Fluoroquinolone Resistance in the Clinical Isolates of Kashmir Valley and In-Vitro Efficacy of Fosfomycin in Multiple Infections

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ABSTRACT

BACKGROUND: Antibacterial resistance is a major concern in healthcare associated infections. Longer hospital stay, empirical treatment without antimicrobial stewardship policy and ineffective infection control practices are making it difficult to treat infections. Kashmir valley lacks surveillance data and awareness about antibiotic resistance among clinicians, thus complicating patient care. The aim of this study was to investigate the prevalence of antibacterial resistance among various clinical isolates.

METHODS: The study was conducted between February 2013 and April 2014 in the Srinagar city. Samples of urine, pus, and other body fluids (N=500) were examined for clinically important pathogens. Standard laboratory protocols were followed for the isolation, identification and susceptibility tests. Recent CLSI guidelines for antimicrobial susceptibility were followed using Kirby-Bauer method.

RESULTS: Predominant pathogens identified in urine cultures were *Escherichia coli* (42.1%), *Klebsiella pneumoniae* (31.57%), *Proteus species* (7.8%), *Pseudomonas aeruginosa* (13.1%) and *Enterococcus faecalis* (5.26%). *Methicillin resistant Staphylocoocus aureus* (14.8%) and *Methicillin sensitive Staphylococcus aureus* (22.2%) were common isolates found in pus. Other frequent clinical isolates found in pus cultures were *Proteus* species (28.1%), *Enterococcus faecalis* (18.5%) and *Pseudomonas aeruginosa* (14.8%). *Klebsiella pneumoniae* and *Staphylococcus aureus* were prevalent isolates in semen cultures. All clinical isolates screened in this study were resistant to quinolones, aminoglycosides, cephalosporins, carbepenems and sulphonamides. Glycopeptide resistance was observed in one of the urinary isolates. All clinical isolates were sensitive to fosfomycin.

CONCLUSION: We found high quinolone resistance in pathogens isolated from community acquired urinary tract infection (UTI) and nosocomial infections. Fosfomycin can be a good alternative choice in the treatment of urinary tract infection with greater efficacy & lesser antibiotic resistance. The drug has not been so far prescribed in routine clinical practice in the valley.

Keywords: Antimicrobial Resistance; Extended Spectrum Betalactamsaes; Multidrug Resistance; Fluroquinolones; Surgical Site Infections; Urinary Tract Infection

INTRODUCTION

Antimicrobial resistance (AMR) is a worldwide concern among public health authorities [1]. Data on AMR in Kashmir is limited. Broad spectrum antibiotics are indiscriminately used in hospitals and other health care settings in Kashmir with a potential for the emergence of superbugs. An understanding of AMR in Kashmir is an urgent need to develop cost-effective strategies. Therefore, our aim was to determine the resistance patterns of the most common bacterial species isolated from various clinical sources received in our laboratory.

METHODS

The study was conducted between February 2013 and April 2014 at the Transworld Muslim University diagnostic center in Barbarshah, Srinagar, India. Five hundred clinical specimen of urine, pus, blood and other body fluids (such as semen) from the males attending the fertility clinics were processed for the isolation of pathogens.

Sample collection and Bacterial identification:

1. Blood culture and sepsis: Appropriate volume of blood sample (20ml) was collected using aseptic precautions for blood culture. Samples were inoculated in brain heart infusion broth (BHI) using blood culture bottles (Himedia Laboratories Pvt. Ltd. Mumbai, India). Cultures were incubated under aerobic conditions with and without CO_2 at $37c^0$. Culture bottles were then examined for growth after 24 hours of incubation and a subculture was performed on 5% sheep blood agar, chocolate agar and MacConkey agar. Plates were then examined for bacterial growth.

2. Urine Culture and Sensitivity: Midstream urine was obtained from patients in sterile urine container vials following the recommendations of Kass [2]. The urine samples were subjected to routine urine analysis by recording the turbidity, pH, color, volume and microscopy following the wet mount to detect pyuria. Samples were inoculated on a chromogenic media (Hichrome UTI Agar, M1353 Himedia Laboratories Pvt. Ltd. Mumbai, India) using a sterile inoculation loop. Plates were incubated at $37c^0$ for 24 hours. Growth was examined after 24 hours of incubation. Presence of more than 10^5 CFU/ml bacteria was taken as significant for urinary tract infection (UTI). Bacterial colonies were then identified on the basis of the color on the chromogenic medium and using Hi-IMVIC (KB001) biochemical identification kit for Enterobacteriaceae and non-lactose fermenters HiStaph Identification Kit (KB004) (Himedia Laboratories Pvt. Ltd. Mumbai, India) for Gram positive bacteria.

3. Semen culture and sensitivity: Semen samples from male patients attending the fertility clinics were obtained in sterile container vials. Patients were instructed to collect samples as per the WHO recommendations for semen collection [3]. For culture, semen samples were allowed to liquefy for 30 min after ejaculation; 0.1 ml of each specimen was aseptically inoculated in a test tube containing 20 ml of brain heart infusion broth, 0.1 ml of the specimen was directly inoculated on solid media plates of sheep blood agar, chocolate agar, MacConkey and Hichrome UTI agar. Chocolate agar plates were incubated anaerobically using an anaerobic jar for the isolation of *Neisseria* species. A loopfull of the culture was then sub cultured from BHI broth on solid media and incubated at 37^0C aerobically for 24 hours. Bacterial colonies were identified by Hi-IMVIC biochemical test kits for *Enterobactericae* (Himedia Laboratories Pvt. Ltd. Mumbai, India).

5. Laboratory detection of Methicillin-resistant Staphylococcus aureus (MRSA): Staphyloccus aureus was identified using standard methods based on colony morphology, Gram's stain, catalase test, mannitol fermentation and coagulase test. A total of 20 isolates were confirmed as *Staphyloccus aureus.* Cefoxitin disc diffusion test was performed for the detection of *Methicillin resistant Staphyloccus aureus (MRSA)* using a 30µg disc. An inhibition zone diameter of ≤19 mm was reported as Cefoxitin resistant and ≥20 mm was considered as Cefoxitin sensitive.

Antibiotic susceptibility tests: Antibiotic sensitivity testing was performed using Kirby Baeur disc diffusion method on Muller Hinton Agar (Himedia Laboratories Pvt. Ltd. Mumbai, India) and as per the Clinical Laboratory Standards Institute. Phenotypic detection of ESBL producing bacteria was performed using ESBL identification kit I SD238 containing Cefotaxime 30 mcg/disc [4].

Statistical analysis: Data was described in percentages.

RESULTS

All the blood cultures (N=25, 5%) were negative for microbial growth. Of the 190 urine samples, *Escherichia coli* was the most predominant organism with 80 isolates (42.1%), followed by 60 isolates of *Klebsiella pneumoniae* (31.6%), 15 isolates of *Proteus species* (7.9%), 25 isolates of *Pseudomonas aeruginosa* (13.2%) and 10 isolates of *Enterococcus faecalis* (5.3%). *E.coli* showed maximum resistance to various antimicrobials (Table I).

Maximum antibiotic resistance was observed in 25 isolates of *E.coli* (31.25%) when tested against nalidixic acid and fifteen isolates against ciprofloxacin. Seven isolates (8.7%) were resistant to gentamicin, 5 isolates (6.25%) were

Table 1: Antimicrobial profiles of Urinary isolates

Antimicrobial Agents Total n(190)	*E.coli* n(8) (42.1%)	*Klebsiella species* n(60) (31.6%)	*Proteus species* n(15) (7.9%)	*Pseudomonas aeruginosa* n(25) (13.2%)	*Enterococcus faecalis* n(10) (5.3%)
Ampilcillin/sulbactam	1 (1.25%)	5 (8.3%)	1(6.6%)	NR	NR
Ceftraixone	5 (6.25%)	7 (11.6%)	2 (13.33%)	3 (12%)	NR
Ceftazidime	2 (2.5%)	2 (3.3%)	0 -	2 (8%)	NR
Cefotaxime	2 (2.5%)	6 (10%)	1 (6.6%)	5 (20%)	NR
Ciprofloxacin	15(18.75%)	12 (20%)	2 (13.33%)	5 (20%)	5 (50%)
Nalidixic acid	25(31.25%)	8 (13.3%)	2 (13.3%)	0	NR
Gentamicin	7 (8.75%)	3 (5%)	2 (13.3%)	3 (12%)	0
Amikacin	5 (6.25%)	5 (8.33%)	1 (6.6%)	2 (8%)	0
Tobramycin	3 (3.75%)	2 (3.3%)	1 (6.6%)	1 (4%)	NR
Imipenem	1 (1.25%)	0	0 -	1(4%)	NR
Meropenem	4 (5%)	0	1 (6.6%)	1(4%)	NR
Polymixin B	0	0	1(6.6%)	NR	NR
Co.trimoxazole	8 (10%)	9 (15%)	1(6.6%)	2	0
Doxycycline	1 (1.25%)	1 (1.66%)	0 -	1	0
Nitrofurantoin	1 (1.25%)	0	0 -	-	0
Ticarcillin	0	0	NR	2 (8%)	0
Erythromycin					4 (40%)
Clarithromycin					0
Rifampcin					0
Linezolid					0
Teicoplanin					0
Vancomycin					1 (10%)

NR: Not recommended as per CLSI

were resistant to amikacin. Low level of resistance was found in one isolate of *E.coli* (1.25%) to Imipenem. Only 5 semen samples were received in our laboratory. *Klebsiella pneumoniae* was isolated from two patients and *Staphylococcus aureus* was isolated from one patient. Among 25 isolates of *Pseudomonas aeruginosa*, two isolates (13.3%) were resistant to gentamicin, amikacin and nalidixic acid, and one isolate (6.6%) was resistant to polymyxin B. Five isolates of *Enterococcus faecalis* (50%) were resistant to ciprofloxacin.

Of the 135 pus samples, 30 (22%) were methicillin-sensitive and 20 (15%) were methicillin-resistant *Staphylococcus aureus (MRSA)*. Eight *MRSA* isolates were resistant to ciprofloxacin (40%), seven isolates were resistant to co-trimoxazole (35%) and three (15%) isolates were resistant to erythromycin, and all 20 isolates were resistant to penicillin. The percentage of resistance to ampicillin-sulbactam in two *MSSA* isolates was 6.6%. Five (16.6%) of *MSSA* isolates were predominantly resistant to ciprofloxacin. All *Klebsiella pneumoniae* strains were completely resistant to ceftriaxone, ceftazidime, ciprofloxacin and levofloxacin (100%) including meropenem & imipenem. Six *Proteus* isolates were predominantly resistant to ceftriaxone (15.7%), 5 isolates to ciprofloxacin (13.1%) and 6 isolates to co-trimoxazole (15.7%). Two isolates of *Pseudomonas aeruginosa* out of 20 (10%) were resistant to ceftazidime and aztreonam (10%), 5 isolates were resistant to gentamicin (25%), two isolates (10%) were resistant to amikacin and piperacillin-tazobactam, one isolate (5%) was resistant to imipenem and three isolates (15%) were resistant to ciprofloxacin. High level of resistance to gentamicin (32%) was observed in 8 isolates of *Enterococcus faecalis*, these strains were also resistant to levofloxacin (24%), ciprofloxacin (16%) and tobramycin (8%) (Table 2).

The in-vitro activity of fosfomycin in urinary isolates *E. coli* was present in 77 (96.3%). Out of 60 *Klebsiella pneumoniae* isolates, 55 (91.7%) were susceptible to fosfomycin. The susceptibility of fosfomycin against *Pseudomonas aeruginosa* was observed in 21 (84%) isolates. The highest susceptibility of fosfomycin was observed in *Enterococcus faecalis*; all the 10 isolates were susceptible to fosfomycin.

Table 2: Antimicrobial profiles of pus isolates

Antimcrobial Agents Total n(130)	*MRSA* n(20) (14.8%)	*MSSA* n(30) (22.2%)	*Klebsiella species* n(2) (1.48%)	*Proteus species* n(38) (28.1%)	*Pseudomonas aeruginosa* n(20) (18.5%)	*Enterococcus faecalis* n(25) (14.8%)
Ampicillin/ Sulbactam	NR	2(6.6%)	2(100%)	5(13.1%)	NR	NR
Aztreonam	-	-	-	0	2(10%)	-
Ceftriaxone	NR	-	2(100%)	6(15.7%)	NR	-
Ceftazidime	NR	-	2(100%)	1(2.6%)	2(10%)	-
Cefotaxime	NR	-	1(50%)	5(13.1%)	-	-
Ciprofloxacin	8(40%)	5(16.6%)	2(100%)	5(13.1%)	3(15%)	4(16%)
Levofloxacin	NR	-	2(100%)	1(2.6%)	NR	6(24%)
Gentamicin	0	-	1(50%)	2(5.2%)	5(25%)	8(32%)
Amikacin	0	-	0	3(7.8%)	2(10%)	4(16%)
Tobramycin	0	-	0	2(5.2%)	-	2(8%)
Imipenem	NR	-	2(100%)	0	1(5%)	
Meropenem	NR		2(100%)	2(5.2%)	2(10%)	
Polymyxin B	NR		0	0	0	
Co-trimoxazole	7(35%)	8(26.6%)	2(100%)	6(15.7%)	0	
Doxycycline	0		0	0	0	
Nitrofurantoin	NR		0	0	0	
Netillin	-	-	0	0	1(5%)	
Ticarcillin	0				-	
Piperacillin/ Tazobactam					2(10%)	
Erythromycin	3(15%)	5(16.6%)				
Clarithromycin						
Rifampicin						
Penicillin	20(100%)	10(33.3%)				
Linezolid	0					
Teicoplanin	0					
Vancomycin	0					1(4%)

NR: Not recommended as per CLISA

We found good in-vitro efficacy of fosfomycin in pus culture isolates. Thirty-eight *MRSA* isolates were sensitive to fosfomycin out of the 52 isolates (73.1%). Twenty-two isolates of *Proteus* species were sensitive to fosfomycin (57.9%). Seventeen isolates of *Enterococcus faecalis* (68%) tested with fosfomycin were found sensitive while 14 isolates (70%) of *Pseudomonas aeruginosa* were also sensitive to fosfomycin.

The prevalence of extended spectrum β-lactamases (ESBL) positive *E. coli* in urinary isolates was 57 (71.3%) out of 80 isolates. Among the *Klebsiella pneumoniae* isolates 33 (55%) were ESBL positive. The percentage of *Proteus* isolates positive to extended spectrum β-lactamases (ESBLs) was 66%; out of fifteen strains, 10 were positive for ESBLs. Seventeen isolates (68%) of *Pseudomonas aeruginosa* were positive for ESBLs. Among the surgical site infection isolates, two strains of *Klebsiella pneumoniae* (100%) were ESBL positive. Twenty-two *Proteus* isolates (57.9%) were also ESBL positive. Out of twenty isolates of *Pseudomonas aeruginosa* strains, 12 (60%) were found to be ESBL positive.

DISCUSSION

We found extensive fluoroquinolone resistance among urinary and pus collections. We also found significant presence of methicillin resistance among *Staphylococcus aureus* isolates and presence of ESBL among gram negative isolates. In addition, we also found *Escherichia coli and Klebsiella pneumoniae* isolates to be

resistant to all quinolones. Fluoroquinolone resistance in urinary pathogens has increased globally [5]. Urinary catheterization, hospitalization, and previous history of UTI have largely contributed to fluoroquinolone resistance [6]. Among the bacteria isolated from semen specimens, *Klebsiella pneumoniae* was the predominant pathogen followed by *Staphylococcus aureus*. Semen culture assists in the diagnosis of bacterial prostatitis and infection related to other male reproductive glands. Any infection in semen can compromise the sperm quality; semen culture can thus prove advantageous in identifying early infection before In-Vitro fertilization and intra uterine insemination procedures [7].

Our study shows increased quinolone resistance in all the pus isolates. In addition, among surgical site infections (SSI) the prevalence of *Methicillin-resistant Staphylococcus aureus (MRSA)* was 14.8%. *MRSA* has gained considerable significance as a hospital pathogen across Kashmir valley in recent times [8]. The high prevalence of *MRSA* has led to the increased use of vancomycin which may prove dangerous in future years [9]. Its indiscriminate use without in-vitro susceptibility in *MRSA* infections can lead to the emergence of vancomycin resistance [10]. Two isolates of *Klebsiella pneumoniae* isolated from SSI were resistant to ampicillin/sulbactam, ceftriaxone, ceftazidime, ciprofloxacin, levofloxacin, imipenem and meropenem. Complete multidrug resistance in two isolates of *Klebsiella species* in our results suggest that Antimicrobial resistance (AMR) can be a serious problem.

The problem of AMR was further complicated by extended spectrum β-lactamase (ESBL) producing isolates. The highest ESBL producing organisms in our study were *E. coli*, *Pseudomonas aeruginosa Proteus* species and *Klebsiella pneumoniae* from patients with UTI. ESBL producing bacteria are a significant cause of morbidity and mortality in patients with UTI and SSI [11, 12].

Fosfomycin is recommended as the first line of drug in uncomplicated UTI [13]. We found that most of the *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and all *Enterococcus faecalis isolates* were sensitive to fosfomycin. UTI is a common infection in Kashmir. As per our knowledge, fluoroquinolone resistance has never been reported from this region [14]. We found limited alternatives for fluoroquinolone resistant uropathogens. Therefore, close monitoring of AMR patterns may prove benefic-ial in directing therapy for the effective treatment of UTI. Fluoroquinolone resistance among gram negative bacteria has led to an increased dependency on carbapenem. Carbapenems are the only choice of treatment for serious infections due to ESBLs. The recent emergence of *Klebsiella pneumoniae carbapenemases (KPCs)* have created a difficult situation in treating this life threatening infection [15-17]. Polymyxins could be a choice of treatment for treating serious infections due to ESBLs and KPCs [18]. Ertapenem can be administered in the empirical treatment of community acquired infections due to ESBLs [19,20].

Insufficient attention has been given to emerging multidrug-resistant (MDR) gram-negative organisms in Kashmir valley. For instance, data collected in our study shows the increased prevalence of MDR *Klebsiella*, *Proteus* and *Pseudomonas* species .The most common resistance pattern was co-resistance to quinolones, third-generation cephalosporins and aminoglycosides. These organisms can cause multiple infections, including pneumonia, bacteremia, meningitis, urinary tract infection, skin and soft-tissue infections [21]. Present guidelines including contact precautions for colonized or infected patients, alert codes, active surveillance, chlorohexidine baths and wipes should be implemented in order to minimize the infections caused by MDR gram-negatives [22-24].

Our study has several potential limitations. Our study was not inclusive of all centers in Kashmir, the study sample was collected based on convenience and not randomly, and patients may not be true reflection of type of patients in general population, all factors that limit the generalizability of our study. Further, the study gives data at one point in time and changing trends in infection and antibiotic resistance were not captured.

We found high antimicrobial resistance in pathogens causing most common infections. Further studies are needed to understand the factors responsible for the prevalence of AMR and to evaluate interventions that can help to control the spread of AMR in Kashmir.

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