Section: Microbiology

Original Research Article

INCREASING ANTIMICROBIAL RESISTANCE AMONG UROPATHOGENS: IS FOSFOMYCIN A RIGHT ANSWER?

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Received : 20/02/2023
Received in revised form : 27/03/2023
Accepted : 13/04/2023

Keywords:
multidrug resistance, Fosfomycin, urinary tract infection.

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DOI: 10.47009/jamp.2023.5.3.46

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 208-212

Abstract

Background: Introduction: Urinary tract infection (UTI) is one of the most common infectious diseases in clinical practice. The choice of antibiotics for the treatment of UTI is limited by the rising trend of antibiotic resistance. There is an urgent need to discover new effective treatment solutions. Fosfomycin may be an interesting alternative to the currently used treatments for UTIs. The current study was undertaken with a dual purpose: to provide insight into the current scenario of the microorganisms causing UTIs and their antimicrobial sensitivity patterns, and to evaluate the activity of Fosfomycin against uropathogens of the Enterobacteriaceae family. **Materials and Methods:** A retrospective study was conducted for one year, from July 2021 to June 2022, in a tertiary care hospital in North India, to evaluate the in-vitro sensitivity pattern of Fosfomycin and other commonly used antibiotics against uropathogens of the Enterobacteriaceae family. Identification of organisms causing significant bacteriuria was done by conventional biochemical tests. Antimicrobial susceptibility testing was performed against these pathogens by the Kirby-Bauer disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** A total of 6480 urine samples were submitted for culture during the study period, which yielded 907 significant bacterial isolates. Among these, 752 (82.9%) isolates were from the Enterobacteriaceae family, and 155 (17.1%) were gram-positive cocci, Pseudomonas, Acinetobacter, and budding yeast cells. Among the Enterobacteriaceae isolates, 530 (70.5%) were Escherichia coli, followed by Klebsiella pneumoniae 136 (18.08%), Proteus mirabilis 38 (5.05%), Citrobacter spp 30 (3.98%), Morganella morganii 8 (1.06%), Providencia rettgeri 7 (0.93%), and Serratia marcescens 3 (0.39%). Only isolates of Enterobacteriaceae were further processed for antimicrobial sensitivity testing during the study. Antibiotics like colistin (11.1%) and polymyxin (11.1%) showed the least in-vitro resistance against all the isolates of Enterobacteriaceae, barring Proteus, Morganella, and Providencia, where they showed 100% in-vitro resistance. Fosfomycin (14.77%) is the other antimicrobial agent that also showed less in-vitro resistance against all the isolates of Enterobacteriaceae. Whereas, the highest resistance was observed for cefixime (78.33%), ceftriaxone (77.27%), levofloxacin (76.73%), followed by amoxicillin/clavulanic acid (69.55%), cefepime (64.23%), and imipenem (62.37%). Fosfomycin has shown very good in-vitro activity against all the tested isolates when compared with many other antibiotics. **Conclusion:** Fosfomycin has emerged as a promising option, especially in cases involving multi-drug resistant uropathogens, in which previous antibiotics have been found resistant in in-vitro antimicrobial sensitivity testing.

INTRODUCTION

Urinary tract infections (UTIs) are the most commonly encountered infectious disease faced by clinicians and affect a large part of the world's population, particularly in developing countries.[1,2] The increasing prevalence of multidrug-resistant (MDR) pathogens has considerably contributed to the increasing proportion of UTIs, as they limit treatment options.[3,4] Most of the Gram-negative organisms causing UTI are known to harbor multiple drug resistance mechanisms, both inherited or transmissible and chromosomal or extrachromosomal, against the most commonly used oral antimicrobial agents such as fluoroquinolones, trimethoprim-sulfamethoxazole, nitrofurantoin, and second and third-generation cephalosporins.[5]

Clinicians often face challenges in selecting appropriate antibiotic therapy for treating UTIs caused by multidrug-resistant Gram-negative bacteria.[6] The emergence of a plethora of multidrug-resistant Gram-negative organisms has prompted a re-evaluation of non-traditional antibiotics. The introduction of antimicrobial agents that are not widely used in clinical practice may provide a ray of hope. One such drug that has recently caught the attention of clinicians is Fosfomycin, a broad-spectrum antimicrobial agent active against both Gram-positive and Gram-negative bacteria. It is available in both oral and systemic forms. Although known for more than four decades, clinical data regarding the use of Fosfomycin for the treatment of UTIs due to various pathogens is very limited.[7]

The current study was undertaken with a dual purpose: to provide insight into the current scenario of microorganisms causing UTI, their in-vitro antimicrobial resistance patterns, and to evaluate the in-vitro activity of Fosfomycin against uropathogens of the Enterobacteriaceae family.

MATERIALS AND METHODS

A retrospective study was conducted from July 2021 to June 2022 in the Department of Microbiology at a tertiary care hospital in North India. A total of 6480 freshly collected mid-stream urine samples were submitted from adult patients from both inpatient and outpatient departments, with all aseptic precautions [8]. The urine samples were processed immediately (within 30 min) after collection. The urine samples were plated by semi-quantitative method on CLED agar (Hi-Media) and incubated aerobically at 37°C overnight and if required, till 48 h. The growth of organisms and colony count were taken into consideration.[5]

The isolates obtained from samples with significant bacteriuria as per the Kass criteria (single species count of more than $\mathbf{10^5}$ organisms per ml of urine) were processed further, and the isolates were identified up to the species level by using standard biochemical tests.[9-12] Inadequate urine samples (<10 ml urine), urine collected from urine bags, specimens collected more than 2 h before submission, specimens submitted in leaking or dirty unsterile containers, and specimens revealing growth of more than two types of bacteria on culture were excluded from the study. The significant pathogens were identified by standard biochemical procedures.[10]

Antimicrobial susceptibility testing was performed on all isolates on Mueller Hinton agar using the Kirby-Bauer disk diffusion method for Fosfomycin (200µg). In addition, susceptibility testing for the following antimicrobial agents (with drug concentrations in µg) was also performed in accordance with the Clinical Laboratory Standards Institute guidelines (CLSI 2020): amikacin (30), gentamycin (10), ceftrizone (30), cefixime (5), cefepime (30), levofloxacin (5µg), nitrofurantoin (300), imipenem (10), Fosfomycin (200), colistin (10), cefaperazone/sulbactam (75/30), piperacillin/tazobactam (100/10), amoxicillin/clavulanic acid (20/10), and so on. All discs and media were obtained from Hi Media Laboratories in Mumbai, India.[13]

RESULTS

Out of a total of 6480 urine samples submitted, 907 (13.99%) were found to be culture positive. Among these, 752 (82.9%) were Enterobacteriaceae isolates, and 155 (17.1%) were other organisms, such as Pseudomonas, Acinetobacter, gram-positive cocci, and budding yeast cells. The distribution of Enterobacteriaceae isolates is shown in [Table 1].

Table 1: Distribution of various urinary pathogens (n=752)

Species	Number	Percentage
Escherichia coli	530	70.5%
Klebsiella pneumoniae	136	18.08%
Proteous species	38	5.05%
Citrobacter species	30	3.98%
Morganella morganii	8	1.06%
Providencia rettgeri	7	0.93%
Serratia marcescens	3	0.4%
Total	752	100%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 2: Resistance pattern of the organisms of Enterobacteriaceae family

Causative agent	Escherichia coli N=530(%)	Klebsiella pneumoniae N=136	Proteus species N=38	Citrobacter species N=30	Morganella morgagnii N=8	Providencia rettgeri N=7	Serratia marcescens N=3	Average resistance	percentage
Amikacin	262 (49.43)	60(44.11)	13(34.21)	7(23.33)	2(25)	6(85.71)	0(0)	350	46.54
Gentamycin	253(47.73)	75(55.14)	22(57.89)	7(23.33)	5(62.5)	7 (100)	0(0)	371	49.33
Levofloxacin	431(81.32)	98(72.05)	23(60.52)	12(40)	7(87.5)	6(85.71)	0(0)	577	76.72
Nitrofurantoin	135(25.47)	71(52.20)	38 (100)	8 (33.33)	8(100)	7(100)	3(100)	317	42.15
Cefixime 3rd	434(81.88)	110(80.88)	22(57.89)	5(16.66)	8 (100)	7(100)	3(100)	589	78.32
Ceftriaxone 3rd	432(81.50)	102(75)	23(60.52)	6(20)	8 (100)	7(100)	3(100)	581	77.26
Cefepime 4th	359(67.73)	91(66.91)	15(39.47)	8(26.66)	3(37.5)	6(85.71)	1(33.33)	483	64.22
Imipenem	334(63.01)	83(61.02)	22(57.89)	15(50)	7(87.5)	6(85.71)	3(100)	470	62.5
Amoxycillin +Clavulanic acid	376(70.94)	97(71.32)	18(47.36)	14(46.66)	8(100)	7(100)	3(100)	523	69.54
Piperacillin+ Tazobactam	241(45.47)	85(62.5)	13(34.21)	9(30)	3(37.5)	6(85.71)	3(100)	360	47.87
Cefoperazone + Sulbactam	261(49.24)	78(57.35)	9(23.68)	9(30)	2(25)	3(42.85)	1(33.33)	363	48.27
Colistin	21(3.96)	7(5.14)	38(100)	1(3.33)	8 (100)	7(100)	3(100)	85	11.30
Fosfomycin	53(10)	53(38.97)	4(10.52)	1(3.33)	0(0)	0(0)	(0)	111	14.76

DISCUSSION

This study was conducted to evaluate the activity of Fosfomycin and its comparable efficacy with other commonly used antimicrobials against Enterobacteriaceae Uropathogen. This study was conducted in the Microbiology department of a tertiary care hospital in North India. 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 a prescription. The resistance pattern varies from one country to another.

In the present study, a total of 6480 urine samples were submitted from various outdoor patient departments (OPDs) and indoor patient departments (IPDs) of a tertiary care hospital and medical college, and from these samples, 752 (82.9%) Enterobacteriaceae isolates and 155 (17.1%) other isolates were obtained. Out of 752 (82.9%) isolates of Enterobacteriaceae, 530 (70.47%) were Escherichia coli, followed by 136 (18.08%) Klebsiella pneumoniae, 38 (5.05%) Proteus mirabilis, 30 (3.98%) Citrobacter species, 8 (1.06%) Morganella morganii, 7 (0.38%) Providencia rettgeri, and 3 (0.39%) Serratia marcescens. Similar studies were conducted by Sabharwal ER et al.[7] and Sujatha R et al.[14] Their findings were 68.8% Escherichia coli, 24.9% Klebsiella spp., 5.28% Proteus species and 57.02% Escherichia coli, 26.75% Klebsiella, 8.99% Proteus species, 3.29% Citrobacter species, respectively, which are quite similar to the present study.

In the present study, the majority of the Enterobacteriaceae isolates were resistant to most of the antibiotics. Among the third-generation cephalosporins, cefixime was found to have 78.32% resistance, and ceftriaxone was 77.26% resistant to Enterobacteriaceae isolates. Our results are well-corroborated with the result of Sardar A et al.[15] which showed 83.6% resistance to cefixime and 74.2% to ceftriaxone against Enterobacteriaceae isolates. Identical findings were also shown by Niranjan B et al.[16] as they found 71% resistance of Enterobacteriaceae isolates for ceftriaxone.

"Fluoroquinolones are the most commonly used urinary antibiotics whose resistance was found to be 76.87% (levofloxacin) in the present study. Similar results (75.3% resistant) were also shown in a study done by Sardar A et al.[15] Enterobacteriaceae isolates were also found to be 64.23% and 62.5% resistant against cefepime and imipenem, respectively. These findings are comparable to the study conducted by Amamoria PM et al in Jaipur.[17], where observations were 70.2% and 38.7%, respectively, against these two drugs. A study conducted by Sabharwal ER et al.[7] showed 61.13% of Enterobacteriaceae isolates were resistant to Amikacin. Identical results were also found in this study where 46.55% of isolates were found to be resistant to amikacin. Combination drugs of beta-lactams with beta-lactamase inhibitors like piperacillin/tazobactam and cefoperazone/sulbactam are increasingly being used nowadays in healthcare settings, especially when nosocomial infections are suspected.[18,19] In the present study, 48.28% of isolates were resistant to cefoperazone/sulbactam, 47.88% to piperacillin/tazobactam, and 69.55% were to amoxicillin/clavulanic acid. Similar results were also found in a study done by Niranjan B et al.[16], in which 74% of Enterobacter isolates were resistant to amoxicillin/clavulanic. In another similar study conducted by Sardar A et al.[15], they found that 18.9% of isolates were resistant to piperacillin/tazobactam and 17.7% to cefoperazone/sulbactam. Oral antibiotic

ISSN (O): 2687-5365; ISSN (P): 2753-6556

nitrofurantoin has comparatively shown good activity against the majority of the Enterobacteriaceae isolates as these isolates showed only 35.90% resistance. A similar study done by Banerjee S et al. found that 29.33% of the Enterobacteriaceae isolates were resistant to nitrofurantoin.[20] Nitrofurantoin, an age-old antibiotic, has been forgotten with the availability of more user-friendly cephalosporins and fluoroquinolone group of drugs. With the advent of multidrug resistance, this antibiotic has gained importance once again. Its availability in oral form and ability to attain high levels in the urine with less resistance makes this drug a preferred drug over the others.[21,22]."

In the current in-vitro antibiotic susceptibility pattern, we found that 14.77% of Enterobacteriaceae isolates were resistant to Fosfomycin. The results of other studies such as by Neunar et al.[23], Nandita Pal et al.[24], and Sabharwal ER et al.[7] in their in-vitro antibiotic susceptibility testing also found that 14%, 6.71%, and 6.04% of isolates, respectively, were resistant to Fosfomycin. Fosfomycin is a novel antibiotic with good in vitro activity against the common pathogens causing UTIs, particularly toward the Enterobacteriaceae. Fosfomycin is active against both Gram-negative and Gram-positive pathogens, including E. coli, Salmonella spp., Shigella spp., Klebsiella, Enterobacter spp., Serratia spp., Citrobacter spp., P. mirabilis, Enterococcus species, and Staphylococcus aureus.[25] Fosfomycin has a low molecular weight and a relatively long half-life (mean half-life-SD, 5.7-2.8 h) and therefore penetrates various tissues with ease, achieving the minimum inhibitory concentrations needed to inhibit the growth of most pathogens.[6] Resistance rates are low and most frequently acquired by chromosomal mutations that do not spread easily.[26]

In the present study, Enterobacteriaceae isolates showed the least in-vitro resistance (11.31%) against colistin, which was well corroborated with the results (3.87%) of Nandita Pal et al.[24], whereas the finding of Sayantan Banerjee et al. (29.62% resistance) was higher than our finding.[20]

In the current study, we found that Enterobacteriaceae isolates were almost equally resistant against both colistin and Fosfomycin, but as Fosfomycin is cheaper in comparison to colistin and it can be taken orally, it is a better option for patients suffering from UTIs caused by Enterobacteriaceae.

Limitation

This study evaluated the in vitro activity of Fosfomycin and not its clinical efficacy. There were very few Enterobacteriaceae isolates other than E. coli and K. pneumoniae found in the study, so more isolates of other pathogens are needed to draw any conclusions about the efficacy of Fosfomycin.

CONCLUSION

The present study observed an increase in drug resistance of Enterobacteriaceae isolates to commonly used antibiotics such as fluoroquinolones, cephalosporins, and other beta-lactams. The inappropriate use of antibiotics has always been a threat for the emergence of multidrug-resistant pathogens. As the antibiotic pipeline is nearly empty with only a few alternative drugs available for these resistant pathogens, testing for old and forgotten antibiotics like Fosfomycin is recommended. It is active in vitro against a large percentage of urinary isolates, and due to its unique mechanism of action, low incidence of resistance, and availability in oral form and single-dose administration, it can be a potential therapeutic alternative over many other antibiotics in the treatment of UTIs. However, more studies and clinical trials are needed before clinicians and infectious disease specialists can wholeheartedly and comfortably use this drug. At the same time, it should be remembered that inadvertent use of Fosfomycin may also lead to the development of resistance to this drug.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556