin, Antibiotic Susceptibility Testing, Multidrug Resistance(MDR).

Abstract

Introduction: Urinary tract infection (UTI) is one of the most common clinical entities encountered by physicians globally. Most of the uropathogens exhibit Multiple Drug Resistance(MDR) mechanisms especially in the members of Enterobacteriaceae. As there is increase in resistance rates, there is necessity in opting alternative treatment Strategies in cases of UTI like Fosfomycin which seems to be a good option attracting the clinician's interest globally. Objective: To evaluate the Fosfomycin susceptibility in bacterial isolates from uropathogens.

Methodology: A total of 624 urine samples were included in the study, out of which 84 isolates satisfying Kass concept were processed and identified by standard protocol and all the isolates were tested for Fosfomycin susceptibility by using Kirby Bauer disc diffusion and E-test, interpreted as per Clinical Laboratory Standards Institute(CLSI) guidelines.

Results: Among the isolates majority were *Escherichia coli*(60.71%) followed by *Klebsiella species*(26.19%), *Enterococcus species*(8.33%), *Staphylococcus saprophyticus*(2.38%) and *Proteus species*(2.38%) out of which 54.76% were Extended-spectrum-beta-lactamase-producing(ESBL) organisms, 40.47% were Multi Drug Resistant Enterobacteriaceae(MDRE) and 15.47% were Carbapenem Resistant Organisms(CRO). Fosfomycin susceptibility was observed highest in *Staphylococcus saprophyticus*(100%), *Proteus species*(100%) followed by *E.coli*(96.7%) *Klebsiella species*(95.45%) and *Enterococcus species*(85.71%).

Conclusion: Our study mainly focussed on Fosfomycin susceptibility in various uropathogens where Fosfomycin was found to have a promising role in treating UTI with both Gram positive and Gram negative organisms and can be considered as a drug of choice for empirical oral treatment in cases of UTI.

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Introduction:-

Urinary tract infection(UTI) is one of the most common clinical entities encountered by physicians globally in both primary and secondary health care settings. Nearly 150 million people suffer with UTI globally every year which has 50-60% of life time incidence in adult women 75%effected are of age group 24 or below.[1,2]

UTI is an infection of bladder(cystitis) or the kidneys(pylonephritis) which may be hospital acquired or community acquired leading to personal,social and economic burden to the patients.Several antibiotics have been in use for treatment of UTIs, most commonly Co-trimoxazole(Trimethoprim-Sulfamethaxazole), Nitrofurantoin, Fluoroquinolones and Beta-lactam antibiotics.Most of the uropathogens exhibit Multiple drug resistant(MDR) mechanisms including Quinolone resistance, broad spectrum Beta-lactamase production and Carbapenemase production. Extended spectrum Beta-lactamase(ESBL) is commonly seen in most of the uropathogens especially in the members of Enterobacteriaceae and also there is increase in MDR uropathogens which can be defined as the organisms exhibiting resistance to three or more antibiotic classes.[1,2]

As there is increase in resistance rates in both Gram positive and Gram negative organisms, there is increased necessity for opting alternate treatment strategies in cases of UTI, but as there is only limited availability of novel antibiotics, reevaluating the older antibiotic agents like Fosfomycin which was discovered in 1969, which is a Phosphoenol Pyruvate(PEP) analogue produced by *Streptomyces species* namely *Streptomyces fradiae* (ATCC21096), *Streptomyces viridochromogenes*(ATCC21240) and *Streptomyces wedmorensis* (ATCC21239). It is available in two oral formulations(PO) Fosfomycintromethamine and Fosfomycinvaluum and one intra-venous(IV) formulation Fosfomycin disodium which have bactericidal activity by inhibiting an enzyme catalyzed reaction in the first step of cell wall synthesis and also has good activity against Gram positive and Gram negative organisms and also have good ability to penetrate biofilms, seems to be an appealing option attracting the clinician's interest globally.[3,4]

This study is carried out with an objective to determine the invitro Fosfomycin susceptibility of common uropathogens isolated with special reference to ESBL producers and MDR organisms.

Materials and Methods:-

This is a prospective observational study conducted in the department of microbiology, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Andhra Pradesh from Mar 2021 to Aug 2021 attaining approval from Institutional Ethics committee.

Out of 624 urine samples from both inpatient and outpatient setting from patients with signs of UTI were included in the study and fresh clean catch midstream urine samples were collected aseptically from the patients after informed consent and were submitted to the clinical microbiology laboratory.

All the urine samples after subjecting microscopy for appreciation of bacteria, pus cells and casts were inoculated on to Cystine Lactose Enzyme Deficient (CLED) medium (HIMEDIA Labs, Mumbai, India) and after aerobic incubation of 24 hrs at 37^{o}C growth was observed and 84 isolates showing significant growth as per Kass concept (single species count of > 10^5 CFU/ml of urine) were processed further and the organisms were identified further by microscopy, colony morphology and standard biochemical reactions.[5,6,7]

All the isolates including Gram positive and Gram negative organisms were further subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method using Muller-Hinton agar(MHA) according to Clinical Laboratory Standards Institute(CLSI) guidelines[5,8] with all the drugs routinely tested for urinary isolates along with Fosfomycin (200mcg)(HIMEDIA Labs, Mumbai, India) containing glucose-6-phosphate and zone of inhibition is noted for all the isolates and interpreted as Susceptible(S), Intermediate(I), Non-Susceptible or Resistant(R) as per CLSI guidelines.[8]

Among the Gram negative organisms isolated ESBL producers are identified by screening method using Ceftriaxone(CTR)(30mcg) discs (HIMEDIA Labs, Mumbai, India), Cefotaxime(CTX)(30mcg) discs(HIMEDIA Labs,Mumbai, India) and Ceftazidime(CAZ)(30mcg) discs (HIMEDIA Labs, Mumbai, India) and those isolates tested positive were subjected to confirmatory test by combined disc method using CAZ(30mcg) discs(HIMEDIA Labs, Mumbai, India) alone and CAZ(30mcg) and Clavulinate(CAL)(10mcg) discs(HIMEDIA Labs, Mumbai,

India) in which the two discs were placed 20mm apart on the surface of Muller-Hinton agar with lawn culture of the tested isolate and was incubated at 35-37^{o} C for 16-18 hours and ESBL production was reported if there is ≥ 5mm increase in zone diameter of CAZ/CAL disc verses the zone of diameter of CAZ alone according to CLSI guidelines[8,9]

In addition we have also considered multi drug resistant Enterobacteriaceae (MDRE) isolates showing resistance to any three of the antibiotic groups tested like Cephalosporins, Fluoroquinolones, Aminoglycosides, Folate pathway inhibitors(TMP-SMX) and Nitrofurantoin and Carbapenem resistant organisms (CRO) were identified by using Meropenem disc(10 mcg)(HIMEDIA Labs, Mumbai, India).

E-strips of Fosfomycin (HIMEDIA Labs, Mumbai, India) are used to determine Minimum Inhibitory Concentration(MIC) of the isolates like Escherichia coli(E.coli) and Klebsiella species and Enterococcus faecalis(E.faecalis) strains with E-strips having gradient concentrations ranging from 0.04mcg/ml to 1,024mcg/ml along with added 50mcg/ml of Glucose-6-phosphate are used and the zone diameter break points of ≤ 64mcg interpreted as Susceptible(S), 128mcg as Intermediate(I) and ≥ 256mcg as Non-susceptible or Resistant as per CLSI guidelines.[8]

As a part of routine quality assurance system, the quality control measures are followed in this study with the aid of quality control strains like *E.coli* (ATCC25922), *K.pneumoniae* (ATCC27736), *E.faecalis* (ATCC29212), *S.saprophyticus* (ATCC15305)[10]

Results:-

A total of 84 isolatesfrom 624 urinary samples fromvarious outpatient departments (OPDs) and In Patient Departments (IPDs) of our hospital which satisfied the Kass concept were processed out which 38(45.23%) were from male patients and 46(54.77%) were from female patients. (Table 1)In which majority of the isolates were *E.coli*(60.71%) followed by *Klebsiella species*(26.19%), *Enterococcus species*(8.33%), *Proteus species*(2.38%) and *Staphylococcus saprophyticus*(2.38%) (Table 2)Out of the isolates 54.76% were ESBL producers, 40.47% were MDRE and 15.47% were CRO.(Table 3)

Fosfomycin susceptibility pattern of various isolates showed highest susceptibility to Proteus species and *S.saprophyticus*(100%) followed by *E.coli*(96.7%), *Klebsiella species*(95.45%) and *Enterococcus species*(85.71%).(Table 4)Out of the isolates 24 were tested by E-test in which 10 isolates were *E.coli*, 10 isolates were Klebsiella species and 4 isolates were Enterococcus species in which 90% of isolates of *E.coli* and *Klebsiella species* and 75% of *Enterococcus species* were sensitive to fosfomycin, where totally 87.5% of isolates tested by E-test are sensitive and 12.5% were resistant (Table 5)

Out of isolates of *E.coli* randomly 10isolates were of ESBL(3), MDRE(2), ESBL+MDRE(2), ESBL+MDRE+CRO(1) and No pattern(2)and 10 isolates of *Klebsiella species* were ESBL(4), ESBL+MDRE(2), ESBL+MDRE+CRO(2) were tested for Fosfomycin susceptibility by E-test where all(100%) isolates were sensitive(Table 6)

Table No 1:- Showing sex wise distribution of Isolates:

Total number of Isolates	Males	Females
84	38	46
In Percentage	45.23%	54.76%

Table No 2:- Showing different isolates from cases of UTI:

Organisms	Number of isolates	Percentage(%)
Escherichia coli	51	60.71
Klebsiella species	22	26.19
Enterococcus species	7	8.33
Proteus species	2	2.38
Staphylococcus saprophyticus	2	2.38
Total number of Isolates	84	100

Table No 3:- Showing Isolates of ESBL/MDRE/CRO:

Total No. of samples (84)	ESBL	%	MDRE	%	CRO	%
Escherichia coli	33	39.28%	21	25%	5	5.95%
Klebsiella species	13	15.47%	12	14.28%	7	8.33%
Proteus species	NIL	Nil	01	1.19%	Nil	Nil
Total with Percentage	46	54.76%	34	40.47%	13	15.47%

Table No 4:- Showing Fosfomycin susceptibility by disc diffusion in various isolates:

Organisms list	Fo sensitive by disc diffusion	Fo resistant by disc diffusion
Escherichia coli	49(96.07%)	2(3.92%)
Klebsiella species	21(95.45%)	1(4.54%
Proteus species	2(100%)	NIL
Enterococcus species	6(85.71%)	1(14.28%)
Staphylococcus saprophyticus	2(100%)	NIL
Total	80(95.23%)	04(4.76%)

Table No 5:- Showing Fosfomycin susceptibility by E test in various isolates:

Organisms list	Fo E-test sensitive	Fo E-test resistant
Escherichia coli	9(42.85%)	1(33.33%)
Klebsiella species	9(42.85%)	1(33.33%)
Proteus species	NIL	NIL
Enterococcus species	3(14.28%)	1(33.33%)
Staphylococcus saprophyticus	NIL	NIL
Total	21(87.50%)	3(12.50%)

Table No 6:- Showing ESBL/MDRE/CRO isolates which were sensitive to Fosfomycin by E-test:

Drug resistance pattern	*Escherichia coli* (10)	*Klebsiella species* (10)
ESBL only	3	4
MDRE only	2	2
ESBL + MDRE	2	2
ESBL + MDRE + CRO	1	2
No Pattern	2	NIL

Discussion:-

UTI is one of the most common infectious disease effecting both out patients and hospitalized patients and the life time incidence among adult and sexually active women is estimated to be 50-60% and the incidence increases by 20% in females with increasing age.[1,11,12,13] The current study was conducted at our institute to evaluate the Fosfomycin susceptibility patterns in bacterial isolates from urine samples.

A total of 84(100%) urinaryisolates satisfying Kass concept (Single species count of $\geq 10^5$ CFU/ml of urine) were processed in the laboratory in which majority of the isolates were from females(54.76%) followed by males(45.24%) correlating with findings of Syed Mehmood et al.[14] Out of the total isolates majority were *E.coli*(60.71%) followed by *Klebsiella species*(26.19%), *Enterococcus species*(8.33%), *Proteus species*(2.38%) and *S.saprophyticus*(2.38%) correlating with findings of Sadia et al.,Miroslow et al.[2,15]

Out of the isolates ESBL producers were 54.76% MDRE were 40.47% and CRO were 15.47% correlating with the findings of Sayanthan Benarjee et al.[16]

When tested for Fosfomycin susceptibility for various isolates by Kirby Bauer disc diffusion method *S.saprophyticus* and *Proteus species* showed 100% susceptibility followed by *E.coli*(96.07%), *Klebsiella species*(95.45%) and *Enterococcus species*(85.71%) correlating with findings of Yosra MH et al.,[10]Sharlee et al.,[17]Dasaraju Rajesh et al.,[18]Fajfr M et al.,[19]Maraki S et al.,[20]Saeed NK et al.[21] who showed Fosfomycin susceptibility up to 100% for various isolates.

A total of 24 isolates comprising *E.coli*(10), *Klebsiella species*(10) and *Enterococcus species*(4) were tested by E-test which showed that 90% *E.coli* and 90% *Klebsiella species* isolates were susceptible for Fosfomycin correlated with findings of Zeeshan F et al.[14] and 75% of Enterococcus species isolates were susceptible for Fosfomycin correlated with findings of Sharmin S et al.[2]

Out of the *E.coli* isolates randomly, produces 3 isolates of ESBL producers, 1 isolate of ESBL+MDRE, 1 isolate of ESBL+MDRE+CRO were tested for Fosfomycin susceptibility by E-test where all these isolates(100%) were susceptible to Fosfomycin and similarly from *Klebsiella species* isolates 4 ESBL producers, 2MDRE, 2ESBL+MDRE and 1 ESBL+MDRE+CRO were tested for Fosfomycin susceptibility by E-test where all these isolates(100%) were also susceptible to Fosfomycin correlating with findings of Yosra MH et al.[10]

The present study highlights the high rate of sensitivity of Fosfomycin against urinary isolates of *E.coli*, *Klebsiella species*, *Proteus species*, *Enterococcus species* and *S.saprophyticus* and the overall resistance rate of these isolates against Fosfomycin is very low.

Conclusion:-

Although empirical antimicrobial therapy for UTI needs to be consistent with antibiogram of the hospital which should be based on the sensitivity data of various uropathogens isolated, Fosfomycin has a promising role in treating cases of UTI with both Gram negative and Gram positive organisms and may be considered as a drug of choice for empirical oral treatment option until the culture and sensitivity report is awaiting.

To conclude in the present scenarioof increasing drug resistance and scarcity of newer antibiotics there is a great need in evaluating the use of older antibiotics and guide the physicians. Although United States food and drug administration (FDA) has approved the usage of some older antibiotics like Fosfomycin, Temocillin,Pevmecillinam etc. many countries have withdrawn the older antibiotics from their market.

Finally it is advisable to include the old antibiotics in surveillance programmes, data regarding resistance rates and determination of MICs and include antibiotic susceptibility testing for old but effective antibiotics and usage of these can be incorporated into routine clinical practice which may be a promising step in order to fight against antimicrobial resistance (AMR) by revival of older antibiotics.

Conflict of interest:
The authors declare no conflict of interest regarding the publication of this paper.

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