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Original Article

Antimicrobial susceptibility and resistance profiling of uropathogens to Fosfomycin and Ciprofloxacin in patients visiting tertiary care hospitals of Peshawar

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ABSTRACT

Background: Urinary tract infections (UTIs) are amongst the most common infections encountered globally and are usually treated empirically based on bacterial resistance to antibiotics for a given region.

Aim: To determine the antimicrobial susceptibility pattern of Fosfomycin and Ciprofloxacin against uropathogens in patients visiting tertiary care hospitals of Peshawar.

Settings: The study was carried out at Department of Pathology, Khyber Medical College/Khyber Teaching Hospital and Rehman Medical College, Peshawar.

Methodology:

This was a prospective cross-sectional study using consecutive sampling of patients with clinical symptoms of UTI. From February to June 2019, bacteria isolated from urine specimens of both inpatient and outpatients with pyuria were evaluated for susceptibility to Ciprofloxacin and Fosfomycin. Midstream urine samples were cultured on MacConkey and Cysteine Lactose Electrolyte Deficient (CLED) media. The isolated uropathogens underwent antimicrobial susceptibility testing as per CLSI guidelines.

Results: Among 820 uropathogens isolated, gram-negative bacilli were identified in 549 (66.9%) of the samples, while gram positive cocci were found only in 271 (33.0%) of the isolates. Invitro susceptibility testing of uropathogens against Fosfomycin and Ciprofloxacin revealed that 811 (98.9%) of the isolates were susceptible to Fosfomycin and 136 (16.6%) were susceptible to Ciprofloxacin. Resistance to Fosfomycin and Ciprofloxacin was observed in 9 (1.1%) and 684 (83.4%) of the isolates respectively.

Conclusion: The uropathogens in both medical facilities had higher susceptibilities to Fosfomycin as compared to Ciprofloxacin.

Keywords: Fosfomycin, Ciprofloxacin, Susceptibility, Uropathogens, UTIs

Introduction

Urinary tract infections are amongst the frequent infectious diseases encountered in the community as well as in the hospitals and represents an important public health concern in terms of medical and economic implications.[1] Management is usually empirical without urine culture or susceptibility testing because the contributing bacterial pathogens and their antimicrobial susceptibility profiles are considered predictable.

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However, recent studies indicated an increasing evidence of antimicrobial resistance against uropathogens including the production of Extended Spectrum Beta Lactamases (ESBLs), Carbapenemases and the acquisition of resistant genes on mobile genetic elements.[2] In developing countries like Pakistan, the situation is further complicated due inappropriate health care practices, inadequate dose and duration of antibiotics and free access to medications without prescription.[3] Globally, identification of uropathogens and their regional resistance profiling has been an area of constant research, upgrading the medical management of patients with UTIs.[4]

In general, *Escherichia coli* predominates among uropathogens accounting for approximately 80% of UTIs, while non-*E.coli* infection occurs more frequently in patients suspected of having complicated

UTIs.[5] A growing concern in the management of patients especially those having acute uncomplicated cystitis is the emergence of Multidrug Resistant (MDR) pathogens. MDR uropathogens confer resistance not only to commonly used beta-lactam antibiotics including all generations of Cephalosporins but also to other beta lactamase inhibitor combinations (Piperacillin/Tazobactam), Trimethoprim, and Cotrimoxazole. Emerging resistance among uropathogens has resulted in a shift of therapy to Fluoroquinolones, relatively short antibiotic courses, and early switch practices. Among available therapeutic options fluoroquinolones remain the only oral antibiotics of choice to treat UTIs, however one of the principles of antibiotic stewardship is to reduce fluoroquinolone use because of its selective properties for antibiotic resistance and associated side effects.[6] (6). Resistance to Quinolone and Fluoroquinolone antibiotics is multifactorial and can be via one or a combination of target-site gene mutations, increased production of multidrug-resistance (MDR) efflux pumps, modifying enzymes, and/or target-protection proteins.

Alternatively, Fosfomycin is an old broad-spectrum bactericidal antibiotic agent that inhibits bacterial cell wall synthesis and prevents bacterial adherence to uroepithelial cells. Previous reports indicated that Fosfomycin is effective against both gram-positive cocci (including methicillin resistant *Staphylococcus aureus* and vancomycin resistance *Enterococcus*) and gram-negative bacilli, (including *Pseudomonas aeruginosa*, ESBL producing bacteria, and carbapenem-resistant *Enterobacteriacea*.[7,8] The pharmacokinetic mechanism of fosfomycin emboldens its practicing use for UTIs, with an oral single dose of 3 g Fosfomycin tromethamine, mean peak urinary concentration occurs within 4 h, which is the concentrations adequate to inhibit most of the urinary pathogens for approximately 24 to 48 hours.[9] We aimed to evaluate the in vitro activity of Fosfomycin and Ciprofloxacin against urinary isolates isolated from patients in at two tertiary care hospitals of Peshawar.

Methodology

A prospective study was conducted from February 2019, to June 2019 in the Department of Pathology, of two tertiary care hospitals, Khyber Medical College/Khyber Teaching Hospital and Rehman Medical Institute, Peshawar. After approval from Institutional ethical review committee, a total of 1268 urine samples were collected from patients who had clinical features suggestive of UTI from both inpatients' and outpatients' departments. The symptoms included one or more of the following: dysuria, urgency, frequency and suprapubic pain. Freshly collected mid-stream clean catch urine samples were collected from the non-catheterized, conscious patients with indications for urine culture as evaluated by the clinicians from the different departments. Surgically collected urine specimens and suprapubic urinary bladder aspirates were collected from specific patients as recommended. If the patients were catheterized; then, urine samples were collected from the catheter with proper aseptic techniques with needle and syringe by standardized technical guidelines.

The urine samples were straight away processed within 30 min after collection. Uncentrifuged urine sample were observed on direct microscopy for pus cells and bacteria. Centrifuged urinary deposits were scanned under microscope for the crystals and casts. The urine samples were plated by semi-quantitative method on Blood agar, MacConkey agar, CLED Agar (bioMe´rieux, Marcy l'E´toile, France) and incubated at 37°C overnight and if required, till 48 hours. After incubation, the Colony Forming Units (CFUs), greater than 10^5/mL was considered as significant bacteriuria, according to Kass.[10] The isolated samples with significant bacteriuria in the backdrop of supportive UTI clinical manifestation and significant presence of pus cells on direct microscopy, as formulated in standard guidelines, were incorporated in the study.

Identification of the bacteria was performed by conventional biochemical tests, Analytical Profile Index (API), (bioMe´rieux, Marcy l'E´toile, France) (API 20E, API 20 NE, API-Staph). For antimicrobial susceptibility the isolates were platted on Mueller-Hinton agar by Kirby-Bauer disc diffusion method. The inhibitory zones diameters after 16-18 hours of incubation, around the antibiotic discs were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines M100-S25 version published in 2019. Due to the nonexistence of acknowledged breakpoints of Fosfomycin for bacteria other than *Enterococcus faecalis* and *Escherichia coli*.[11] the Fosfomycin breakpoints for *E. coli* proposed by the CLSI for the remaining evaluated gram-negative isolates were used, a practice that was also monitored by authors of parallel studies.[12] (Reference strains of *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603) were used as controls for the Gram-negative bacteria, for gram-positive bacteria *S. aureus* (ATCC 25923) was used and

were incorporated in all daily runs. The panel of antimicrobial agents that were tested for each organism are given in Table 1. The collected data was analyzed using SPSS version 20.

Table 1: Antibiotics panel used for UTI causing bacteria

Enterobacteriaceae	Non-Enterobacteriaceae	Enterococcus spp.	Staphylococcus aureus
Cefazolin	Ceftazidime	Ampicillin	Penicillin
Ceftriaxone	Cefepime	Ciprofloxacin	Cefoxitin
Cefotaxime	Tazobactam-piperacillin	Levofloxacin	Tetracycline
Augmentin	Ciprofloxacin	High-level Gentamicin	Ciprofloxacin
Ciprofloxacin	Levofloxacin	Nitrofurantoin	Levofloxacin
Levofloxacin	Amikacin	Vancomycin	Vancomycin
Cotrimoxazole	Gentamicin	Linezolid	Linezolid
Nitrofurantoin	Imipenem	Fosfomycin	Nitrofurantoin
Amikacin	Meropenem	Teicoplanin	Amikacin
Gentamicin	Colistin	-	Gentamicin
Meropenem	Fosfomycin	-	Cotrimoxazole
Imipenem	-	-	Fosfomycin
Colistin	-	-	-
Fosfomycin	-	-	-

Results

A total of 1268 urine samples were collected at Pathology Laboratory of two tertiary care hospitals of Peshawar. Based on inclusion criteria a total of 820 (64.7%) clinical urinary isolates were analyzed. Out of total 820 isolates 480 (58.5%) originated from adult outpatients clinics, 105 (12.8%) from patients hospitalized in medical wards, 91 (11.1%) from adult patients hospitalized in surgical wards, 78 (9.5%) from Pediatric units, 31(3.9%) from intensive care unit adult patients, and 35 (4.3%) from patients from other units. Majority of the infected patients were females 517 (67%) as compared to male patients with a frequency of 303 (37%).

The total 820 tested urinary bacterial isolates represented 549 (66.9%) gram-negative isolates and 271 (33.0%) gram-positive isolates (Figure 1). The 549 (66.9%) gram-negative isolates represented 487 (88.7%) members of the *Enterobacteriaceae*, 231(47.4%) of which were *Escherichia coli*, 90 (18.4%) *Klebsiella spp*., 66 (13.6%) *Enterobacter spp*., 31(6.4%) *Citrobacter spp*., and 69 (14.2%) *Proteus spp*., *Non- Enterobacteriaceae*, *Pseudomonas spp*. represented 62 (11.3%) of gram negative isolates. The 271 (33.0%) tested gram positive isolates consisted of 132 (48.7%) *Enterococcus faecalis* isolates, 44 (16.2%) *Staphylococcus aureus* isolates, 95 (35.0%) *Enterococcus faecium* isolates (Table 2).

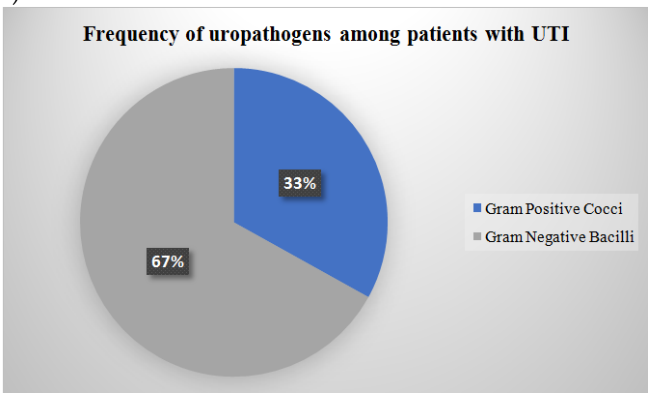
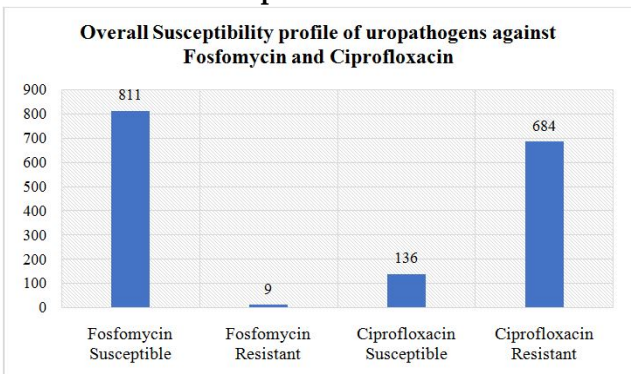


Figure-1: Represents the frequency of uropathogens isolated from patients with urinary tract infection

In total, 811 (98.9%) of the total 820 (64.7%) tested urinary isolates were found to be susceptible to Fosfomycin; of which 542 (66.1%), were gram negative, and 269 (32.8%), were gram positive. Ciprofloxacin susceptibility was observed only among 136 (16.6%) of the studied isolates while 684 (83.4%) of the uropathogens turned out to be resistant to ciprofloxacin (Figure 2).

Figure 2. Represents the overall susceptibility profile of isolated uropathogens against Fosfomycin and Ciprofloxacin



The pathogen specific susceptibility results to Fosfomycin and Ciprofloxacin from different uropathogens isolated are shown in Table 3.

Table 2. Represents uropathogens isolated from the cultured samples collected from patients with UTI at tertiary care hospitals of Peshawar.

Uropathogens isolated	Total No of Urine specimens (n=1268)	Frequency (%)
Total number of urine samples without uropathogens	448	(35.3%)
Total number of urine samples with uropathogens	820	(64.7%)
Escherichia coli	231	(28.2%)
Staphylococcus aureus	44	(5.4%)
Pseudomonas spp.	62	(7.6%)
Enterococcus faecalis	132	(16.1%)
Enterococcus faecium	95	(11.6%)
Proteus spp.	69	(8.4%)
Klebsiella spp.	90	(10.8%)
Enterobacter spp.	66	(8.0%)
Citrobacter spp.	31	(3.8%)

Table 3. Susceptibility profiles of nine common uropathogens to Fosfomycin and Ciprofloxacin

Isolated Uropathogens	**Samples n=820 (64.7%)**	**Fosfomycin**		**Ciprofloxacin**	
		Susceptible n= (%)	**Resistant n= (%)**	**Susceptible n = (%)**	**Resistant n= (%)**
Escherichia coli	231	231 (100%)	0 (0%)	42 (18.1%)	189 (81.8%)
Proteus spp.	69	69 (100%)	0 (0%)	36 (52.2%)	33 (48.4%)
Enterobacter spp.	66	64 (98%)	2 (20%)	2 (3.0%)	64 (96.9%)
Citrobacter spp.	31	31 (100%)	0 (0%)	13 (41.9%)	18 (58.0%)
Klebsiella spp.	90	87 (97%)	3 (3%)	0 (0%)	90 (100%)
Staphylococcus aureus	44	44 (100%)	0 (0%)	27 (61.4%)	17 (38.6%)
Enterococcus faecalis	132	130 (98%)	2 (2%)	12 (9.1%)	120 (90.9%)
Enterococcus faecium	95	95 (100%)	0 (100%)	3 (3.2%)	92 (96.8%)
Pseudomonas spp.	62	60 (98%)	2 (2%)	1 (1.6)	61 (98.4%)
Total	820	811 (98.9%)	9 (1.1%)	136 (16.6%)	684 (83.4%)

Discussion

Widespread antibiotic resistance among bacterial pathogens that causes UTIs is considered a major public health threat that impacts therapeutic strategies in health care settings. One of the most important scientific advancements of 20th century in controlling bacterial infections was the discovery of antibiotics. Unfortunately, antibiotics are now becoming one of the biggest medical issues of the 21th century as bacteria evolve resistances even to more potent antimicrobial agents jeopardizing the successful outcomes of critically ill patients (9). The situation is worrisome in developing countries like Pakistan. Due to lack of standard treatment guidelines antibiotics are often overprescribed by health care workers and overused by the public(10). Free availability of antibiotics and lack of practice of bacterial culture and sensitivity profiling prior to commencing therapy, uropathogens like *Escherichia. Coli., K. Pneumoniae, Pseudomonas spp., proteus spp., Enterobacter and Citrobacter* spp. have developed complex and creative strategies to circumvent antibiotic attack (11).

In the present study frequency of UTIs was high among outpatients as compared to inpatients indicating a high prevalence of UTIs in the community settings. More females were infected as compared to male patients which could be explained by the anatomical structure and position of the urethra in close proximity to the anus in female patients as described earlier (12, 13). Globally, MDR Gram-negative bacteria especially *E. coli and K. pneumoniae* have been implicated as major pathogens in hospital as well as in the community acquired infections. Majority of the uropathogens identified in the present study were gram-negative isolates (66.9%) representing88.7% members of the *Enterobacteriaceae* which correlates with other studies in Pakistan and other countries (14-16). Among family *Enterobacteriaceae*, *E. coli* (47.4%,) and *Klebsiella spp.*, (18.4%) were major pathogens encountered followed by *Enterobacter spp.*, *Citrobacter spp.*, and *Proteus spp.*, in

13.6%, 6.4%and 4.2% of the isolates respectively. These findings are in line with a recently published data reporting *E.coli* (80.9%) as the most prevalent pathogen followed by *Klebsiella* (4.5%) and other species identified in the current study (17). Presence of *E. coli* (47.7%) and other enterobacteria in urine isolates might be the result of colonization of the periurethral area by members of the colonic flora. *Non-Enterobacteriaceae*, *Pseudomonas spp.* represented 11.3% of gram-negative isolates as reported by previous studies as well (18). Members of the genus Pseudomonas are not only intrinsically resistant to multiple antibiotics but also capable of acquiring new resistance mechanisms making management of infected patients more complicated especially in hospital settings (19). Gram positive cocci were present in 33% of the studied isolates with *Enterococcus Faecium, Enterococcus faecalis and Staphylococcus aureus* present in variable frequencies. Gram-positive cocci have been recognized as potential uropathogens in various studies. Recently, *Kim et al.* ,reported a higher frequency (58.9%) of gram positive bacteria among patients infected with uropathogens(20). It should be noted that the frequencies and sequence of bacterial isolates causing UTIs after *E. coli* differs not only from region to region but is also dependent on other factors including age and gender of the patients.

Worldwide, identification of locally endemic uropathogens and their susceptibility profiling has been an area of constant research, contributing towards management of patients with UTIs. In 2011, The Infectious Diseases Society of America suggested the use of Cotrimoxazole, Nitrofurantoin and Fosfomycin in case the infecting uropathogens are susceptible to these drugs or reported local resistance is less than 20%, while Fluoroquinolones were recommended as second line alternative drugs (21). Recent reports indicated that Fosfomycin, a natural antimicrobial agent has been the drug of choice for acute uncomplicated cystitis caused by gram-negative bacteria, especially *Enterobacteriaceae* as well as gram-positive bacteria that are resistant to traditionally used antibiotics (22).

In the present study we attempted to determine the invitro activity of Fosfomycin and Ciprofloxacin against uropathogens. Findings of the current study revealed that Fosfomycin has best activity (98.9% susceptible) against most of the uropathogens isolated with only limited resistance (1.1%) among studied isolates. In contrast, a high level of resistance (83.4%) was observed among both gram- negative bacilli and gram-positive cocci with inhibition of only 16.6% of the isolates. Ciprofloxacin has been one of the most widely used fluoroquinolones and has a well-known safety profile, as well as excellent activity against most of the common uropathogens. One of the commonly prescribed antibiotics (accounting for 49% of antibiotic prescriptions) for uncomplicated UTIs in last few decades was Ciprofloxacin, a Fluroquinolone with excellent activity against uropathogens (23). Worldwide, there is now an increasing trend of antibiotic resistance among evolving bacterial strains to Ciprofloxacin with a shift to other treatment regimens (24). Importantly, in developing countries like Pakistan lack of standard medial practices, self-medication, inadequate dosage and duration of antibiotics are main factors involved in the spread of antibiotic resistance phenomenon. Against uroathogenic *E. coli,* Ciprofloxacin and Cotrimoxazole resistance was observed in 74.5% and 48.5% of the isolates (25). Similarly, in other species like *Klebsiella* the proportion of fluoroquinolones resistance has steadily increased, with nearly fivefold increase from 11% in 2005 to 50% in 2012 in Italy (26). Consistent with their results *E. coli and Klebsiella spp.,* turned out to be resistant to Ciprofloxacin in 81.8% and 100% of the isolates respectively. Other uropathogens also revealed decreased susceptibility to Ciprofloxacin necessitating the use of alternate agents with demonstrable high activity like Fosfomycin.

Fosfomycin, a novel antibiotic with broad spectrum of activity against the common pathogens causing UTI, has drawn attention of clinicians due to advantage of being administered in a single dose and its low resistance profile (27). Fosfomycin susceptibility rates observed in the present study were comparable to colistin, imipenem, and meropenem for most of the gram-negative urinary isolates.

Notably in our study, all the *E. coli* tested isolates were susceptible to Fosfomycin. A study in Thailand, on uropathogenic *E. coli and Klebsiella,* the susceptibility to Fosfomycin was noted in 98.7% of isolates (28). Similarly, *Ko et al* investigated the Fosfomycin susceptibility in 97 ciprofloxacin resistant *E. coli* urinary isolates and detected Fosfomycin resistance in only one strain. Findings of current study supports the evidence that Fosfomycin could be a hope for patients suffering from various drug resistant uropathogens with high invitro efficacy against uropathogens. Local data on identification and antimicrobial resistance patterns of resistant pathogens will assist in appropriate choice of antibiotics and reduce therapeutic costs. It will also help in assessing the local trends of resistance and provide essential

epidemiological information about circulating bacterial strains with development of effective molecular diagnostic methods and novel drugs against infection and most importantly implementation of effective infection control policies.

Conclusion

Fosfomycin has high *in vitro* activity against common uropathogens. It bears a high potential to surface as a safe substitute and favorable oral agent for both inpatient and outpatient treatment of UTIs. Further studies are required to assess the underlying molecular mechanisms of Fosfomycin resistance among common uropathogens encountered are needed.

Limitations

One of the limitations of this study was a non-probability consecutive sampling technique which might have resulted in selection bias.

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- Amina Gul participated in conception, study design planning, study conduction, analysis, interpretation and discussion
- Maria Khan participated in Amina Gul participated in conception, study design planning, study conduction