Journal of Infection and Molecular Biology. 1 (4):xxxxx In Press
http://www.nexusacademicpublishers.com/journal/2

Research Article

Multiple Drug Resistance Pattern in Urinary Tract Infection Patients in Peshawar; Khyber Pukhtunkhwa (KPK), Pakistan

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ARTICLE HISTORY
Received: 2013–07–16
Revised: 2013–11–20
Accepted: 2013–11–27

Key Words: Urinary tract infection, Multi drug resistant, *E. coli*, CLED

ABSTRACT

Urinary tract infection (UTI) is the most common problem in hospitalized and outdoor patients. It is mainly found in females because of the shortness of the urethra and its closeness to anus which facilitate entrance of fecal micro–flora to urinary tract. Aim of the study was to investigate the bacterial uropathogens and their antibiotic susceptibility in a tertiary care hospital, Peshawar, Pakistan. A total of 200 urine samples were analyzed and cultured on Cysteine Lactose Electrolyte Deficient (CLED) medium. All the bacterial isolates were identified by biochemical tests. Out of 200 samples, 113 were positive and 87 were negative. In positive samples, 36(31.9%) were male and 77(68.1%) were female. Out of 113 patients 80(70.8%) were hospitalized and 33(29.2%) were walk in patients. Out of those 113 positive samples, *E. coli* was the dominant uropathogen 77(68.1%) followed by *Staphylococcus aureus* 13 (11.5%), Proteus spp. 9(8.0%), Pseudomonas spp. 6(5.3%), Klebsiella spp. 4(3.5%), and methicillin resistant *Staphylococcus aureus* 4(3.5%). Antibiotic susceptibility test was performed by disc diffusion method according to Clinical Laboratories Standard Institute (CLSI). Bacterial isolates showed resistance to ampicillin (72.0%), ciprofloxacin (53.1%), norfloxacin (51.3%) and trimethoprim–sulfamethaxozole (53.1%). Bacterial spp. resistant to other antibiotics was also prevalent. Meropenem was the most effective antibiotic against all the bacterial isolates. In conclusion, high incidence of single and multiple antibiotic resistant bacterial strains is matter of enormous concern. Meropenem was the drug of choice to control urinary tract infections..

ARTICLE CITATION: Shahzad KA, Ullah F, Muhammad K, Khatoon F, Qazi MH and Ahmed I (2013). Multiple drug resistance pattern in urinary tract infection patients in Peshawar, Khyber Pukhtunkhwa (KPK), Pakistan. *J. Inf. Mol. Biol.* 1 (4):xxxxx In Press

INTRODUCTION

Urinary tract infections (UTIs) are amongst the most common infections described in outpatients setting. Urinary tract infections are most common health problem for both sexes (males and females). However females are more prone to urinary tract infections because of the shortness of the urethra and its closeness to anus which facilitate entrance of fecal flora to urinary tract (Stamm and Norrby, 2001; Ribeiro *et al*., 2002). The main symptoms of UTI include urgency, increased frequency, pain on urination and a foul odor of urine. Normal urine is sterile, but it may become contaminated with microbiota of the skin near the end of its passage through the urethra. Urinary system infections are most frequently initiated by an inflammation of the urethra, or urethritis. Infection of the urinary bladder is called *cystitis*, and infection of the ureters is urethritis. Urinary tract infections (UTI) are among the most frequent bacterial infections encountered both in the outpatient units and in nosocomial infections. Urinary infections are frequently caused by Enterobacteriaceae (Gales et *al*., 2000; Akram *et al*., 2007). Among Enterobacters, *E. coli* accounts for 75 to 90 percent urinary tract infections (Stamm and Norrby, 2001). *E. coli* remain the predominant uropathogen followed by *S. saprophyticus*, *K. pneumonia*, Enterobacter, Proteus Spp. and Enterococcus Spp. (Garofalo *et al*., 2007; Raz *et al*., 2005; Lavanya and Jogalakshmi, 2002; Honkinen *et al*., 1999; Mohanty *et al*., 2003; Taneja *et al*., 2010). Urinary tract infections (UTIs) represent one of the most common diseases encountered in medical practice today (Rashedmarandi *et al*., 2008). Worldwide 150 million per annum people suffer from urinary tract infections (Stamm and Norrby, 2001). Different causing species of urinary tract infections are showing resistant not only to conventional antibiotics but also to new more potent antibiotics (Taneja *et al*., 2010). Uropathogens have shown a changed pattern of susceptibility to antibiotics results in increase resistance to commonly used antibiotics over the last decade (Magalit *et al*., 2004).

In many parts of Khyber Pukhtunkhwa (Kpk), because of usage of uropathogens contaminated spring water and lake of facilities for urine culture and antimicrobial susceptibility testing, leading to improper diagnosis and irrational antibiotic treatment (e.g. self–medication) of UTI (Shafiq *et al*., 2013). The present study is therefore designed to isolate and identify bacterial causes of UTIs and to determine their susceptibility to antibiotics.

ISSN: 2307–5465 (Online); ISSN: 2307–5716 (Print)

MATERIALS AND METHODS

A total of two hundred suspected urine samples were collected from the walk in and hospitalized patients in a tertiary hospital at Peshawar, Pakistan. The samples were processed for urinalysis and culturing after labeling each sample. If delay was to be suspected then boric acid was used (0.1g/10ml of urine) for preservation to prevent multiplication of bacteria. Uncentrifuged urine was taken in a dropper and put a drop on clean slide for microscopy. Urine samples with more than normal WBCs were considered as susceptible for UTIs.

Aseptically collected samples were placed on suitable culture medium like Cysteine lactose electrolyte deficient (CLED) agar (Oxoid, UK), Blood agar (Oxoid, UK) and MacConkey's agar (Oxoid, UK). Each plate was examined for colony count and etiological agent after 18–24 hours of incubation at 37°C, which are the main criteria for defining true urinary tract infection by bacterial culture. Each urine sample culture yielding 10^5 organisms per milliliter of urine was considered significant bacterial growth for the diagnosis of true urinary tract infection. Isolates from positive samples were identified based upon standard laboratory procedures including, morphological characteristics, Gram's stain, rapid tests (catalase, oxidase, coagulase, bile solubility), and biochemical tests indole, methyl red, Voges–Proskauer and citrate (IMViC), triple sugar iron (TSI), oxidation/fermentation (O/F), urease and nitrate reduction (Harley and Prescott, 2002).

Antibiotic susceptibility test were performed by Clinical and Laboratory Standards Institute (CLSI)–recommended modified Kirby–Bauer disc diffusion method on Mueller–Hinton agar with commercial antibiotic discs (Oxoid Ltd, UK) (CLSI, 2006). Isolates showing an intermediate level of susceptibility were classified as resistant. Antibiotic concentrations used to determine antibiotic susceptibility for bacterial pathogens were: ampicllin (25µg), ciprofloxacin (5µg), nitrofurantoin (300µg), norfloxacin (10µg), Chloramphenicol (30µg), meropenem (10µg), tygacil (15µg), cefazolin (30µg), vancomycin (30µg), methicillin (10µg), Tazocin (30µg), trimethoprim / sulfamethaxozole (1.25µg) and fusidic acid (10µg). As reference strains *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as controls for Gram positive and Gram negative bacteria, respectively.

In our study, 200 suspected urine samples were screened for UTIs and results about the prevailing pathogens from outdoor and admitted patients are presented in the Table 1. Out of these, 113 were positive for bacterial infection (33 from walk in patients and 80 from hospitalized patients). *E. coli*, the predominant pathogen, was more in admitted patients 55 (71.4%) as compared to walk in patients 22 (28.6%). *Staphylococcus aurous* in admitted patients were 8(61.5%) and outpatient were 5(38.5%) while 100% methicillin resistant strains of *Staphylococcus aurous* were recovered from admitted patients. *Pseudomonas aeruginosa* was 5(83.3 %) in admitted patients and 1(11.11%) in outpatients. *Proteus mirabilis* and Klebsiella spp. were 5(55.6%) and 3(75%) in admitted patients and were 4(44.4%) and 1(25%) in walk in patients, respectively.

Distribution of UTI patients with respect to medico demographic characteristic (age 1–15, 16–30, 31–45, 46–60, ›61years) is presented in Table 2. Among total 113 patients, ten (8 male and 2 female) were in the range of 1–15 years of age, thirty (6 male and 24 female) in range of 16–30 years of age, 26 (8 male and 18 female) in range of 31–45 years of age, 3(3 female) were in range of 46–60 years of age and one female above 60 years of age was found positive. It showed that women were more prone to urinary tract infection in these ages.

Table 1: Distribution of UTI Pathogens in Outpatient and Admitted Patients

Sr. No	Isolated Organism	Outpatients	Inpatients
1	*Escherichia Coli*	22 (28.57 %)	55(71.42%)
2	*Staphylococcus aureus*	5(38.46%)	8(61.53%)
3	*Pseudomonas aeruginosa*	1(11.11%)	5 (83.33 %)
4	Proteus species	4 (44.44%)	5(55.55%)
5	Klebsiella species	1(25%)	3(75%)
6	MRSA	0(0%)	4(100%)

Table 2: Distribution of *E. coli* with Age and Sex

Age	No of Patients	Male	Percentage	Female	Percentage
1–15	8	6	75	2	25
16–30	33	6	18.18	27	81.81
31–45	26	8	30.76	18	69.23
46–60	9	3	33.33	6	66.66
›61	1	1	100	0	0

Table 3: Distribution of UTI Pathogens with Sex of Patients

Sr. No	Bacteria	Total	Male	% age	Female	Percentage
1	*E. coli*	77	24	31.16	53	68.83
2	*Proteus mirabilis*	9	3	33.33	6	66.66
3	*Staphylococcus aureus*	13	8	61.53	5	38.46
4	MRSA	04	2	50	2	50
5	Klebsiella spp.	04	0	0	4	100
6	*Pseudomonas aeruginosa*	06	3	50	3	50

Note: Methicillin Resistant *Staphylococcus aureus*

Sex wise distribution of bacterial pathogens of UTI patients is shown in Table 3. Among 113 patients, *E. coli* was isolated from 24 (31.2%) male and 53 (68.8%) females. *Staphylococcus aurous* was isolated from 8(61.5%) male and 5(38.46%) female. However, four methicillin resistant strains of *Staphylococcus aurous* were also isolated from 2(50%) male and 2(50%) female patients. *Proteus mirabilis* was recovered from 3 male and 6 female patients. Four Klebsiella and six *Pseudomonas aeruginosa*

ISSN: 2307–5465 (Online); ISSN: 2307–5716 (Print)

were also isolated from UTI patients. Antimicrobial sensitivity pattern of bacterial pathogens isolated from UTIs patients is shown in Table 4. Among bacterial pathogens, 72% isolates were resistant ampicillin, 53% were resistant to ciprofloxacin, 51.3% to norfloxacin, 53.1% to tmp/smz, 52.2% to nitrofurontoin, 40.2% to aztreonam, 53.3% to chlorophenicol, 43.4 % to cefaclor, 50.4% to cefazolin,38% to tazocin, 30.1% to tygacil, 21.2% to meropenem, 36.1% to nalidix acid, 5.9% to fusidic acid, 24% to methicillin and 5.9% to vancomycin, respectively. Four *Staphylococcus aurous* species were resistant to methicillin and 13 were sensitive to vancomycin and fusidic acid.

Antibiotic	Resistance age%	Antibiotic	Resistance age%	Antibiotic	Resistance age%
AMP	71.96	TMP/SMZ	53.09	CEC	43.36
NOR	51.3	F	52.21	CZ	50.4
NA	36.08	MEM	21.23	ATM	40.2
CIP	53.1	TZP	38.05	FA	11.8
C	53.3	Tygacil	30.08	VA	5.88
MET	24				

Note:AMP:Ampicillin;Cip:Ciprofloxacin;F:Nitrofurantoin.N:Norfloxacin;C:Chloramphenicol(C);MEM:Meropenem,TYG:Tygacil;CZ:cefazolin;VA:vancomycin;MET:methicilln; TZP:Tazocin; TMP / SMZ :Trimethoprim / Sulfamethaxozole; FA:Fusidic acid

Table 4: Antimicrobial Sensitivity Pattern against UTIs

DISCUSSION

Urinary tract infection in human beings is common problem all over the world (Gupta, 2002). The present study was undertaken to ascertain the causative bacteria of urinary tract infection (UTI) and their antibiotic susceptibility in Kpk, Pakistan. Our study showed that Enterobacteriaceae are predominant causative organisms to cause UTI, followed by Gram–positive cocci, a finding consistent with the results of Pankaj (2012). In present study urine specimens were collected from 200 suspected UTI patients, out of which 113 (56.6%) were positive and 87(43.5%) were negative. Acharya *et al.* (2011) conducted similar kind of study by collecting 950 samples. Out of these 237 (24.94%) samples grew potential pathogens causing UTI and 713(75.05) were negative.

It was observed that UTI was more in females than male. Our finding match with Bashir *et al.* (2008) .They analyzed 109 positive samples in which 36 samples were in male and 73 were in females. UTIs were mainly found in females, the reason might be the shortness of the urethra and its closeness to anus which facilitate entrance of fecal micro–flora to urinary tract. In our study *E. coli* was more in hospitalized patients 55(71.42%) as compared to walk in patients 22 (28.57 %). Naeem *et al.* (2010) studied two groups, outpatient Group I and admitted patients group II in which *Escherichia coli* was the most common isolate in both groups (60% and 53% in Group I and II respectively). Our study is in correlation with Pankaj *et al.* (2012). In 219 positive samples prevailing organism was *Escherichia coli* (81.3%, 178 isolates). Pseudomonas species was 1(11.11%) in hospitalized and 5(83.33%) in walk in patients. Our findings are in fair correlation with the other study in which Pseudomonas spp. was (5.17%) (7.3%), (7%) found to be the causative agent of UTIs respectively (Ojo and Anibijuwon, (2010); Bashir *et al.*, (2008); Naeem *et al.*, (2010)). It gives the impression of extensive dissemination of MDR uropathogens in both community and hospital environment. The contributing factors for the appearance of such high bacterial antibiotic resistance in UTIs from both groups of patients may be numerous. In the present study, the incidence of *proteus mirabilis* was also observed in admitted patients 5(55.55%) and in walk in patients was 4(44.44%). Ojo and Anibijuwon, (2010) also reported Proteus spp. (17.25%) in a finding. Among gram negative bacilli, *klebsiella pneumonia* 5(55.55%) in admitted patients, 4 (44.44%) in walk in patients was also isolated. The results also well correlate with the study carried out Manikandan *et al.* (2011) reported 15.8% incidence of *Klebsiella pneumonia* among uropathogens and Mashouf *et al.* (2009) also reported 10.2% incidence of *Klebsiella pneumonia*. *Staphylococcus aureus* and MRSA was also recovered 5(38.46%), 0(0%) in hospitalized and 8(61.53%),4(100%) in walk in patients respectively. and the results of the present study are comparable with study of Mashouf *et al.* (2009) who reported the incidence of methicillin resistant *staphylococcus* and *staphylococcus aurous* in UTI.

Antibiotic susceptibility tests were performed. Bacterial isolates showed highest rate of resistance against ampicillin (71.96%), ciprofloxacin (53. %1), norfloxacin (51.3%), tmp/smz (53.09%), nitrofurontoin (52.21%), aztreonam (40.2%), chloramphenicol (53.3%), cefaclor (43.36%), cefazolin (50.4%), tazocin (38.05%), tygacil (30.08%), meropenem (21.23%), nalidixic acid (36.08%), fusidic acid (5.88%), methicillin (24%) and vancomycin (5.88%). Our findings match with another study conducted by Naeem *et al.* (2010). Bacterial isolates resistance to two groups outpatient Group I and admitted patients group II was; amoxycillin, amoxycillin/clavulanate (55% and 24%), Ciprofloxacin (63% and 24%), Levofloxacin (73% and 43%), cefixime, cefotaxime (70% and 38%), ceftriaxone (75% and 37%), cefoperazone/sulbactum (93% and 76%), amikacin, imipenem (95% and 86%) and pipracillin/tazobactum (95% and 86%). Another study conducted by Mowla *et al.* (2011) in which 92% strains were resistant to ampicillin (n=33), 52% of the isolates (n=19) were resistant against sulfamethoxazole–trimethoprim, 50% (n=18) to tetracycline, 25% (n=9) to chloramphenicol, 50% against azithromycin, 8.33% (n=3) isolates showed resistance against to mecellinum. Among the strains, 72% were resistant to nalidixic acid (n=26) and 50% (n=18) were resistant to ciprofloxacin.

Our study concludes that *E. coli* is one of the important causative agents of urinary tract infection especially in females. High incidence of single and multiple antibiotic resistant bacterial strains is matter of enormous concern. Meropenem was the drug of choice to control urinary tract infections.

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ISSN: 2307–5465 (Online); ISSN: 2307–5716 (Print)